PIG COMMERCIAL TRAILER VIBRATION PROFILES DURING SUMMER AND A

POTENTIAL METHODOLOGY TO ESTIMATE REAL-TIME MUSCLE FATIGUE

by

DANIELA ALEJANDRA ALAMBARRIO GONZALEZ

(Under the Direction of John Michael Gonzalez)

ABSTRACT

The objectives of these studies were to collect and quantify three-axis acceleration data from six locations within commercial pig transport trailers during summer and determine if electronic muscle stimulation is a valid new methodology to replicate physical activity and estimate muscle fatigue using electromyography. Data indicated that pigs transported in the bottom deck were exposed to hotter temperatures than pigs in the top deck of trailers during all stages of transportation. Data also indicated that pigs on the bottom deck were exposed to greater horizontal accelerations (x- and y-axis). For the second objective, data indicated that electronic muscle stimulus replicates physical activity and stimulates muscle fatigue, which was detected through metabolic analyses of ATP, ADP, NAD+, and NADH. Even though the objectives of these studies were achieved, other tools are still needed to estimate muscle fatigue in real-time and during transportation. In the future, these studies can be used to create vibration comfort levels and provide specific pig health risks because of exposure to vibrations during transportation.

INDEX WORDS: *vibration patterns, transport loss, muscle fatigue, electronical muscle stimulus, electromyography*

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DEDICATION

I would like to dedicate this thesis to my parents, Leidis Gonzalez and Danilo Alambarrio. Without their support through my studies, I would not be where I am today. They inspire me to achieve my goals and go above and beyond. Los amo!

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emotional support, and for making it more exciting to come home from work after a long day. I could make this more personal but all of them know that I don't like people or emotions, so this is it.

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CHAPTER 1-LITERATURE REVIEW

Transportation and Stress

Transport losses

The term “transport loss” describes pigs that die or become nonambulatory at any point of transportation from farms to stunning at the processing plants (Fitzgerald et al., 2009). Pigs are classified upon arrival at the processing plant according to movement and structural appearance. The industry classifies dead pigs are depending on when during transportation process pigs were found dead (dead on arrival or dead in a pen). Nonambulatory pigs are pigs that can move but are unable to keep up with other pigs after transportation. Nonambulatory pigs can be further classified into two different categories. First, injured pigs refer to pigs that possess a compromised ability to ambulate because of a physical injury. The second classification, fatigued pigs are pigs without a physical injury which refuse to move or cannot keep up with other pigs and display open mouth breathing, skin discoloration, muscle tremors, and abnormal vocalization (Bertol et al., 2005).

Transport loss is not a new problem in the pig industry. Data on dead and nonambulatory pigs were first published in 1930 (Smith, 1937). Over time, death and nonambulatory rates have increased. For example, Ritter et al (2020) reported a 0.33% and 0.19% increase from 2005 and 2009, respectively (Ritter er al., 2009) In economic terms, Ritter et al. (2020) estimated transport loss cost the swine industry around $89 million. Additionally, transport loss reduces the pork industry ability to contribute to the U.S food chain and feed a growing population (Morris et al., 2021).

Fatigued pig syndrome

Pig producers attribute the term "fatigued pig syndrome" to describe nonambulatory pigs (Ritter et al., 2009; Fitzgerald et al., 2009; and Doonan et al., 2014). The term is used to loosely classify pigs that arrive at the abattoir without obvious physical signs of disease, trauma, or injury and still are unable to stand on their own or move. Muscle tremors, skin discoloration, abnormal vocalization, increased body temperature, and increased oxygen demand are other signs of fatigued (Anderson et al., 2002). Hamilton et al. (2004) reported physical exercise and physiological or emotional stress as some factors causing fatigue. Fatigued pigs undergo metabolic acidosis characterized by increased lactic acid, decreased bicarbonate values, and decreased blood pH (Bhagavan, 1992; Edwards et al., 2011).

Environmental Condition/Stressors

Transport loss is a multifactorial process (Fitzgerald et al., 2009). Market-weight pigs can become stressed or fatigued by being mixed with other pigs, environmental changes, food and water deprivation, season, transportation period, loud noise, and trailer design. Bligh (1985) and Machado et al. (2021a) identified the thermal environment as the most significant contributor to increased injured, nonambulatory, and death-on-arrival rates. Additionally, pigs are sensitive to heat stress because they have limited energy dissipation through evaporation due to their minimal sweating efficiency (Bligh, 1985). Hence, Machado et al. (2021b) observed pig heat stress severity, specifically, internal heat zones developed within trailers at different transportation stress. The study was conducted in northeastern Brazil with temperatures around 28.2 and 31.2°C and relative humidity between 62 and 68%. The bottom fore and bottom center trailer sections had greater heat than the remaining trailer compartments and pigs transported in these compartments had greater rectal temperatures and respiration frequencies than the rest of the pigs. In other studies, pigs transported in the same compartments showed greater creatine kinase than pigs in other compartments (Pereira et al., 2018; Moak et al., 2022). Trailer ventilation dynamics may explain temperature variation throughout trailers. Compartments directly behind truck cabins showed greater temperatures associated with the restricted air flow and proximity to the truck's engine (Brown et al., 2011). Plitcher et al. (2011) and Moak et al. (2022) observed temperature, relative humidity, and temperature heat index (*THI*) increases while stationary because of heat stagnation and reduced exchange rate or internal airflow caused by the presence of a solid front wall and imbalance of air pressure gradients. During stationary times, studies reported that ventilation or water mist systems can refresh trailers under warm conditions. Pereira et al. (2018) reported lower trailer temperature and THI values during stationary by 5.3 and 4.8 °C, respectively, in trailers with fan-misting banks, but relative humidity increased by 8.6%. The combined effect of increased water vapor and decreased temperature explained Pereira et al. (2018) results. Environmental conditions variation inside trailers suggests the need for improvement in trailer designs.

During transportation, trailer design has potential implications to ease pig movement and environmental conditions inside the trailer (dalla Costa et al., 2007). Potbelly and straight deck trailers are the primary designs used in the U.S. The major difference between a potbelly and a straight deck trailer is the number of internal ramps. Conte et al. (2015) demonstrated that steep internal ramps up to 32° of inclination in potbelly trailers required increased pigs' physical effort. In the same study, pigs that walked the steep ramps to the top and bottom trailer compartments had greater rectal temperature after loading and during transportation than pigs in other trailer sections. Similarly, Ritter et al. (2008) observed that pigs transported on potbelly trailers had greater rates of open mouth breathing and skin discoloration rates than pigs unloaded from straight decks in different seasons.

In the U.S. transport loss rates vary throughout the year; this variation may be accredited to seasonal changes. Fitzgerald et al. (2009) observed data from an integrated pork producer in the Midwest, in the study, fatigued pigs contributed more to transportation loss during the winter, whereas dead pigs contribute more during the summer. Sommaville et al. (2017) measured blood metabolite stress indicators and found pigs transported in the winter had greater blood lactate, but pigs transported during the summer had greater blood cortisol and creatine kinase. Many studies have reported that transport losses are greater in summer; however, the increasing number of nonambulatory pigs during winter is a growing concern (Ritter et al., 2008; Torrey et al., 2013).

Sutherland et al. (2006) observed that nonambulatory rates were impacted by transportation length and journey variation. In the same study, dead-on-arrival pig numbers increased as transportation time increased from 30 minutes to 4 hours, but decreased for transportation time longer than 4 hours. In addition, the rate of nonambulatory pigs decreased as the transportation time increased. Results suggest that pigs with shorter transportation time have insufficient time to recover from stressful situations such as loading and handling. Rademacher and Davies (2005) observed similar results where transportation times longer than 3 hours did not influence transportation loss rates. The previous results agree with Hamilton et al. (2004), and Ritter et al. (2006) suggested that fatigued pigs usually recover within 2 or 3 hours of rest, but many pigs die before they are allowed to rest.

Vibrations

Vibration standard

Pigs are exposed to vibrations during transportation and vibration transmission from the trailers to pigs can be significant and interfere with pigs' comfort and health. The International Organization for Standardization (**ISO**) provides health tolerance limits for human whole-body exposure to vibrations, probability of vibration perception, and motion sickness incidence. The standard provides vibrations magnitudes for comfort, varying from uncomfortable to extremely uncomfortable. In addition, the standard indicates that vibrations between the estimated action value (**EAV**) and the estimated limit value (**ELV**) may pose health risks, and vibrations above ELV are likely to cause health risks. Human whole-body reaction to different vibration magnitudes depends on individual composition and characteristics. Similarly, comfort expectations and annoyance tolerances are different in many trailers (ISO 2631-1, 1997). Currently, no standard methods are available to evaluate animal exposure to vibrations, but attempts have been made to apply human vibration levels and response data to animals. The standard values are yet the best values available; however, values may not apply to animals because of morphology, body size, and stance differences.

Althougth the ISO guideline does not provide specific health problems related to vibrations, musculoskeletal disorders or muscle fatigue, reduced stability, motion sickness, breath shortness, displacement of the gravity center, and back pain are some of the side effects of vibrations above EAV of agricultural tractor operators (Cvetanovic and

Zlatkovic, 2013; Adam and Jalil, 2017). Additionally, floor vibrations in a transporter depends on suspension systems and rigidity. Suspensions commonly used in off-road vehicles and trailers consist of low stiffness suspensions with poor damping that attenuate vibrations in some environments (Randall, 1992; Peeters et al., 2008). Seating systems (floor and seats) are considered the primary contact point for transmission and amplification of vertical vibrations; however, conditions may vary according to agricultural field conditions, truck fabrication, and structure (Adam and Jalil, 2017).

Vibration analysis

The ISO defines how to objectively evaluate human whole-body exposure to vibrations, including exposure parameters, duration, amplitude, and frequency (ISO 2631-1, 1997; Machado et al., 2017). Vibration magnitude calculation depends on which parameters are used to estimate accelerations. The basic evaluation method of ISO guidelines includes the measurement of weighted root mean square (**RMS**) and the fourth power vibration dose value (**VDV**). Weighted acceleration refers to RMS and is often used to estimate vibrations transferred to individuals rather than environment physical vibrations (Morris et al., 2021). Root mean squared calculations include measurements of occasional shock and transient vibrations (ISO 2631, 1997). The RMS is determined by

$$\bar{a}_{RMS} = \left(\frac{1}{N} \sum_{0} a_n^2 \, (n) \right)^{1/2}$$

Where $\bar{a}_w$ is the instantaneous frequency-weighted acceleration, and n represents the number of samples. Root mean squared acceleration is generally the preferred method to estimate the severity of individual human exposure (Griffin, 1998; Gebresenbet et al., 2011). Root mean squared is measured in meters per second to the power 1.75 (m/s^2). In cases where the basic evaluation underestimates the total effect of vibrations, VDV is determined as an alternative measure (ISO 2631, 1997.) Vibration dose value is used to assess vibration exposure over time (T) or accumulative measurement of total vibrations experienced by individual (Griffin 1998; Aradom & Gebresenbet, 2013; Morris et al, 2021). The VDV is based on the fourth power of acceleration, then VDV is more sensitive to shock peaks vibrations (ISO 2631, 1997). Power VDV is measured in meters per second to the power 1.75 ($m/s^{1.75}$) or in radians per second to the power 1.75 ($rad/s^{1.75}$) and is given by

$$VDV = \left\{ \int_{0}^{T} [a_w(t)]^4 \right\}^{1/4}$$

Vibration magnitudes are measured depending on how vibrations reach individual bodies. Technology advances allow the estimation of movement with accelerometers or transducers. Vibrations transmitted to individuals are measured on surfaces between individuals and the source of vibrations (ISO 2631, 1997). The ISO suggests that an accelerometer should be mounted to record motion, but the mount should not alter the standing surface or environment. Even though ISO measurements are based on human comfort and health, the techniques used to quantify the vibration environment do not reference exposure to vibrations for specific species (Morris et al., 2021)

Livestock exposure to vibrations

Vibration levels transferred from trailers to animals during transport and frequencies of a typical cattle trailer were investigated by Gebresenbet et al. (2011). The study measured accelerations on chassis, trailer floor, and animals. Transmissibility was estimated using RMS and VDV accelerations, first between the chassis and floor, and ratio from the floor to cattle. Root mean squared acceleration of animal exposure to vibration in the study were 0.63, 0.68, and 0.75 m/s^2 for vertical, horizontal, and lateral axes, respectively. Floor RMS vibration were 0.67, 0.63, and 0.51 m/s^2 for vertical, horizontal, and lateral axes, respectively. All reported values were between RMS EAV and ELV levels provided by ISO. In addition, vibrations transmitted from floor to animal were amplified by 100-154% VDV and 95-143% RMS. Results indicated that vibration magnitude might increase when transmitted from trailer floors to animals, and transmission to animals may vary depending on trailer sections.

Other factors affecting vibrations besides the trailer suspension system are engine speed, driving speed, road surface, driver, and driving style. Transport companies whose drivers have received formal training in animal transportation are more likely to deliver pigs in good condition (Doonan et al., 2014). In a study, Peeters et al. (2008) observed the effect of driving style on pigs transported in different trailers using VDV as comfort value. Accelerations showed no difference between driving style or drivers, but one driver had a numerically greater VDV in the horizontal direction with a wild driving style. In general, VDV were greater in the vertical direction than in the horizontal direction.

Factors under the driver's control, such as cornering and aggressive braking, cause aggressive shocks from acceleration motion that influence trailer vibrations (Randall, 1992). Poor driving is also associated with bruising (Tarrant, 1990). Thus, Kehler et al. (2022) observed trailer compartment accelerations and its effect on carcass bruising in market cows. Data were recorded on potbelly trailers with five compartments and accelerometers located inside trailers. Root mean squared values were around 1.01, 0.72, and 0.97 m/s^2 for vertical, horizontal, and lateral directions, respectively. During the study, cows were exposed to accelerations above EAV and ELV RMS ISO levels. Results showed that cows transported in the fore compartment had more bruises than cows in other compartments; however, none of the individual trailer compartment acceleration affected bruising. Even though there were no statistically differences, lateral accelerations numerically increased the number of bruising per carcass. In the study, no conclusions were obtained in the study, previous research showed that lateral and horizontal accelerations are impacted by driving style (Peeters et al., 2008). The study also reported uncomplete data; hence, result could vary and differ in RMS values during transportation.

Implementing ISO techniques of human exposure to whole-body vibrations, Morris et al. (2021) collected three-axis accelerations from six locations within commercial transport trailers. Data were collected from six trailer sections on split deck and pot belly trailers. Transportation data were averaged over an entire trip for each pig load and used to calculate RMS and VDV. Vibration patterns observed almost always exceeded EAV and frequently exceeded ELV. Regarding trailer sections, the bottom aft compartment experienced more vibrations by 152% and 181% for RMS and VDV compared to other trailer compartments. Similarly, the bottom fore compartment had greater acceleration than other trailer compartments. In the study, data suggest that compartments located directly above the chassis and wheels of the truck and trailer are exposed to larger vibrations compared to the rest of the trailer sections. In addition, ISO levels suggest that trailer vibrations may be enough to affect pig muscle function.

Tonic vibration reflex

Whole-body exposure to vibrations is detrimental in humans. Muscle reflex contractions are affected by vibrations in humans and animals (Eklund and Hagbarth, 1966; Burke et al., 1976). Tonic vibration reflexes are involuntary muscle contractions induced by exposure to vibrations (Park and Martin, 1993; Kiguchi and Maemura, 2021). Tonic vibration reflex (**TVR**) is vibrations' most observed motor effect. Gregory et al. (1988) suggested that vibration's shaking effects directly affect intrafusal muscles that stimulate the release of cross-bridges and promote the thixotropic process (Nakajima et al., 2009). Additionally, mechanical vibrations influence the neurological network by stimulating sensory receptors within cutaneous, muscular, and articular structures and affect spinal reflexes (Burke et al., 1976).

Muscle sensitivity to vibrations has shown that TVR is mainly attributable to muscle spindle Ia (Granit and Henatch, 1956; Burke et al., 1976). Vibrations to elicit TVR, first activate the muscle spindle and produce an Ia afferent discharge signal. Then, muscle spindle discharges are sent to the spinal cord, activating monosynaptic and polysynaptic circuits, causing the muscle to contract (Burke et al., 1976; Nakajima et al., 2009). Tonic reflexes are observed in contracting and relaxed muscles and increase progressively with time exposure to vibration and can persist after vibration ends. In addition, TVR is associated with specific soft tissue disorders, contributes to muscle fatigue, and increases the risk of cumulative trauma disorders (Park and Martin, 1993). Most research on TVR has been done using direct electronic muscle stimulation and measured with surface EMG (Romaiguere et al., 1991; Park and Martin, 1993; Nakajima et al., 2009;).

Muscle Fatigue

Muscle contraction

Muscle contraction involves regulatory and contracting myofibrillar proteins. During contraction, regulatory proteins actin and myosin form cross-bridges directly between their filaments generating contractile force. Therefore, regulatory proteins tropomyosin and troponin facilitate the contractile process depending on the availability of calcium ions concentration in the fiber's sarcoplasm (Arbele, 2001).

Muscle contractions can be initiated through several means; however, most contractions are initiated through stimuli arrival at the sarcolemma of the muscle fiber. In skeletal muscle, contractions are often initiated by electrical stimulus from the brain or spinal cord and transmitted by nerve fibers. Electrical potential for stimulus exists between the inside and the outside of cells under resting conditions. The electrical potential is maintained by positive ions on the inside and negative in the outside fluids of nerve and muscle fibers. Intracellular fluids are high in potassium ions (K+), while extracellular fluids contain high concentrations of sodium (Na+) and chloride (Cl-) ions. Ideal concentrations of Na+ and K+ across the plasma membrane are maintained by the active transport of Na+ out of the cell and K+ into the cell. In order to move Na+ and K+ across concentration gradients, adenosine triphosphate (**ATP**) is required. In addition, nerve and muscle fibers can transmit electrical impulses or action potentials along their membrane surface. The action potential is initiated by increasing membrane permeability caused by depolarization. Action potentials are transferred from the motor nerve to muscle fiber via T-tubules responsible for releasing Ca2+ from the sarcoplasmic reticulum (Arbele, 2001).

The immediate energy source during muscle contraction is ATP. During contraction, ATP is hydrolyzed into adenosine diphosphate (**ADP**) and inorganic phosphate (**Pi**). Calcium enhances the splitting activity of myosin ATP that resides in the head region of myosin. Thus, the increased Ca2+ concentration initiates the contraction process by moving and binding tropomyosin and troponin, which allows the formation of cross-bridges between actin and myosin. Cross-bridges develop contractile force when the angle filament attachment changes and actin filaments are pulled to the center of the sarcomere. During contraction, actin and myosin length and A band are not affected; however, sarcomere length and the width of I and H zones are influenced (Arbele, 2001).

Energy Sources for Muscle Contraction- ATP, ADP, NAD+ and NADH

Muscle contraction is an energetically demanding process; however, the amount of ATP in muscle is insufficient. Hence other metabolic pathways must be available for the resynthesis of ATP. The faster energy source for ATP is synthesis throughout ADP phosphorylation with the result of ATP and creatine. Phosphorylation happens in the sarcolemma; therefore, ATP is broken down through contraction; however, in this pathway, ATP is subjected to depletion during long periods of contraction (Arbele, 2001; Zumbaugh et al., 2022).

Aerobic metabolism is the most efficient mechanism of ATP synthesis. During aerobic metabolism, glycogen is broken down into glucose molecules (glycolysis) that are further broken down to produce pyruvic acid. From glycolysis, ADP is re-phosphorylated to ATP, where hydrogen ions are transported to the mitochondria. Tricarboxylic acid (**TCA**) is the second part of aerobic metabolism and occurs in the mitochondria (Arbele, 2001; Enoka and Duchateau, 2008). Mitochondrial ATP requires the coenzyme nicotinamide adenine dinucleotide (NAD+). As an essential coenzyme, NAD+ gains two electrons and protons from glycolysis and multiple TCA steps and is reduced to NADH. Pyruvate and NADH provide reducing equivalents for the TCA and the electron transport chain (**ETC**), which are responsible for ATP production. In the ETC, mitochondrial NADH is oxidized and donates its protons, reducing oxygen to water (Stein and Imai, 2012).

Anaerobic metabolism can supply energy to muscles when the oxygen supply is limited. During oxygen limitation, the hydrogen ions released from glycolysis and the TCA accumulate in the muscle and are used to reduce pyruvic acid to lactic acid, which allows glycolysis (Arbele, 2001; Westerblad et al., 2010). From anaerobic metabolism, Latic acids accumulate in the muscle, which in abundance reduces glycolysis rate, which reduces ATP resynthesis; therefore, the muscle fatigues, slowing muscle contraction rate because of insufficient energy and the acidic environment (Arbele, 2001).

Muscle Fatigue

Skeletal muscle intense activation results in decreased contractile function. Muscle denotes the failure of contractile proteins to maintain force output leading to decreased physical activity or performance. The site of muscle fatigue depends on the task being performe. Numerous mechanisms have been suggested as causative of muscle fatigue; however, most are related to alterations in excitation or cell metabolisms (Enoka and Stuart, 1992; Enoka and Duchateou, 2008). Although the causes of muscle fatigue are not clearly understood, limited energy supply, specifically the ATP generating process, accumulation of waste products and the depletion of muscle glycogen and low blood glucose appears to be important contributing factors (Fitts, 1994; Sahlin et al., 1998). In addition, studies have demonstrated that levels of NAD+ and NAHD are also affected by muscle fatigue. Duboc et al. (1988) and White and Schenk (2012) observed that NAD+ increase and NADH decrease with fatigue of skeletal muscle.

Muscle fatigue is a complex process that involves multiple factors and is relatively dependent on the fiber type composition of contracting muscle and the intensity and duration of contractile activity. Adult mammalian skeletal muscle contains four distinct fiber types (type I, IIA, IIX, and IIB). Fiber types are classified on their functional and metabolic properties as fast glycolytic, fast oxidative glycolytic, and slow oxidative. Each fiber type contains a specific isozyme for contractile protein myosin, and fibers are identified on their histochemical-determined myosin ATPase activity (Fitts, 1994). Thus, the fast type IIb and slow type I fibers contain the greatest and lowest myofibrillar ATPase activity, respectively. The fast type IIb fiber had the greater fatigability due to their low mitochondrial content, low efficiency, and dependence on anerobic metabolisms. Therefore, contractile properties are dependent on specific cellular associated with cross-bridge cycle (Fitts, 1994; Aberle et al., 2001; Westerblad et al., 2010).

Electrical Muscle Stimulation

Electromyostimulation or electrical muscle stimulation (**EMS**) consists of applying discontinuous electric impulses to activate upper motor neurons through electrodes on the skin close to dermis tissue (Kemmler et al., 2012). Electrical muscle stimulation impulses antidromic transmission of the signal and replace action potentials causing involuntary muscle contractions (Hsu et al., 2011). Electrical muscle stimulation has been extensively used as a promising approach for treating humans of nearly every age who suffer from muscle mass impairment, extended bed rest, and movement restrictions because of musculoskeletal complications, neurodegenerative diseases, and respiratory or cardiac diseases (Sabut el al., 2010; Kemmler et al., 2012).

Electrical stimulus involves body movement produced by repetitive muscle contractions that increase energy expenditure because the more muscle contracts, the more energy consumed. Research has shown that electrically stimulated muscle had different metabolic responses than voluntary muscle contractions (Kemmler et al., 2012; Kortiaunou et al., 2021). During EMS, fast-twitch fibers are the first ones to be activated because of their large nerve axons with low-input resistance against external stimulation and their superficial location. When studying metabolic responses of EMS, Kortiaunou et al. (2021) observed greater oxygen consumption and greater glycolysis in Type II fibers than in Type I in EMS contracted muscle. Similarly, Eijsbouts et al. (1997) and Banerjee et al. (2005) stated that EMS contracted muscle increases energy expenditure and oxygen consumption by 0.7 and 0.11 per minute, respectively, compared to voluntary muscle contraction.

Muscle contractions induced by EMS can also increase muscle fatigue. The increase in impulse frequency potentially leads to quick-setting muscle fatigue. The EMS frequency refers to the pulses produced per second during stimulation measured in Hertz (Hz; Kesar and Binder-Macleod et al., 2006). Electrical muscle stimulation frequencies vary depending on the intervention and type of muscle, but most clinical implementation uses 20-50 Hz patterns. Jones et al. (1979) observed that muscles stimulated with low frequencies (10-30 Hz) had a progressive decline of muscle forces. Therefore, low frequencies are used to avoid muscle exhaustion. On the contrary, Bigland-Ritchie et al. (1979) and Vivodtzev et al. (2006) observed a rapid decline in muscle forces and high fatigue with high frequencies; therefore, electrically stimulated muscles produce more fatigue and need more time to recover than voluntary muscle contractions (Bigland-Ritchie et al., 1979; Downey et al., 2016).

Electromyography

Electromyography (**EMG**) studies motor unit action potentials associated with muscle activity during movement (Al-Mulla et al., 2011). With EMG, muscle fibers are stimulated to contract by action potentials triggering depolarization and polarization within individual muscle fibers creating magnetic fields (Williams, 2018). Then, the recorded data represents the sum of myoelectrical activity for a defined event in the selected muscle. In humans, this muscle evaluation is commonly used to collect muscle function during specific movement postures, muscle function activity during sports, occupational and rehabilitation movements, and maternal muscle activity. In veterinary medicine, EMGs have become a practical clinical examination of skeletal muscle activity.

There are two methods of EMG, indwelling EMG and surface EMG. Surface EMGs are a non-invasive technique used on conscious animals during locomotion to study normal, and pathological muscles and measure muscle fatigue (Williams, 2018). The use of surface EMG is limited to superficial musculature and signal interference because of skin displacement associated with poor electrode attachment. Similarly, muscle signals are affected by other muscles being integrated into the signal, and the depth of subcutaneous muscles reduces the reliability of EMG data (De Luca et al., 2010; Williams, 2018). The most accurate measurements of EMG data are the root mean square (**RMS**), median power frequency (**MdPF**), and mean power frequency (**MPF**). The RMS represents the muscle load in time to measure amplitude or electrical activity associated with muscle fiber recruitment (Bartuzi et al., 2007; Noel et al., 2016). Then, the MdPF and MPF are signal power parameters and refer to the velocity at which motor unit action potential travels along muscle fibers during contraction (Soares et al., 2015; Noel et al., 2016). When evaluating muscle fatigue, Noel et al. (2016) determined that RMS increases and MdPF and MPF decrease as muscle fatigues. Additionally, muscle fatigue in EMG signals is reflected by an increase of amplitude and a decrease of spectral frequencies (Enoka and Duchateou, 2008).

CHAPTER 2-COMMERCIAL STRAIGHT-DECK TRAILER VIBRATION AND MICROCLIMATE CONDITIONS DURING MARKET-WEIGHT PIG TRANSPORT DURING SUMMER[1]

[1]D. A. Alambarrio, B. K. Morris, R. B. Davis, K. K. Turner, L. A. Motsinger, T. G. O'Quinn, and J. M. Gonzalez. Accepted by *Frontiers in Animal Science*.

ABSTRACT

The objective of this study was to collect and quantify three-axis acceleration data from six locations within commercial pig transport trailers during summer. Two trucks with straight-deck trailers transporting two loads per day were observed for five consecutive days (N = 20). Accelerometers were placed under trailers' top and bottom decks' (**DEC**) floors, in the center, of three sections (**SEC**; fore, middle, aft). Data from each trailer section were processed to calculate z- and x-y-axis root-mean-squared (**RMS**) and vibration dose value (**VDV**) during loading, transport, and unloading. There were no DEC × SEC interactions or SEC main effects for z-axis RMS or VDV during any transportation stages ($P > 0.06$). The bottom deck had greater x-y axis RMS than the top deck during all transportation stages ($P < 0.01$). The bottom deck had greater x-y-axis VDV than the top deck during loading and transport ($P < 0.03$), but no difference ($P = 0.52$) during unloading. The bottom deck had greater z-axis RMS and VDV than the top deck during loading and transport ($P < 0.01$), but there were no differences during unloading ($P > 0.07$). There were no SEC effects for x-y- and z-axis RMS and VDV during all transportation stages ($P > 0.06$). Acceleration values were compared to exposure action values (**EAV**; injury possible) and exposure limit values (**ELV**; injury likely) vibrations thresholds. Over the five observation days during all transport stages, a greater percentage of compartments violated both RMS and VDV thresholds in the x-y orientation (average 90%) more than the z-oritentation (average 76%). Overall, these data indicate straight-deck trailer bottom decks experience greater three-axis vibrations than top decks and pigs may

experience discomfort during transportation that could contribute to fatigue or the non-ambulatory condition.

Keywords: *accelerations, animal wellbeing, environment, fatigued pig syndrome, stress, transport loss*

INTRODUCTION

The animal food industry is under social pressure to adhere to acceptable animal welfare standards. From a social perspective, animal welfare standards account for individual livestock needs and provisioning stress-free environments. Pig are transported more the once in their lives because of the U. S. multisite pork production, imposing stress that potentially causes sickness or death (Kephart et al., 2014). Thus, transportation welfare is a growing social concern. During transportation, livestock are exposed to multiple stressors including handling, steep loading ramps, poor driving skills, feed and water deprivations, and temperature and humidity variation (Hamilton et al., 2004; Peeters et al., 2008; Schwartzkopf-Genswein et al., 2012). Many of these factors and other transportation related factors contribute to the resulting meat quality (Correa, 2011; Correa et al., 2013), but more importantly, the animal's welfare (Pilcher et al., 2011).

In the pig industry, transport loss is the term which refers to pigs that die or become non-ambulatory at any stage from trailer loading at the farm to entering harvest at the commercial abattoir (Ellis et al., 2003; Fitzgerald et al., 2009). A subcategory, non-ambulatory pigs, cannot move, refuse to walk, and display signs of severe acute stress after transportation (Anderson et al., 2002; Ritter et al., 2009). The non-ambulatory category can further be subdivided into two categories. Non-ambulatory, non-injured or fatigued pigs which refuse to walk or keep up with other pigs, but display no signs of injury, trauma, or disease. Non-ambulatory, injured pigs become non-ambulatory due to a physically compromised ability to ambulate (Ritter and Ellis, 2008; Kephart et al., 2010). The financial burden associated with non-ambulatory and dead pigs increased from $46 million during 2006 (Ritter et al., 2009) to $89 million during the 2012-2015 production window (Ritter et al. 2020). Non-ambulatory and dead pigs not only impact pork industry economics, but also reduce the pork industry's ability to contribute to the food supply and feed a growing population as estimated by Morris et al. (2021).

During transportation, pig's comfort can vary depending on trailer compartment and design (Randall et al., 1996). Different trailer designs are used to transport pigs; some differences between trailers are the number of compartments and ramps (dalla Costa et al., 2007; Ritter et al., 2008). Some trailer designs may increase pig handling difficulty during loading and unloading, which may result in more stress (Ritter et al., 2008; Conte et al., 2015) and an increase in transport loss incidence (Cormier and Doonan, 2008; Correa, 2011). Additionally, divergent trailer designs may have different structures, suspension systems, and damper types; however, suspensions commonly used in off-road vehicles and trailers consist of low-stiffness suspensions with poor damping that attenuate vibrations in some environments (Randall, 1992; Peeters et al., 2008). Thus, trailer design has potential implications that affect pig movement (Ritter et al., 2008) and trailer microclimate (Morris et al., 2021).

While not extensively studied as other microclimate influencers, trailer vibrations could be a stress influencer during transportation. Early research indicated pigs were more sensitive to fast simulated trailer vibrations than road noise of slow vibrations (Stephens et al., 1985). Two studies by Perremans et al. (1998; 2001) reported 15 to 25 kg piglets showed elevated heart rate and plasma cortisol and adrenocorticotropic hormone when vertically vibrated. During transportation, Randall (1992) and Aradom et al. (2013) showed pigs were subjected to vertical, lateral, and horizontal vibrations induced by vehicle motion which displaced the pig's center of gravity, causing discomfort and movement sickness. Vibrations are primarily caused by road surface roughness, undulation curvature, variations in speed, and poor suspension systems (Gebresenbet et al., 2003; Rebelle, 2021). Currently, the International Organization for Standardization (**ISO**) 2631-1 provides exposure action values (**EAV;** injury possible) and exposure limit values (**ELV;** injury likely) as guidance for human whole-body uncomfortable and potentially dangerous vibration levels (ISO 2631-1, 1997; ISO 2631-5, 2018). The ISO does not report pig health risks or performance effects; however, Streijger et al. (2015) demonstrated vibration exposure had profound effects on pig's specific body parts and systems depending on its frequency, amplitude, and duration. In pigs, Morris et al. (2021) suggested potbelly and straight deck trailer vibrations violated ISO EAV and ELV thresholds to be considered uncomfortable and possibly affect pig's muscle function, especially in the bottom aft (**BA**; Figure 1) trailer compartment. These data were collected during winter and the literature demonstrates season strongly influenced vibrations, transportation losses, and distribution within loss categories. Fitzgerald et al. (2009) showed fatigued pigs represented the greatest portion of total loss during winter and dead pigs were greater during summer months. Environmental stressors of pig transportation are well documented in the literature (Ritter et al., 2007; Caulfield et al., 2014; Sommavilla et al., 2017; Driessen et al., 2020). Diemand (1991) reported materials, including rubber and metal, become stiff and/or brittle and vibrations from diesel engines are amplified when exposed to cold conditions. This could indicate vibrations reported by Morris et al. (2021) are not reflective of vibrations experienced by pigs during the warm conditions of the summer. Therefore, the objective was to collect and quantify three-axis acceleration and microclimate conditions from locations within commercial transport trailers shipping market-weight pigs during the summer.

MATERIALS AND METHODS

Before beginning the trial study methods were evaluated by the Institutional Animal Care and Use Committee (**IACUC)** at the University of Georgia and the study was determined not to require IACUC approval. Approval was not required because all procedures occurred before and after client-owned pig transport and researchers did not observe live pigs (see below). Written informed consent was obtained from the owners for record collection on their pigs utilized in this study.

Data Collection

Vibration data were collected over 5 days in August 2021 on two straight-deck trailers (Figure 1) designated and owned by one North Carolina producer. Accelerometer deployment methods of Morris et al. (2021) were followed. Two loads of finished pigs were observed per truck every day (N = 20). Accelerometer devices [Pelican 1200 Case (Pelican, Torrance, Ca), Omars AC power supply (Wellmade, Shenzhen, China), myRIO accelerometer (National Instruments, Austin, TX)] were attached to the under-side of the floor of the top and bottom fore (front) section of the trailer (**TB** and **BF**, respectively), center section (**TC** and **BC**, respectively), and aft (back) section (**TA** and **BA**, respectively) with four rubber tarp straps. One accelerometer was placed in the center of the floor (see Morris et al., 2021 for pictoral representation) with the x-axis pointed forward to the direction of movement, y-axis pointed left/right, and z-axis pointed up/down.

Accelerometer data were recorded on SanDisk Cruzer Fit 16 GB USB flash drivers (Western Digital, San Jose, CA). Temperature and relative humidity of each compartment were recorded every minute with USB data loggers (Model OM-HL-SP-TH; Omega Engineering Inc., Norwalk, CT). Data were not available for the day 1 and 5 BA sensor due to a lost sensor and loss of power.

Trucks and trailer's make, model, and year information were collected prior to trucks leaving their headquarters, but remain confidential for this publication. Trailers were manufactured in 2020, had two decks, air-bag suspensions, no internal ramps, eight compartments, did not possess mechanical ventilation systems, and all side and three roof vents were open to allow maximum airflow. Aside from gravel roads at the farms (less than approximately 5% of total distance traveled), trucks traveled along paved roads, and all trips terminated at the same commercial abattoir. On days one and four, trucks transported pigs from the same farms during each morning and afternoon transport session, but farms were different on each day and session (Farm #1, 2, 3, and 4, 33.0, 48.4, 71.6, and 71.6 km to plant, respectively). On day two, both loads for both trucks originated from the same farm (Farm #5, 32.0 km to plant). On day three, both trucks transported from the same farm during the morning session (Farm #6, 58.3 km from plant), one truck transported from the morning farm during the afternoon session, and the second truck transported from a different farm (Farm #7, 33.3 km from plant). On day five, loads were transported from four different farms (Farms #8, 9, 10, 11, 71.5, 25.7, 86.9, and 82.1 km from plant, respectively). Prior to transporting afternoon loads, trucks returned to headquarters to be washed.

The producer provided documentation of trailer start and end times. Pigs were marked for transport by company personnel prior to shipping, loaded onto trailers by pen, and were mixed with pigs from other pens within a compartment. Pigs were loaded at an average density of 0.47 ± 0.02 m^2 per pig or 252 ± 0.11 kg/m^2. The producer provided livestock receiving tickets that included the number of pigs transported, loaded and unloaded truck and trailer weights, which were used to calculate loaded and average pig weight. Using standards of the producer, pigs were subjectively categorized as "dead" or "cripple/stress" by trained personnel at the abattoir's receiving alley. Pigs found dead or euthanized on the trailer during unloading were classified as dead. A pig not capable of walking under their own power without assistance off the trailer was categorized as cripple or stressed. Crippled animals had a clear injury preventing them from walking off the trailer.The producer provided Samara Vehicle Telematics Information (Samsara Inc., San Francisco, CA) of truck and trailer motion from the farm to the abattoir because passengers were not allowed to track movement. System global positioning system were used to estimate the three stages of transportation process noted below.

Vibration Analysis

The methods of Morris et al. (2021) were followed but data were processed and analyzed within three different stages of the transportation process; loading (**LOD**), transport (**TRA**), and unloading (**ULD**). The LOD stage consisted of the time truck and trailer backed up to the farm's loading dock until they moved away from the dock. The TRA stage consisted of the time truck and trailer left the farm until they arrived at the abattoir's gate. The ULD stage was from the time the truck and trailer arrived at the abattoir until they moved away from the unloading dock.

MATLAB (MathWorks, Natick, MA) was used for vibration analyses. Response variables calculated were root mean squared (**RMS**), used to evaluate pig vibration exposure, and vibration dose value (**VDV**) to quantify total vibration experienced over time. The weights given by ISO 2631-1 were applied to the RMS values to quantify the effective vibration experienced by the pigs instead of physical vibrations in the environment. The acceleration data frequency content was analyzed by calculating power spectral density. Motion in the vertical direction consisted of the z-axis which was the same for the trailer and pigs, and the horizontal direction, which was the x-y axis combined as a general vibrational bulk quantity represented by RMS or VDV. Pig vibration exposure was compared to ISO thresholds of EAV and ELV of RMS 0.43 and 0.86 m/s^2, and VDV 8.50 and 17.00 $m/s^{1.75}$, respectively (ISO 2631-1, 1997).

Temperature and Relative Humidity Data Processing

After each data collection day, temperature and relative humidity data were downloaded using LogPro Software (Omega Engineering Inc.) and imported to Microsoft Excel. Data were averaged within four stages of transportation; LOD, TRA, 30 minutes after transport began (**TRA+30**), and UDL. Temperature was analyzed 30 minutes after transport commenced to determine initial effects of pigs and wind velocity on the environment (Morris et al., 2021). Historical temperature and relative humidity data were collected by time from the nearest national weather station (Fayetteville, NC). These values were utilized to calculate trailer compartment temperature and relative humidity change from ambient (**Delta**). Temperature and relative humidity were also utilized to calculate temperature-humidity index (**THI**) using the equation THI (°C) = 0.8T + (RH/100) × (T-14.3) + 46.4, where T denoted temperature and RH denoted relative humidity. Values less than 74 were considered “safe”, between 74 and 79 were considered “critical”, between 79 and 84 were considered “dangerous”, and greater than 84 were considered “emergency” (National Oceanic and Atmospheric Administration, 1976).

Statistical analyses

The RMS and VDV data were analyzed as a completely randomized design with a 2 × 3 factorial using trailer load as the experimental unit. Fixed effects included trailer Deck (**DEC**; Bottom or Top), Section (**SEC**; Fore, Center, or Aft), and their interaction. No random effects were included in the model. Temperature and humidity were sorted by time of day (Morning or Afternoon) and analyzed as above. All data were analyzed using the PROC GLIMMIX statement with the Kenward-Roger degrees of freedom approximation of SAS 9.4 (SAS Inst. Inc., Cary, NC). Pairwise comparisons between the least square mean of factor level comparisons were calculated with the PDIFF command. All means presented are LSMeans and variation is reported as standard error of the mean. Differences were considered significant at $P < 0.05$.

RESULTS

Load Descriptive Statistics

Across both trucks and all trips, approximately 187 pigs, weighing 122 kg, experienced transportation trips (LOD to UDL) lasting 157 min (Table 2.1). In the present study, morning and afternoon trips had numerically similar LOD, TRA, and UDL durations; however, waits were numerically longer in the afternoon resulting in more extended total trip time and greater variability. Temperature is another well-documented transport loss influencer. As expected, afternoon trips had hotter atmospheric temperatures by 4.7, 6.8, and 6.0°C during LOD, TRA, and ULD transportation stages, respectively.

Morning trips were exposed to more humid environmental conditions differing from afternoon trips by 28.2, 28.3, and 26.3% during LOD, TRA, and UDL transportation stages, respectively.

Trailer location vibration analysis

There were no DEC × SEC interactions for x-y-axis RMS and VDV during all transportation stages ($P > 0.11$; Figures 2.2 and 2.3). There were no SEC main effects at all transportation stages ($P > 0.07$), but the bottom deck had a greater RMS value than the top deck during all stages ($P < 0.01$). There were no SEC main effects for VDV during all transportation stages ($P > 0.12$), but the bottom deck had a greater VDV compared to the top deck during LOD and TRA ($P < 0.01$). There was no SEC effect ($P > 0.52$) during ULD.

There were no DEC × SEC interactions for z-axis RMS and VDV ($P > 0.13$; Figures 2.4 and 2.5). During LOD and TRA stages, the bottom deck had greater RMS and VDV than the top deck ($P < 0.01$); however, there were no DEC effects for RMS and VDV during ULD ($P > 0.07$). There were no SEC main effects on RMS and VDV during all transportation stages ($P > 0.06$).

Compartment EAV and ELV Threshold Violations

During all stages of transport over the five days of observation, 100% of compartments were exposed to x-y axis RMS values above the EAV (0.43 m/s^2; Figure 2.6). The x-y axis ELV (0.86 $m/s^{1.75}$) by 53, 100, and 96% of compartments during LOD, TRA, and ULD, respectively. Z-axis RMS EAV threshold was violated by 96, 100, and 96%, and the ELV threshold was violated by 25, 71, and 63% of compartments during LOD, TRA, and ULD, respectively (Figure 2.7). One-hundred precent of compartments’ x-y axis values violated the EAV threshold (8.5 m/s^2) during all tranpostation stages (Figure 2.8). Compartment x,y axis VDV values violated the ELV threshold (17 $m/s^{1.75}$) by 71, 86, and 78% during LOD, TRA, and ULD, respectively. Z-axis VDV EAV threshold was violated by 96, 100, and 93%, and the ELV threshold was violated by 43, 68, and 64% of compartments during LOD, TRA, and ULD, respectively (Figure 2.9).

Temperature and relative humidity analyses

There were no DEC × SEC interactions for all temperatures and relative humidity measurements recorded in the morning and afternoon ($P > 0.21$; Table 2.2). During the morning at all transportation stages, there were DEC effects ($P < 0.02$) for all environmental measures except relative humidity and delta-relative humidity during LOD ($P > 0.09$). The bottom deck had a greater ambient temperature, delta-temperature, and THI compared to the top deck ($P < 0.02$). The top deck had greater relative humidity and delta-relative humidity than the bottom deck during TRA+30, TRA, and UDL stages ($P < 0.01$). There were no SEC effects for all measures at all stages ($P > 0.10$), except temperature and delta-temperature at LOD and UDL, and THI at LOD. During LOD, the fore section had a greater ambient temperature, delta-temperature, and THI than the center and aft sections ($P < 0.02$), which did not differ ($P > 0.83$). During UDL, the fore section had greater temperature and delta-temperature compared to the aft section ($P < 0.01$), and the center section did not differ from the other sections ($P > 0.18$).

During the afternoon, there were no DEC effects for all measures at all stages ($P > 0.13$), except the top deck had a greater ambient temperature, delta-temperature, and THI compared to the bottom deck during LOD ($P < 0.03$). There were no SEC effects for all measures at all stages ($P > 0.13$) except for temperature and delta-temperature during UDL

(P = 0.04). The fore section had greater temperature and delta-temperature compared to the aft section (P < 0.01), and the center section did not differ in either measure compared to the other sections (P > 0.11).

DISCUSSION

In North Carolina, Morris et al., (2021) observed longer trips (203 min) during winter months compared to the present study. This was a function of a different producer's pigs and farms being observed. Perez et al. (2002) and Ritter et al. (2006) reported pigs exhibited greater fatigue signs after short trips, but most pigs recovered after 180 min. In the present study, 0.11% of total pigs transported were reported dead in the morning. This percentage was below U.S. industry values reported by Ritter et al. (2020) that ranged between 0.22 and 0.24%. Morris et al. (2021) reported North Carolina load's dead pig percentage during winter were equal to the present study. The present study observed 0.11% and 0.05% of pigs were categorized as fatigued during the morning and afternoon, respectively, which were smaller values than the value reported by Ritter et al. (2020; 0.63%). Because Ritter et al. (2020) surveyed a much larger percentage of the pigs harvested, this could be the reason for discrepancy in values compared to the current study and Morris et al. (2021; 0.39%).

Market-weight pig losses during transportation greatly impact animal wellbeing and industry economics (Ellis et al., 2003). Unfortunately, transport is an inevitable event of the modern pig industry and transportation conditions directly impact pig welfare, stress and ultimate meat quality. Trailer design, space allocation during transport, distance traveled, and weather are major stressors associated with transportation losses and are well documented in the literature (Ritter et al., 2007; Fitzgerald et al., 2009; Pilcher et al., 2011).

Because of these studies, pork producers implemented numerous techniques and operating procedures to reduce non-ambulatory pig incidence (Fitzgerald et al., 2009); however, few studies focus on trailer vibration impacts on market pig stress and muscle fatigue and function (Randall et al., 1996; Sutherland et al., 2006).

Randall (1992) and Peeters et al. (2008) concluded livestock experienced greater vibrations than truck drivers and trailers were weakly dampened structures not designed to minimize vibrations. Each trailer section's vibration patterns were analyzed in the present study, but individual compartments did not differ in RMS or VDV accelerations; however, pigs transported in trailers' bottom decks were exposed to greater RMS and VDV accelerations than pigs transported in the top deck by 54.8 to 67.6% on the z-axis and 63.3 to 76.1% on the x-y-axis during LOD and TRA. Multiple studies reported accelerations were more prominent in trailer's bottom because vibrations were transmitted from the tires and chassis directly to the bottom deck and then to the top deck (Arandom and Gebresenbet, 2013; Morris et al., 2021). In contrast to the current study, Morris et al. (2021) reported the bottom aft compartment z- and x-y axis RMS and VDV accelerations were greater by 152 to 181% compared to other compartments. The present study used two straight-deck trailers with newer truck and trailer models compared to those used by Morris et al. (2021), who combined data from various potbelly and straight-deck trailers. Ritter et al. (2007) and Ritter et al. (2008) found pigs transported in potbelly trailers showed increased fatigue signs when compared to straight-deck trailers. In addition to differences in trailer type observed, the present study had the same two drivers throughout data collection, allowing a more consistent driving style. Peeters et al. (2008) reported driving style affected horizontal accelerations, but not vertical accelerations and increased pigs' stress levels.

Furthermore, Arandom and Gebresenbet (2013) demonstrated different drivers produced divergent vibration levels. Therefore, type of trailers observed and consistency in drivers could be the reason for absence of individual compartment differences in the current study. There are no guidelines for evaluating physiological impacts of exposing pigs to vibrations; however, Peeremans et al. (1998) reported piglets experiencing greater accelerations demonstrated heart measures indicative of greater stress. The ISO standard is based on human whole-body exposure to vibrations, but Randall et al. (1996) stated ISO standards were the best available guidance to estimate animal discomfort in animals. Although past studies used the ISO threshold as a reference, Gebresenbet et al. (2011) used ISO to evaluate vibration levels and frequencies in dairy cows and later investigated vibration effects on cattle postural stability and pig behaviors (Aradom and Gebresenbet, 2013). The ISO 2631-1 provides a list of approximate comfort levels with their respective RMS and VDV thresholds, where EAV and ELV values correspond to "injury possible" and "injury likely" comfort levels, respectively (ISO 2631-1, 1997; Morris et al., 2021).

In the presnt study, a greater percentage of compartments observed violated the EAV and ELV in the x-y axis, possibly indicating vibrations in this orientation were more impactful than the z-axis.

Morris et al. (2021) observed that RMS and VDV accelerations exceed ISO thresholds more on the z-axis. On potbelly trailers, trailer compartments' z-axis RMS and VDV accelerations exceeded the EAV by 100% and exceeded ELV by 64.4 and 57.8%, respectively. On straight deck trailers, trailer compartments RMS and VDV exceeded EAV by 96.2 and 94.1%, respectively, and exceeded ELV by 47.2 and 37.2%, respectively. On the x-y axis orientation, EAV and ELV were violated by pot-belly trailer RMS values by

67 and 14%, respectively, while straight deck trailers violated thresholds by 41 and 9%, respectively. It is unknown why the results of the current study differ, but possible factors include trailer type, driver, types of roads, weather, number of curves (more in North Carolina versus Kansas), and trailer age/upkeep. These are all factors that are uncontrollable in an industry study such as the present one.

Gebresenbet et al. (2011) reported vibration exposure values were 0.11 and 0.46 m/s^2 above the European Union EAV 0.5 m/s^2 on the z- and x-y-axis, respectively. Randall (1992) stated random horizontal axis was the greatest contributor to postural instability which increase discomfort. While ISO standards do not provide a specific exposure time period to elicit injury, prolonged (years) and repetative exposure would be required to cause substantial effects. But, this does not mean that vibrations pigs are experiencing are not causing injury or muscle fatigue conditions, especially given the flooring they are transported on. This question should be addressed by subsequent research.

The divergent atmospheric conditions of the current study influenced in-trailer temperature and humidity patterns experienced by transported pigs. Environmental conditions and trailer compartment locations can impact pig comfort and welfare. Thermal variations among compartments inside the trailer occur due to the lack of ventilation because of trailer pressure distribution variation during transportation (Machado et al., 2021a; Machado et al., 2021b). In the current study and during morning LOD, the fore section had 1.5°C greater ambient and delta-temperatures than the average of the center and aft sections. This resulted in the fore section having a 2.3 unit greater THI than these sections. During morning UDL, the fore section had 1.2°C greater ambient and delta-temperatures than the aft section, but there were no effects on THI. By afternoon transport sessions, the fore section only had 0.9°C greater ambient and delta-temperatures than the aft section during UDL. The increased temperatures and THI experienced in the fore compartments is in agreement with Brown et al. (2011), Machado et al. (2021b), and Morris et al. (2021) also reported fore compartments had greater temperature than the other compartments by 2.1, 0.65 and 2.5 °C, respectively. During warm ambient temperatures, Pereira et al. (2018) and Moak et al. (2022) observed similar results where the BF section had a greater THI value than other compartments in potbelly trailers by 1.91 and 1.97 units, respectively. In addition, a greater serum creatine kinase concentration was also observed in pigs transported in the BF section (Moak et al., 2022), associated with greater temperatures and THI values in the trailer section. Brown et al. (2011) attributed greater BF temperature and THI to the section's proximity to external heat sources like the truck engine, floor, and wheels and the restricted ventilation by the truck cabin in front of the compartment. The current study's results may indicate that time of day affects the temperatures pigs experience in various compartments; especially when trailers are not moving and subjected to increased airflow.

In the current study, RH on trailers was greater during the LOD stage for all trips and decreased during TRA and TRA+30 stages. Pilcher et al. (2011) stated that relative humidity increased during stops and waiting times in farm and plant facilities. Relative humidity results indicated that airflow and dynamics during transportation were responsible for the RH decrease during TRA and TRA+30 and increase during ULD, which is typical for passively-ventilated trailers (Dewey et al., 2009). The same trend of RH decrease and increased throughout the transportation process was observed by Morris et al. (2021) with 0.2 to 6.79% decrease during TRA and 2.4 to 2.8% increase during stationary or UDL. Unlike temperature trends in the current study, trailer section did not affect relative humidity measures during morning and afternoon transport. Xiong et al. (2018) also found humidity and humidity index did not vary among different pot-belly trailer sections during mild, warm, and very hot weather conditions. During colder conditions, Morris et al. (2021) found fore and center sections of pot-belly and straight-deck trailers had 4.3% greater relative humidity that the aft section. Therefore, this indicates relative humidity differences within trailers could be influenced by time of year.

While trailer sections' temperatures differed during morning LOD and UDL, there were trailer deck differences in morning temperatures during all stages of transport. Throughout all stages of transport, the bottom deck had on average 1.3°C greater temperatures than the top deck. This could possibly be due to roof vents on the top deck being open which allowed hot air to rise and escape the deck. Thirty minutes after transport through unloading, the bottom deck had 5.8% less morning relative humidity than the top deck. Despite this, the bottom deck's THI was greater through all transportation stages by 1.7 units. Differences seen in the morning were eliminated in the afternoon except LOD top deck temperature measures and THI were greater than the bottom deck by 1.3°C and 1.4 units, respectively. The increased temperature could have been from the direct sun exposure pigs experienced due to roof vents being open and the lack of airflow with no trailer movement. Morris et al. (2021) observed similar results where the bottom decks had greater ambient temperature and relative humidity by 2.7°C and 4.1%, respectively, during winter. Machado et al. (2021a,b) stated bottom decks have greater temperatures due to thermal heat core formation resulting in a reduced heat removal rate. Generally, the trailer's top decks have favorable ventilation conditions for pigs during transportation, but Machado et al. (2021a) stated pigs transported in top decks were more susceptible to physical stress related to direct solar exposure which could add to stress. The authors also reported divergent conditions experienced by pigs transported on straight-deck trailer bottom decks caused them to possess greater rectal temperature and respiratory rate than pigs transported on top decks by 0.69°C and 4.6 breaths/min, respectively. The current study's results indicate that the bottom deck possesses an unfavorable microclimate during transport in the morning most likely due to airflow differences, and the top deck possesses an unfavorable microclimate in the afternoon when the trailer is not moving and pigs are exposed to direct sunlight.

Elevated THI during live transport is considered the most relevant risk factor for injured and non-ambulatory pigs (Machado et al., 2021b). Xiong et al. (2015) stated THI provided a comparative assessment of in-trailer conditions; however, a dispute exists over THI calculation's usefulness in determining emergency severity associated with heat stress (EFSA AHAW Panel, 2022). While deck and section differences were mostly found during morning transport, trailer compartment's THI were in danger or emergency categories throughout the study. Fox et al. (2014) reported as ambient temperature increased, THI increased. Pereira et al. (2018) reported pigs demonstrated more activity when THI was reduced in a compartment's microclimate. Machado et al. (2021b) observed greater skin temperature, rectal temperature, and lactate in pigs transported in compartments with a THI in the danger or emergency zone. Moak et al. (2022) reported serum creatine kinase was 7% greater in the compartment with a THI greater than the alert category. While there are several interventions available for THI mitigation, opportunities do exist to make further improvements in this area.

CONCLUSIONS

The present study provided an evaluation of commercial straight deck trailer vibration profiles and environmental conditions under industry conditions during summer. Study results limitations may lie in the fact two truck/trailer/driver combinations were observed, fewer loads were observed compared to the Morris et al. (2021) study, and routes were not standardized. While the argument could be made observing less truck/trailer/driver combinations could provide less variable results or noisy data, this hypothesis would have to be tested in a controlled study under non-industry conditions. These data indicated pigs transported in bottom deck were exposed to hotter temperatures than pigs in the top deck during all stages of transportation. Moreover, pigs on the bottom deck were exposed to greater horizontal accelerations (x- and y-axis). The ISO guidance implementation demonstrated trailer vibrations during transportation are beyond injury thresholds and may contribute to fatigued pig syndrome or non-ambulatory conditions. In order to further understand the exact effects of vibrations in pigs, further research is required to determined different strategies that allow the quantification of muscle fatigue during transportation.

CONFLICT OF INTEREST

In the present study, there were no real or perceived conflict of interest.

AUTHOR CONTRIBUTIONS

BM, JG and RD contributed to the conception and design of the study. JG and KT contributed with the data collection for the study. BM and DA organized the data base. DA, JG and TO performed statistical analysis. DA wrote the first and final draft of the manuscript. JG, KT, LM and RD contributed with manuscript edits. All authors contributed to manuscript revision, read, and approved the submission.

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DATA AVAILABLE STATEMENT

The data presented in this study are available on fair request from the respective author.

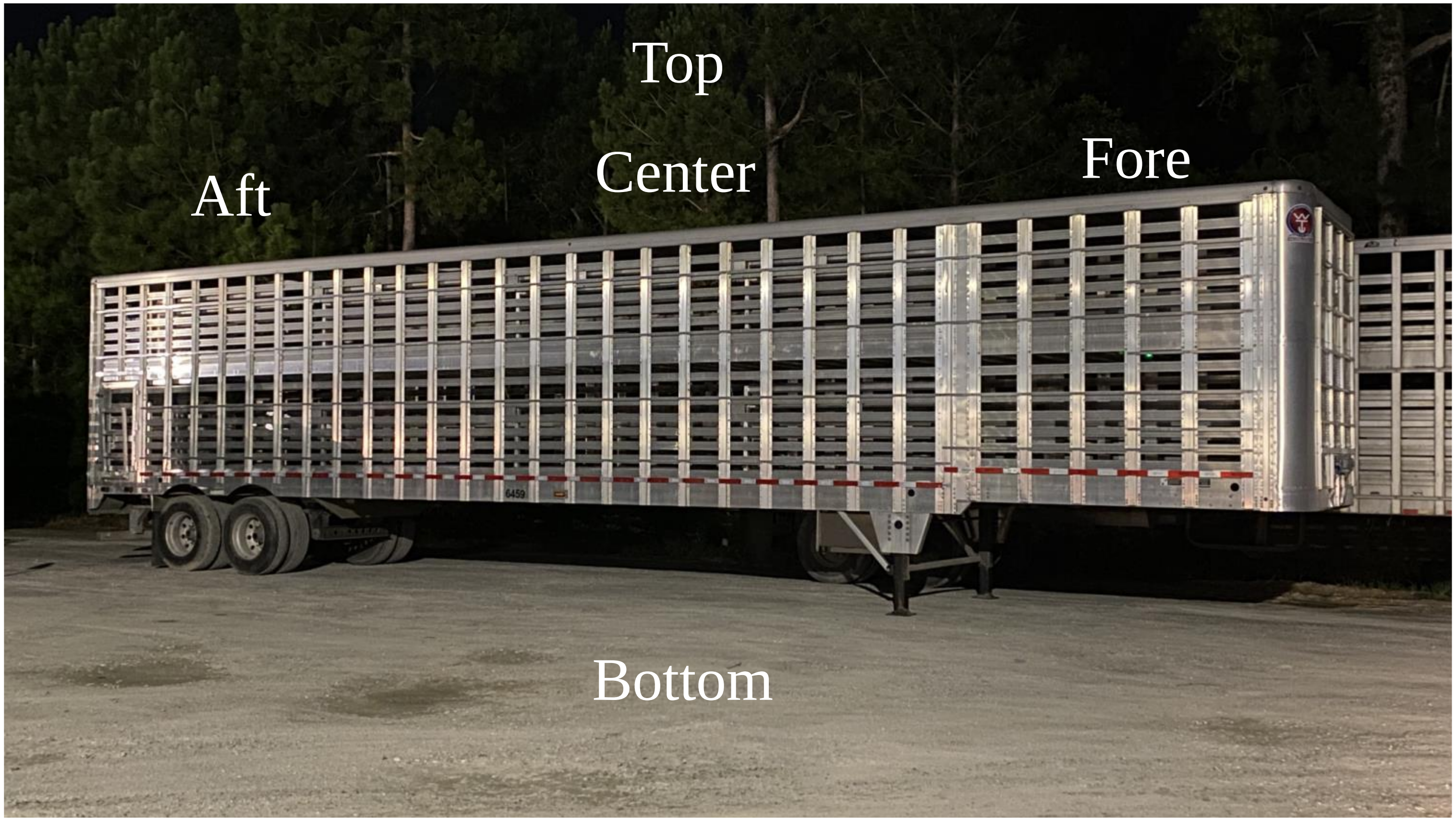

Figure 2.1. Pictoral representation of straight-deck trailers and approximate sensor placement locations with abbreviation in parentheses. Six locations were top aft (rear; **TA**), top center (**TC**), top fore (front; **TF**), bottom aft (rear; **BA**), bottom center (**BC**), bottom fore (front; **BF**),

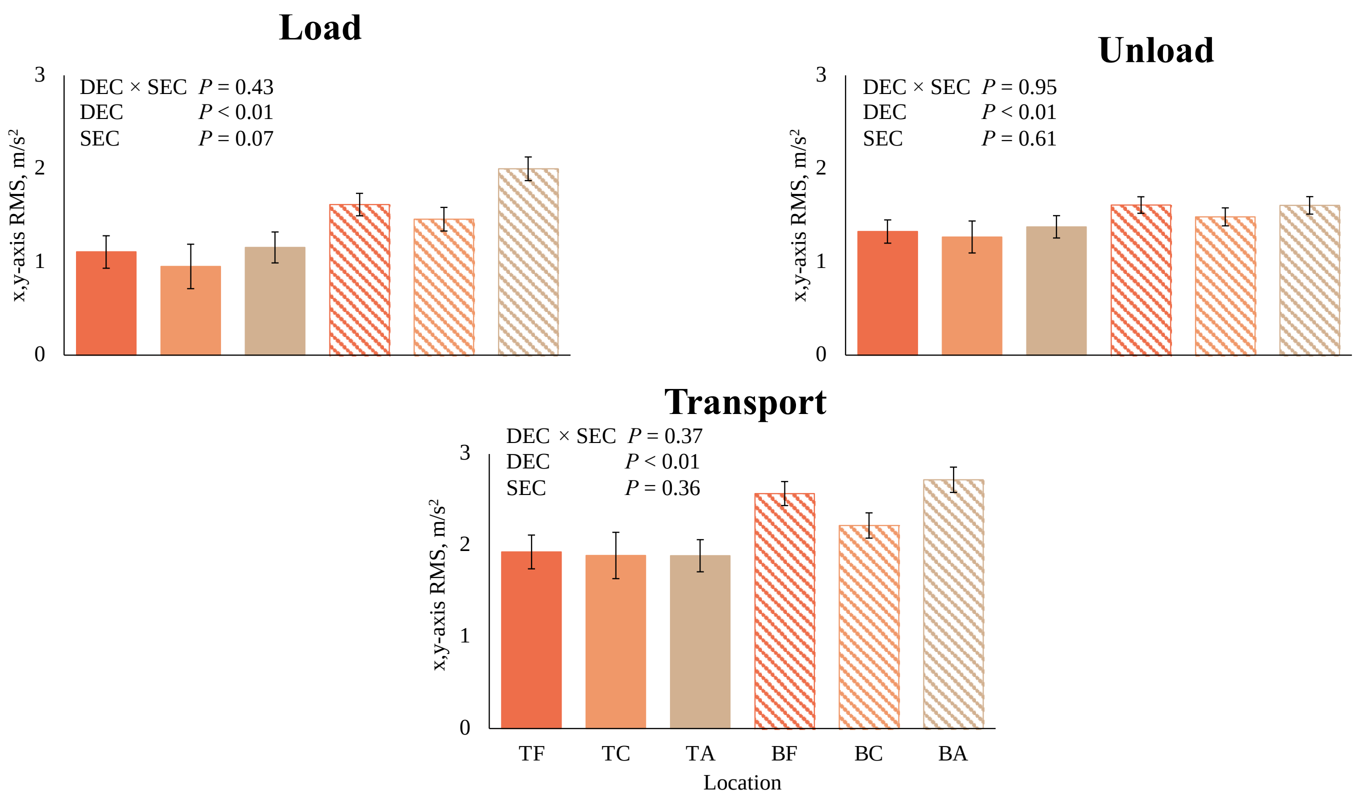

Figure 2.2. Weighted x-y-axis root mean square (**RMS**) experienced by pigs transported within two decks (**DEC**; **T**, Top; **B**, bottom) and three sections (**SEC**; **F**, fore; **C**, center; **A**, aft) located within two trailers during loading, transport, and unloading. Two trailers transported two daily market-weight pig loads over five-consecutive days.

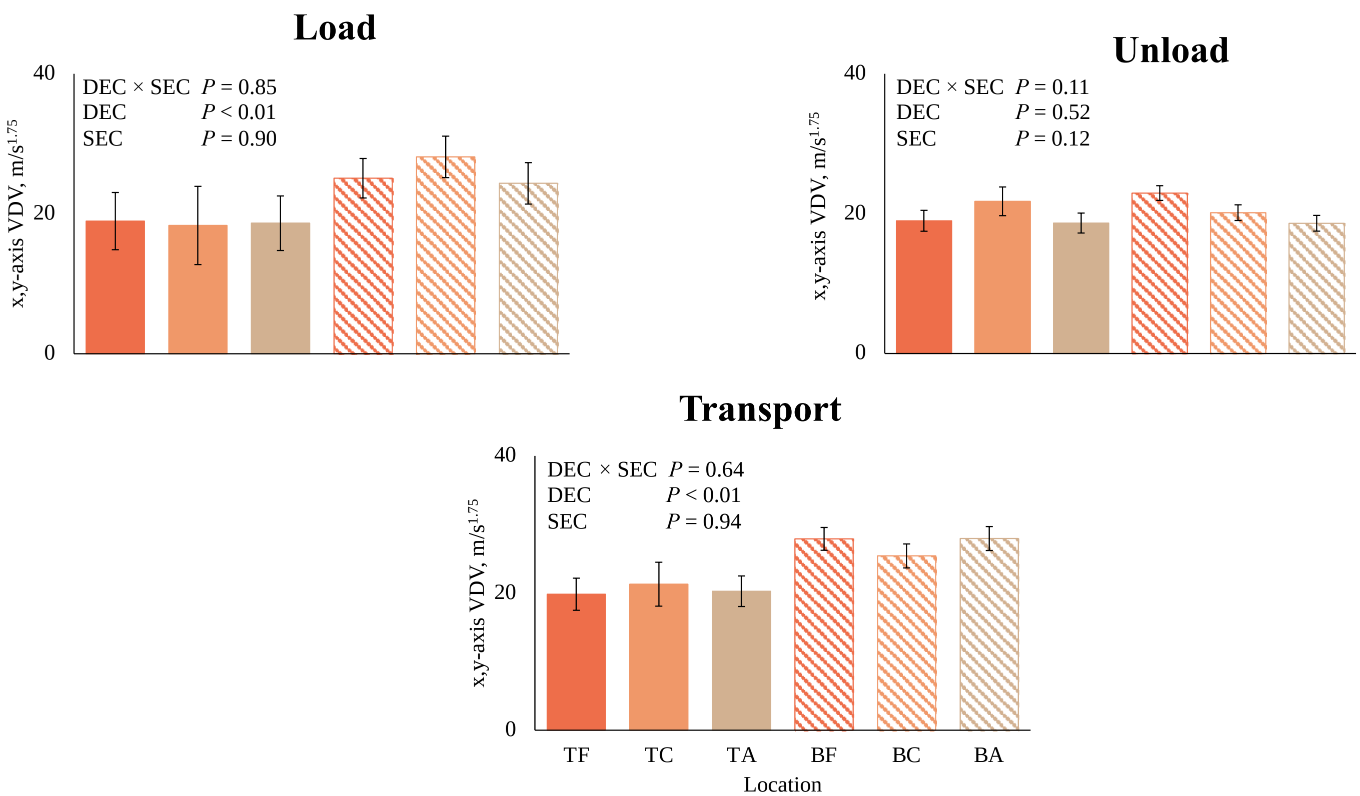

Figure 2.3. Weighted x-y-axis vibration dose value (**VDV**) experienced by pigs transported within two decks (**DEC**; **T**, Top; **B**, bottom) and three sections (**SEC**; **F**, fore; **C**, center; **A**, aft) located within two trailers during loading, transport, and unloading. Two trailers transported two daily market-weight pig loads over five-consecutive days.

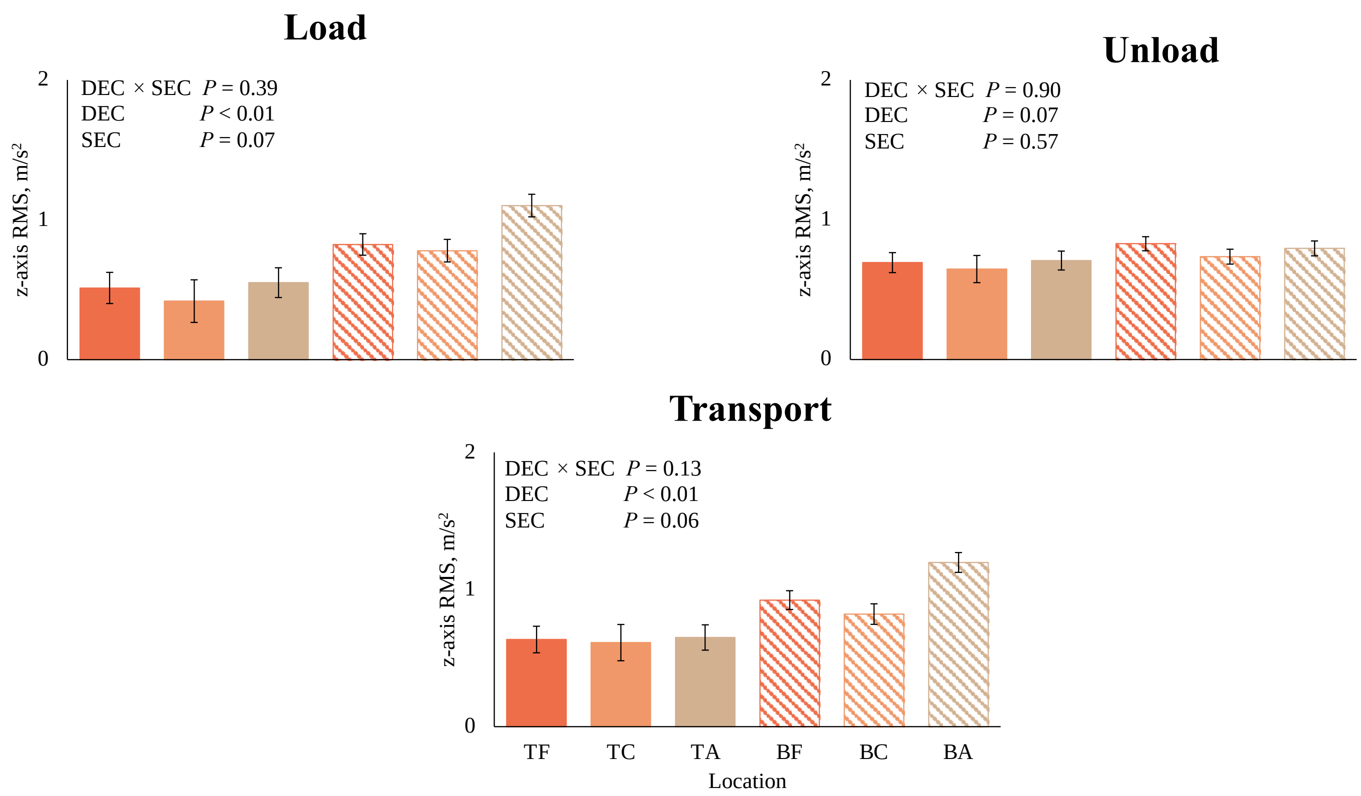

Figure 2.4. Weighted z-axis root mean square (**RMS**) experienced by pigs transported within two decks (**DEC**; **T**, Top; **B**, bottom) and three sections (**SEC**; **F**, fore; **C**, center; **A**, aft) located within two trailers during loading, transport, and unloading. Two trailers transported two daily market-weight pig loads over five-consecutive days.

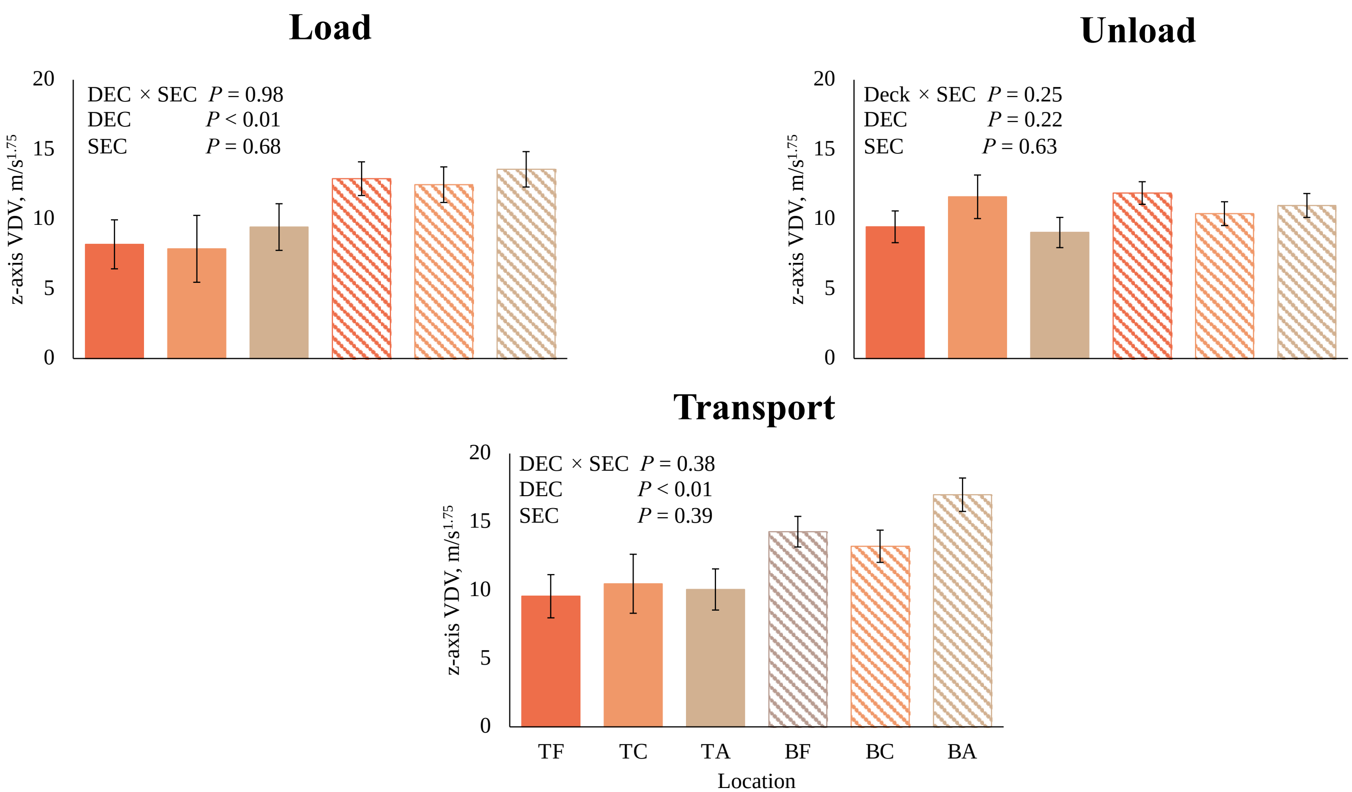

Figure 2.5. Weighted z-axis vibration dose value (**VDV**) experienced by pigs transported within two decks (**DEC**; **T**, Top; **B**, bottom) and three sections (**SEC**; **F**, fore; **C**, center; **A**, aft) located within two trailers during loading, transport, and unloading. Two trailers transported two daily market-weight pig loads over five-consecutive days.

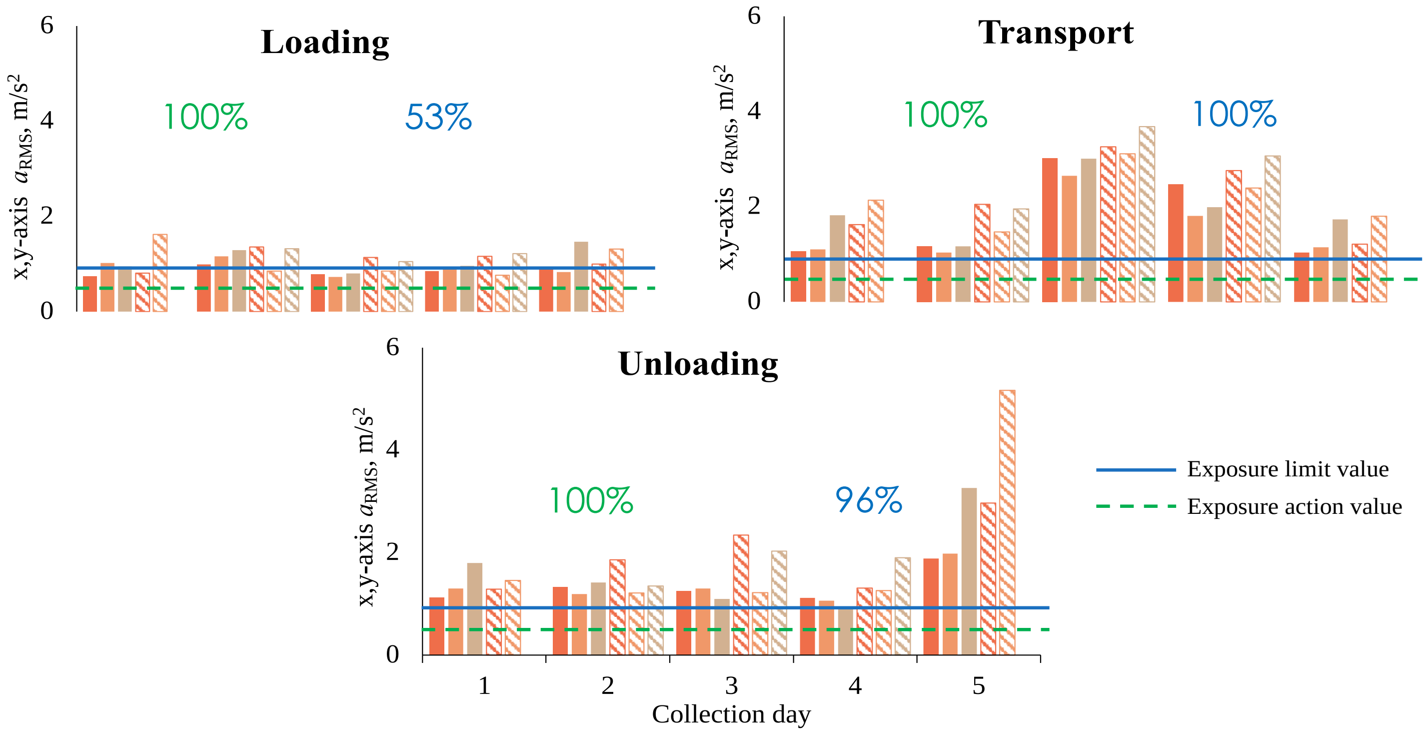

Figure 2.6. x-y-axis root mean square (**RMS**) experienced by pigs transported within two decks (**DEC**; **T**, Top; **B**, bottom) and three sections (**SEC**; **F**, fore; **C**, center; **A**, aft) of two trailers during loading (**LOD**), transport (**TRA**), and unloading (**UDL**). Least squares means encompass two daily market-weight pig loads recorded over five consecutive days. Exposure action value (**EAV**; 0.43 m/s^2; dotted green line) and exposure limit value (**ELV**; 0.86 m/s^2; solid green line) correspond to "injury possible" and "injury likely" comfort levels, respectively.

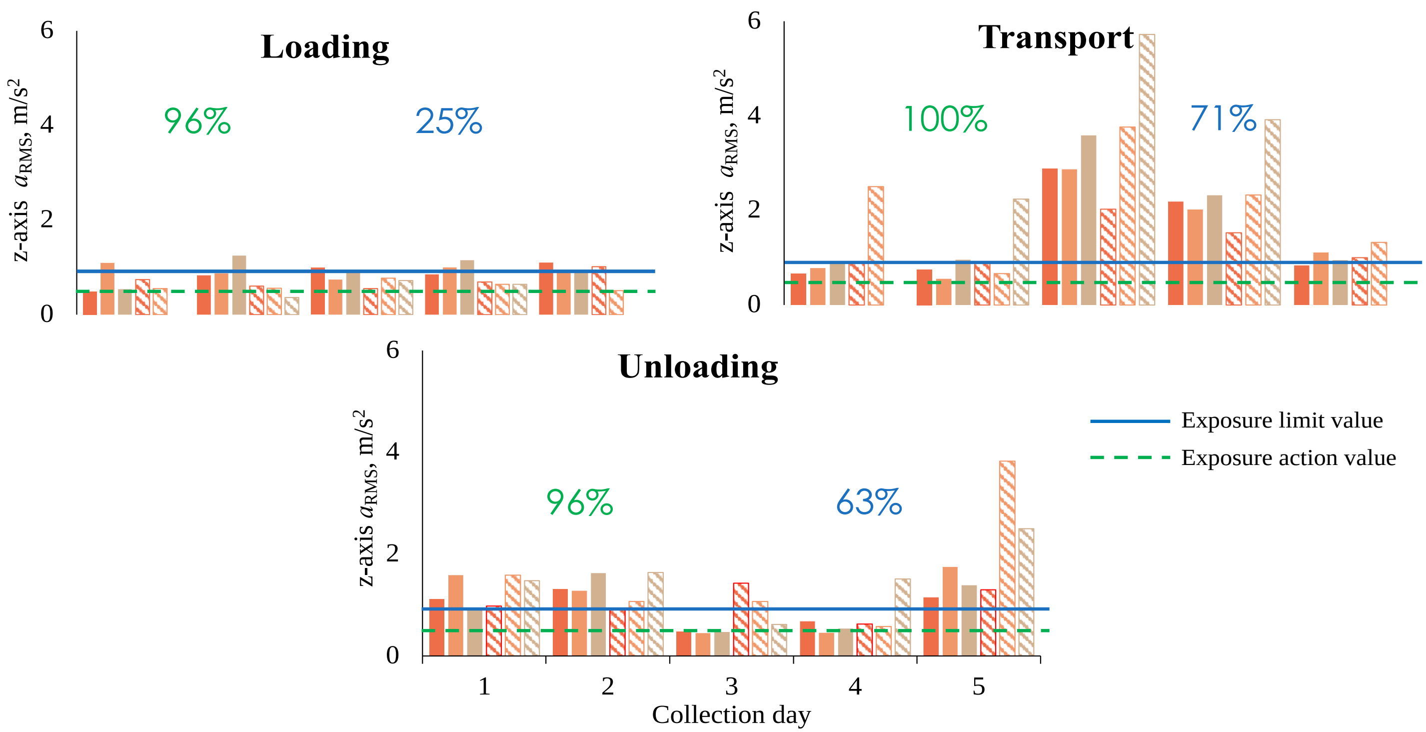

Figure 2.7. z-axis root mean square (**RMS**) experienced by pigs transported within two decks (**DEC**; **T**, Top; **B**, bottom) and three sections (**SEC**; **F**, fore; **C**, center; **A**, aft) of two trailers during loading (**LOD**), transport (**TRA**), and unloading (**UDL**). Least squares means encompass two daily market-weight pig loads recorded over five consecutive days. Exposure action value (**EAV**; 0.43 m/s^2; dotted green line) and exposure limit value (**ELV**; 0.86 m/s^2; solid green line) correspond to "injury possible" and "injury likely" comfort levels, respectively.

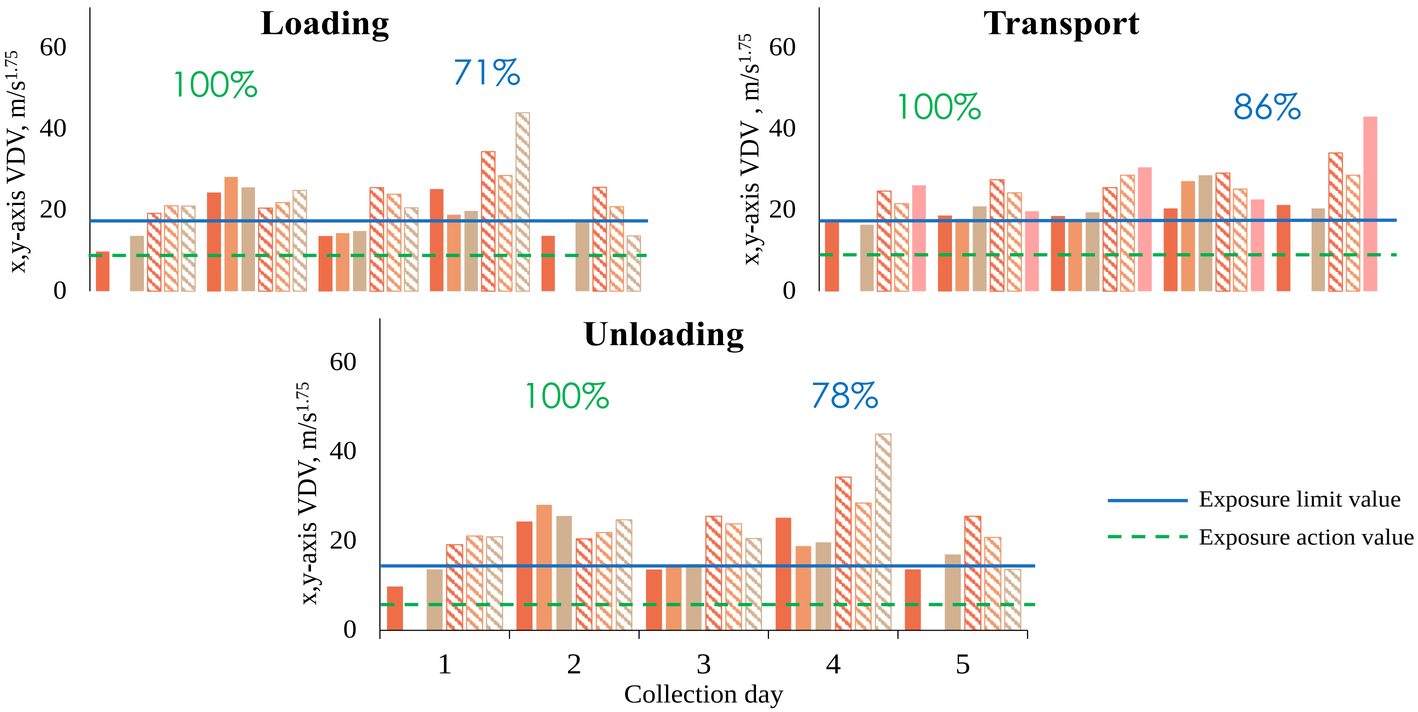

Figure 2.8. x,y-axis vibration dose value (**VDV**) experienced by pigs transported within two decks (**DEC**; **T**, Top; **B**, bottom) and three sections (**SEC**; **F**, fore; **C**, center; **A**, aft) of two trailers during loading (**LOD**), transport (**TRA**), and unloading (**UDL**). Least squares means encompass two daily market-weight pig loads recorded over five consecutive days. Exposure action value (**EAV**; 8.5 $m/s^{1.75}$; dotted green line) and exposure limit value (**ELV**; 17 $m/s^{1.75}$; solid green line) correspond to "injury possible" and "injury likely" comfort levels, respectively.

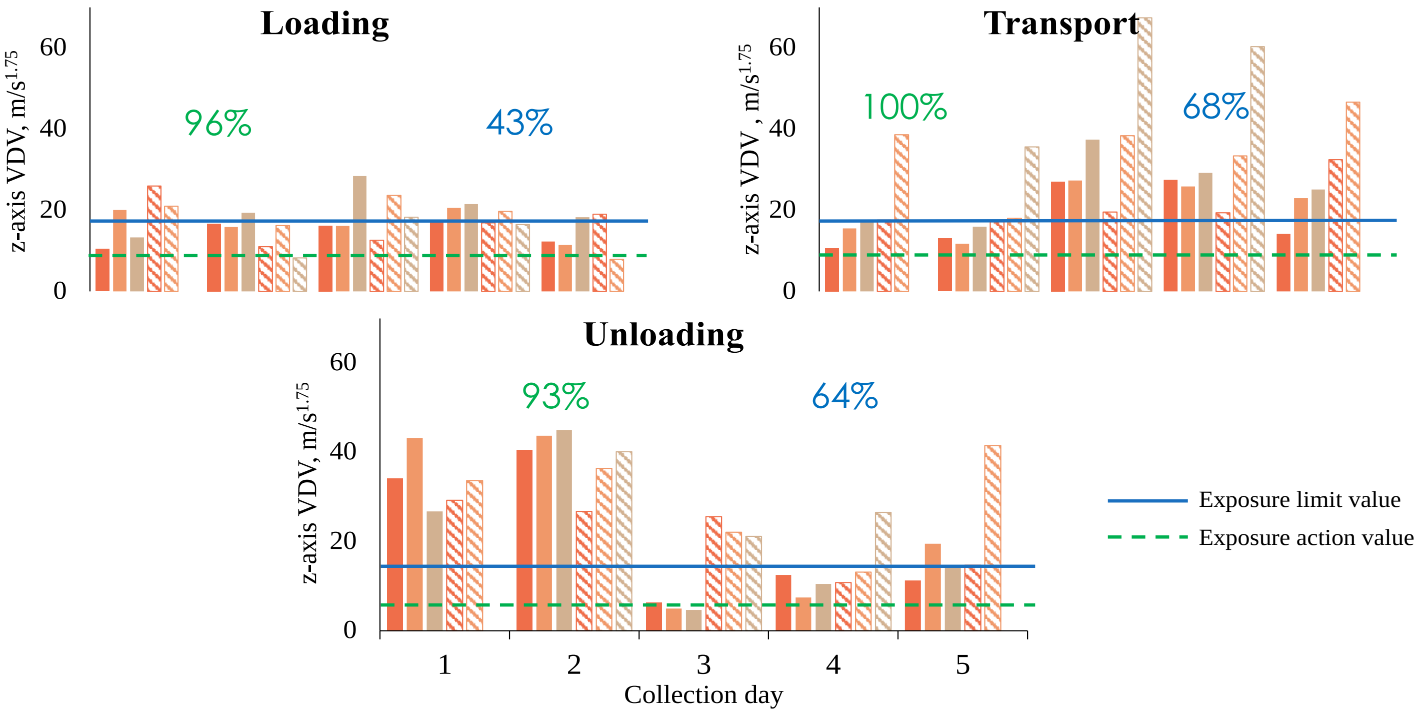

Figure 2.9. *z*-axis vibration dose value (**VDV**) experienced by pigs transported within two decks (**DEC**; **T**, Top; **B**, bottom) and three sections (**SEC**; **F**, fore; **C**, center; **A**, aft) of two trailers during loading (**LOD**), transport (**TRA**), and unloading (**UDL**). Least squares means encompass two daily market-weight pig loads recorded over five consecutive days. Exposure action value (**EAV**; 8.5 m/s$^{1.75}$; dotted green line) and exposure limit value (**ELV**; 17 m/s$^{1.75}$; solid green line) correspond to "injury possible" and "injury likely" comfort levels, respectively.

Table 2.1. Commercial pig transport characteristic means and standard deviations during loading, transport, and unloading

	Morning		Afternoon	
	Mean	Standard deviation	Mean	Standard deviation
Load characteristics				
Pigs per load	187.8	5.8	186.4	9.5
Total load weight[1], kg	35,875	3,556	35,365	3,772
Total pig weight[2], kg	22,053	3,521	21,596	3,911
Average pig weight, kg	123.2	10	120.5	7.7
Dead[3], %	0.11	0.36	0.00	0.00
Fatigued[4], %	0.11	0.22	0.05	0.17
Temperature, °C				
Loading	24	2.3	28.5	3.4
Transport	25	3.8	31.8	2.6
Unloading	26.4	4.6	32.4	3.1
Relative humidity, %				
Loading	92.2	4.7	64.0	5.5
Transport	88.1	8.8	59.8	6.8
Unloading	82.5	11.9	56.3	7.5
Time, min				
Loading	43	7	45	9
Transport	54	13	56	24
Plant waits	15	13	34	29
Unloading	30	7	34	9
Total	144	29	169	68

[1]Weight of truck, trailer, and pigs.

[2]Weight of loaded pigs only.

[3]Pigs categorized as dead by plant personnel. These pigs were found dead on the trailer during unloading.

[4]Pigs categorized as stressed or crippled by plant personnel. A pig not capable of walking under their own power without assistance off the trailer was categorized as cripple or stressed. Crippled animals had a clear injury preventing them from walking off the trailer.

Table 2.2. Average ambient and delta-temperature and relative humidity by deck of straight-deck trailers transporting market pigs in North Carolina during August 2021[1]

	Top			Bottom				*P*-value		
	Fore	Center	Aft	Fore	Center	Aft	SEM	Deck	Section	Deck × Section
Morning										
Ambient										
Temperature, °C										
Load	25.0	23.2	23.2	25.9	24.7	24.6	1.00	<0.01	0.01	0.80
Transport+30 min	25.6	25.4	25.1	27.3	26.3	26.6	0.84	<0.01	0.44	0.96
Transport	26.9	26.0	25.9	28.0	27.7	27.5	1.00	<0.01	0.43	0.84
Unload	29.0	29.0	28.2	30.9	29.6	29.3	1.22	<0.01	0.05	0.43
Relative humidity, %										
Load	93.2	91.7	96.8	89.2	91.9	91.8	2.43	0.11	0.34	0.49
Transport+30 min	92.8	90.5	95.2	84.2	87.6	88.2	1.86	<0.01	0.13	0.22
Transport	87.0	88.8	91.6	91.6	82.8	82.6	2.36	<0.01	0.22	0.51
Unload	94.6	87.4	89.0	80.5	82.9	83.5	3.21	<0.01	0.17	0.93
Delta										
Temperature, °C										
Load	1.6	-0.7	-0.6	1.9	1.1	0.9	0.78	0.02	0.02	0.46
Transport+30 min	0.9	0.6	0.3	2.5	2.0	1.8	0.59	<0.01	0.42	0.97
Transport	1.6	0.6	0.7	2.7	2.4	2.2	0.80	<0.01	0.45	0.82
Unload	2.6	2.5	1.8	4.4	3.3	3.0	0.52	<0.01	0.04	0.49
Relative humidity, %										
Load	1.1	-0.2	4.7	-3.0	-0.5	-0.6	3.12	0.09	0.38	0.54
Transport+30 min	2.2	6.6	3.3	5.5	9.2	7.3	3.31	<0.01	0.10	0.21
Transport	0.2	2.3	4.8	-5.3	-3.9	-4.1	3.51	<0.01	0.36	0.68
Unload	2.1	5.5	6.5	-2.0	0.2	0.8	3.64	<0.01	0.20	0.92
THI[2]										
Load	76.1	72.9	73.5	77.3	75.7	75.4	1.61	<0.01	<0.01	0.59

Transport+30 min	77.3	76.6	76.6	78.9	78.6	79.4	1.32	<0.01	0.65	0.97
Transport	79.6	77.3	77.7	79.8	79.5	79.2	1.44	0.02	0.55	0.82
Unload	81.6	82.3	81.1	84.2	82.6	82.2	1.64	0.01	0.13	0.21
Afternoon										
Ambient										
Temperature, °C										
Load	33.7	32.7	33.0	32.6	31.4	31.6	1.04	0.03	0.26	0.98
Transport+30 min	33.8	33.4	33.4	33.2	32.9	32.9	0.81	0.31	0.84	0.99
Transport	34.1	33.7	33.4	33.6	33.5	33.3	0.76	0.51	0.63	0.90
Unload	35.3	34.7	34.2	34.7	34.2	33.9	0.69	0.13	0.04	0.85
Relative humidity, %										
Load	66.2	69.9	67.3	69.5	73.1	71.2	3.56	0.16	0.46	0.99
Transport+30 min	62.1	63.7	64.2	63.9	66.4	65.6	2.43	0.27	0.57	0.96
Transport	58.0	61.8	61.7	60.6	61.2	60.7	3.18	0.88	0.70	0.77
Unload	62.1	63.7	64.2	63.9	66.4	65.6	3.39	0.87	0.72	0.85
Delta										
Temperature, °C										
Load	3.0	1.8	2.3	1.8	0.6	0.8	0.86	0.01	0.13	0.96
Transport+30 min	2.2	1.4	1.8	1.6	1.3	1.2	0.97	0.49	0.81	0.94
Transport	2.0	1.5	1.3	1.5	1.3	1.2	0.71	0.55	0.66	0.94
Unload	3.0	2.2	1.8	2.3	1.8	1.5	0.71	0.17	0.04	0.85
Relative humidity, %										
Load	2.2	6.6	3.3	5.5	9.2	7.3	3.26	0.14	0.35	0.97
Transport+30 min	1.4	3.9	3.5	3.2	5.8	5.0	3.30	0.40	0.58	1.00
Transport	-1.0	2.9	2.7	1.6	2.7	2.2	5.13	0.83	0.75	0.88
Unload	2.5	2.5	5.4	2.3	3.1	3.4	5.58	0.87	0.85	0.94
THI[2]										
Load	85.9	85.2	85.4	84.9	83.8	83.6	1.15	0.01	0.24	0.90
Transport+30 min	85.1	85.0	85.3	84.7	84.9	84.7	0.89	0.33	0.99	0.89
Transport	85.4	85.1	84.8	84.7	84.7	84.4	0.92	0.26	0.73	0.99

Unload	87.0	85.9	85.9	85.9	85.5	85.2	1.08	0.22	0.41	0.90

[1]A total of 20 loads of pigs transported in straight-deck trailers were observed.

[2]THI: temperature humidity index. Index less than 74 indicate "safe". Index between 74 and 79 indicate "critical". Index between 79 and 84 indicate "dangerous". Index greater than 84 indicate "emergency" (National Oceanic and Atmospheric Administration, 1976).

CHAPTER 3-USE OF ELECTROMYOGRAPHY AND ELECTRONIC MUSCLE STIMULATION TO INDUCE AND MEASURE PIG MUSCLE FATIGUE[2]

[2]D. A. Alambarrio, B. K. Morris, R. B. Davis, K. K. Turner, E. B. Grabarczyk, and J. M. Gonzalez. To be submitted to *Animals*.

ABSTRACT

The objective of this study was to determine if electrical muscle stimulation (**EMS**) is a better methodology to induce muscle fatigue in barrows than performance test. Barrows (*N* = 20) were allocated into two treatments (**TRT**, Control, and Stimulus) to simulate muscle fatigue. Electrical muscle stimulation was used to recreate muscle contractions, and motor signals were recorded using superficial electromyography (**EMG**) on Bicep Femoris (**BF**) and Semitendinosus (**ST**). Muscle tissue was collected before and after EMS stimulation to address energy consumption during muscle contraction associated with muscle fatigue. No TRT × Muscle × Time interaction was observed for any of the metabolites measured ($P > 0.06$). Adenosine triphosphate (**ATP**) showed TRT × Muscle and Time × Muscle interactions ($P = 0.05$). Electromyography data were processed every 4 sec and analyzed to different normalization methods by periods, TRT, and muscle; however, no TRT × Muscle × Period was observed ($P > 0.92$) for any analysis of data normalization implemented. Metabolite and EMG data indicate that EMS is not a better technique to measure pig muscle fatigue.

Key words: *electromyography, electricall muscle stimulation, muscle fatigue*

INTRODUCTION

Fatigued pig syndrome is used to describe pigs that suffer from transport stress without showing physical signs of trauma, disease, or injury (Anderson et al., 2002; Doonan et al., 2014). Fatigued pigs are also referred to as identify as non-ambulatory after tarsportation (Ritter et al., 2006). Fatigued pig syndrome is not a new issue in the U.S. swine industry; however, the rate of fatigued pigs has increased from 0.44% in 2009 to

0.63% in 2019 (Ritter et al., 2009; Ritter et al., 2020). Ritter et al. (2020) reported fatigued pigs cost the industry approximately $41 million during the 2019 production chain. Utilizing Ritter et al. (2020) data, Morris et al (2021) estimated fatigued pigs reduced the swine industry's ability to contribute the food security by accounting for approximately 335 million lost 4-oz boneless meals. Fatigue can encompass several phenomena that are the consequences of different physiological mechanisms; therefore, there is no specific cause of fatigue pig syndrome identified to date.

Asmussen et al. (1979), Enoka and Stuart (1992), and Lorist et al. (2002) state that muscle fatigue includes muscle performance capacity decrease, and acute performance impairment followed by the inability to produce effort to complete an action. Limitations in Adenosine triphosphate (**ATP**) production and supply rate are the most common causes of muscle fatigue. Muscle fatigue is affected by adenosine diphosphate (**ADP**), nicotinamide adenine dinucleotide (**NAD+**), and nicotinamide adenine dinucleotide + hydrogen (**NADH**) because of their role in ATP production (Enoka and Duchateau, 2008; Stein and Imai, 2012). Muscle contractions from electronical muscle stimulation (**EMS**) have been shown to instigate muscle exhaustion and fatigue (Eijsbouts et al., 1997; Banerjee et al., 2005). Electronical stimulation induces antidromic impulses which act as replacement of action potentials causing involuntary muscle contractions; therefore, is used as an alternative to physical activity for sedentary individuals or bed rest conditions (Hsu et al., 2011). Electronical impulses of EMS induce involuntary repetitive muscle contraction; consequently, EMS increases muscle energy expenditure as muscle contracts. Kemmler et al., (2012) observed EMS recruits different muscle fibers compared to voluntary contractions. Electronical stimulus activates fibers under the electrode; therefore, that electrically stimulated muscle had different metabolic responses than voluntary muscle contractions (Fitts, 1994; Kemmler et al., 2012; Kortiaunou et al., 2021).

Different detection methods are applied to muscle signals to detect muscle fatigue; however, surface electromyography (**EMG**) is the primary method to record muscle electrical signals (Al-Mulla et al., 2011). Surface EMGs are a non-invasive technique used on conscious animals during locomotion to study normal and pathological muscles and measure muscle fatigue (Williams, 2018). Fatigue is known to be reflected in EMG signal as an increase of amplitude and a decrease of its characteristic spectral density (Kallenberg et al., 2007; Enoka and Duchateau, 2008). Electromyography has been used in human and animal physiology to directly measure root mean square (**RMS**) as an indicator of action potential conduction velocity and muscle fiber recruitment (Bartuzi et al., 2007; Noel et al., 2016). Electromyography has been used in livestock to measure fatigue (Robert et al., 2000; Noel et al., 2016); however, the use of EMG is limited to superficial musculature, and signal interference because of skin displacement associated with poor electrode attachment (De Luca et al., 2010; Williams, 2018). Muscle fatigue can be partially elaborated with various models of exercise, procedures, and processes applied to quantify muscles' fatigue. Therefore, the objective of this study was to determine if EMS is a better methodology for inducing muscle fatigue in barrows than performance test provided by Alcocer (2022).

MATERIALS AND METHODS

Study methods were evaluated and approved by the University of Georgia (**UGA**) Institutional Animal Care and Use Committee (**IACUC**) #A2022 07-066-Y1-A0

Data Collection

Muscle fatigue data were collected at the UGA Meat Sciece Technology Center (Athens, GA) on barrows (*N* = 20; CG36 × P26, Choice Genetics, West Moines, IA) from the UGA swine facility on 2 different days in August 2022. Upon arrival, barrows stratified by weight, and within each time barrows were allocated to one of two treatments. Treatments consisted of barrows either being subjected to a hog electric stunner super-contraction (Best and Donovan, Cincinnati, OH) postmortem (**ES**) or not (**C**). Barrows were rendered insensible to pain with a captive bolt (Jarvis Product Co., Middletown, CT), stimulus was applied according to each treatment, and barrows were exanguinated.

Muscle Contraction and Muscle Fatigue Analyses

Electrical muscle stimulation was used to stimulate right hind limb bicep femoris (**BF**) and semitendinosus (**ST**) muscle contraction of the using the iStim EV-805 4CH Digital TENS/EMS (Everyway Medical Instruments Co., New Taipei City, Taiwan) with IStim Super Soft squared 2 × 2 tens (Everyway Medical Instruments Co., New Taipei City, Taiwan). Frequency of EMS was set at 70 Hz with a 4:4 duty cycle (4 sec on, 4 sec off) for 20 min. Surface EMG was used to record muscle contraction patterns. Electrodes were positioned in the middle of the positive and negative EMS. Using a custom program in Noraxon MR 3.16 (Noraxon USA, Scottsdale, AZ), EMG data were processed for each electrical burst corresponding to a muscle contraction. The EMG amplitude characteristics were derived as root mean square (**RMS**) processed using MATLAB (MathWorks, Natick, MA). Root mean squared raw data were filtered by using the artifact detection/removal and thresholding algorithms on MATLAB. Through MATLAB coding signals from EMS were deleted and EMG data was reconstructed via cubic interpolation through the gap. A 6^{th}

order Butterworth lowpass and a 6th order highpass filters were also applied to remove high and low frequency components. Negative data points were turned into positive, only absolute values were considered in the dataset.

In Excel (Microsoft 365, Microsoft Corporation, WA), data were organized and processed for statistical analyses by muscle separately. Data were first normalized to the average of the first 30 sec of stimulation of each individual barrow. Each second of stimulation was divided by the normalization value and multiplied by 100 to yield a normalization value percent. Percentages were averaged every four minutes to yield 5 values for statistical analysis. The second data processing method was the same as above for normalization value generation, but a global normalization value was generated by averaging all control pigs' normalization values. This global value was utilized to generate normalization value percentages for all barrows' data and averaging was conducted as previously described. The third and fourth method of processing was conducted as described above; however, normalization values were generated according to Noel et al. (2016) where the entire stimulation period was averaged. A final processing method occurred where values were generated for each individual pig according to Noel et al. (2016), but the last 30 sec of the stimulation period was averaged to yield one percentage value for statistical analysis.

ADP, ATP, NAD+ and NADH Analyses

Muscle biopsies were taken from BF and ST muscles before and after EMS from tissues under the EMG electrode attachment. A 6-gauge piercing needle (Precision Needles, Denver, CO) was used to perforate the skin and access muscle tissue with a Mammotome Elite biopsy gun entry (Mammotome, Cincinnati, OH). From each muscle biopsy, 40±0.5 mg muscle tissue were frozen with liquid nitrogen and sent out from each biopsy to the Metabolic Core Facility at the University of Iowa for an LC-MS redox panel of ADP, ATP, NAD+, and NADH concentrations. Samples were lyophilized, homogenized, dried and reconstituted in acetonitrile. Data of LC-MS were acquired on Thermo Q Exactive hybrid quadrupole Orbitrap mass spectrometer with ZIC-pHILIC guard column. The liquid chromatography used was a Millipore SeQuant ZIC-pHILIC and the mass spectrometer data acquisition was performed in a range of m/z 70-1,000 with resolution at 70,000. Acquired data were processed by Thermo Scientific TradeFinder 4.1 software, and ADP, ATP, NAD+, and NADH were identified based on the University of Iowa Metabolomics Core facility standard-confirmed, in-house library. Data were normalized to the sum of all the measured metabolite ions in that sample.

Statistical Analyses

All four-minute averaged normalized percent and metabolite data were analyzed as a completely randomized design with repeated measures using barrow as the experimental unit. Treatment, period, and their interaction served as fixed effects, while barrow within treatment served as the random effect. Period served as the repeated measure, barrow within treatment served as the subject, and compound symmetry was used as the covariance structure. Final 30 sec data were analyzed as a completely randomized design using barrow as the experimental unit. Treatment served as the fixed effect and barrow within treatment served as the random effect. All models were analyzed using the MIXED procedure of SAS 9.3 (SAS Inst., Cary, NC). Pairwise comparisons between the least squares means of the factor level comparisons were computed using the PDIFF option of the LSMEANS statement. Statistical significance was determined at $P < 0.05$ and trends at $0.15 < P > 0.05$.

RESULTS

Muscle Contraction from Electromyography Analyses.

There were no TRT × Muscle × Period, TRT × Period, or Muscle × Period interactions when data were analyzed using all normalization methods ($P > 0.48$; Table 3.1 to 3.4). There was no TRT × Muscle interaction ($P > 0.88$) for RMS values normalized to individual pig's 20 min of stimulation (Table 3.3); however, there were TRT × Muscle interactions for data normalized to individual pig's first 30 sec of stimulation, normalized to all control pigs' first 30 sec of stimulation, and normalized to all control pigs' 20 min of stimulation ($P < 0.01$; Figure 3.1). When normalized to individual pig's first 30 sec of stimulation, control BF values were greater ($P < 0.01$) than control ST values, but there were no differences ($P = 0.26$) when muscles were super-contracted. When normalized to all control pigs' first 30 sec of stimulation and all control pigs' 20 min of stimulation, there were no differences between control pig muscles ($P > 0.94$), but ST was greater than BF for ES pigs ($P < 0.01$). There were no TRT × Muscle interaction or main effects for last 30 s of stimulation RMS data normalized to individual and control pigs' 20 min stimulation period ($P > 0.18$; Table 3.5). There was Muscle effect for data normalized to individual pig's first 30 sec, all control pig's first 30 sec, and all control pig's 20 min of stimulation ($P < 0.01$), with no Muscle effect for data normalized to individual pig's 20 min and last 30 sec of stimulation ($P > 0.18$). There was a Period effect when data was normalized to individual pig's first 30 sec of stimulation ($P = 0.08$), but there was no period effect for the rest of the normalization analyses ($P > 0.86$).

ADP, ATP, NAD+, and NADH Analyses

There were no Muscle × TRT × Time interaction or TRT effect on muscle ATP level ($P > 0.47$); however, there were TRT × Muscle and Time × Muscle interactions ($P = 0.03$; Figure 3.2). Control BF had greater ($P < 0.01$) ATP concentration than control ST, but concentration did not differ ($P = 0.77$) between ES muscles. There was no difference ($P = 0.64$) between pre- and post-EMS BF ATP concentration, but post-EMS ST had less ($P < 0.01$) ATP than pre-EMS ST. There were Muscle and Time main effects for ATP level where BF had greater ($P < 0.01$) concentration than ST, and pre-EMS muscle had greater ($P < 0.01$) ATP than post-EMS muscle. There were no Muscle × TRT × Time or main effects for ADP, NAD+, and NADH ($P > 0.06$; Figure 3.3). Although, there were Time effects where muscle ADP and NAD+ had lower ($P < 0.13$) concentrations post-EMS, except for ES BF for muscle ADP and control ST for muscle NAD+. There was no Time effect for muscle NADH post EMS ($P = 0.31$). There were no Muscle effects ($P > 0.55$) for ADP, NAD+ and NADH.

DISCUSSION

Surface EMG is a non-invasive tool used for different species clinical examination and assessing muscle fatigue (Bartuzi et al., 2007; Noel et al., 2016; Williams, 2018). When monitoring muscle fatigue, EMG technology is unreliable because is limited to superficial musculature and signals are impacted by subcutaneous fat thickness between electrodes and active muscle fibers, and other muscle signals (De Luca et al., 2010). Additionally, EMG is restricted to superficial musculature; therefore, skin displacement and poor electrode attachment affect EMG data accuracy. Alcocer (2022) used EMG technology to assess muscle fatigue development in barrows during performance test exposure; however, during data collection, prolonged electrode attachment issues were encountered which interfered with EMG signal quality. Grabarczyk et al. (2022) reported 41.57% of EMG data was considered incomplete or flawed because of pig-induced signal interference during performance test. Strikes and other aggressive movements were also recorded during EMG data collection; thus, this resulted in flawed data displaying greater RMS variation. Because of these issues, protocol development to measure muscle fatigue in the absence of movement is warranted.

Electronical muscle stimulation is used in physical therapy and rehabilitation as a method to increase physical fitness and combat a sedentary lifestyle (Banerjee et al., 2005). As a rehabilitation method, EMS is employed to prevent skeletal muscle atrophy in patients with respiratory and cardiac diseases subjected to prolonged bed rest (Banerjee et al., 2005; Hsu et al., 2011). In the current study, EMS was applied on two muscles important to pig ambulatory movement, BF and ST, to resemble physical activity in euthanized barrows.

Muscle contractions caused by EMS are repetitive and involuntary. Jones et al. (1979) observed EMS frequencies between 40 and 50 Hz recruited more fatigue-resistant slow-twitch muscle fibers (Type I), and frequencies greater than 50 HZ recruited more easily fatigued fast-twitch muscle fibers (Type IIA and IIB). In the current study, EMS muscle load was recorded with EMG RMS. Electromyography RMS evaluates muscle fiber recruitment by comparing active muscle fibers to the number utilized during initial movement (Bartuzi et al., 2007; Noel et al., 2016). Muscle fibers directly underneath EMG and EMS electrode attachment were not analyzed; however, Alcocer (2022) reported more Type IIB fibers in BF and ST muscle in barrows of similar age. Type IIB muscle fibers have less oxidative and more glycolytic capacity, which provides quick, powerful movements that last a short time (Gerrad and Grant, 2002); therefore, RMS values first increase because of fiber recruitment and then decrease because of fiber exhaustion and muscle fatigue onset (Kingugasa et al. 2004; Alcocer, 2022). The same was observed in the current study in data normalized to individual pig's first 30 sec of stimulation, and numerically in data normalized to all control pig's first 30 sec, to individual and all control pig's 20 min stimulation. Although there was no specific treatment influenced, EMG data showed that ST was impacted more by super-contraction than BF and may be accredited by muscle size and divided signal between electrodes with each muscle receiving 35 Hz. Van der Wal (1978) reported increased lactate after electrical stunning because electrical stunning is accompanied by muscle contraction. Lactate is a biological indicator of muscle fatigue onset; therefore, ES barrows should have shown a greater decrease in muscle power force.

Limitations in energy supply or reduction of ATP turnover of skeletal muscle are the most common muscle fatigue hypothesis (Sahlin et al., 1998). Skeletal muscle has the unique capability of changing energy expenditure in situations where explosive contractions are required. The transition from rest to exercise has showed to increase energy demand more than 100-fold, compromising the ATP generation process and utilization (Sahlin et al., 1998; Baker et al., 2010; Calbet et al., 2020). Karatzaferi et al. (2001) indicated intracellular ATP does not decrease below 60% of the resting values on whole muscle during intense exercise. In the current study, ATP concertation decreased by 13.68 and 88.59% post EMS compared to pre-EMS of BF and ST, respectively. The previous result indicated EMS muscle increased energy expenditure more than voluntary contraction from physical activity. Additionally, ES barrow's BF muscle ATP concentration of ES barrows decreased by 36.41% compared to CON barrows, suggesting using the stunner electrical impulse could potentially simulate muscle exhaustion; however, this was not observed in the ST. Other studies indicated muscle ATP is constant despite large fluctuations in energy demand in muscle fatigue and indicates that cellular energetics adjust the ATP-generating process to the demand (Green, 1991; Fitts, 1994), observed in C and ES barrow's ST muscle ATP.

During energy regulation, ADP is essential because it regulates glycolytic flux and oxidative phosphorylation rates at the beginning of the TCA cycle (Calbet et al., 2020). Sahlin and Ren (1989) observed an increase in muscle ADP from 18 to 216 μmol after high-intensity exercise and fatigue development, and Sahlin et al. (1997) observed and increase of 138 μmol after prolonged cycling and fatigue development. During exercise, increased ADP was accredited to ADP muscle diffusion restriction (Korge, 1995) because of adenine nucleotides spatial gradients in contracted muscle (Shalin et al., 1998). Additionally, several studies reported increased ADP reduced free energy released during ATP hydrolysis which reduced muscle contraction power output (Yamashita et al., 1994; Allen et al., 1997; Calber et al., 2020). In the current study, ADP muscle concentration showed the opposite, ADP concentration decreased after EMS, but ES BF concentration increased by 5.25%, agreeing with the previous theory ADP increased after fatigue. During intense exercise, NAD+ and NADH are essential nucleotides to maintain oxidoreactive power to the electron transport chain to support ATP regeneration rate from glycolysis and mitochondrial respiration (Bogan and Brenner, 2008; Baker et al., 2010). Jobsis and Stainsby (1968) and Duboc et al. (1988) observed an increase in NAD+ levels accompanied by decreased NADH levels after exercise muscle fatigue onset in dogs and rats. In the current study, the opposite trend was observed. Muscle NAD+ decreased after EMS accompanied by NADH increase; however, control barrow's ST followed the previous theory with a 15.68% decrease in NADH accompanied by a 5.80% increase in NAD+ levels after EMS.

It is important to consider that when the barrow succumbs to exsanguination resulting in anoxia, skeletal muscle still attempts to achieve antemortem ATP and glycogen homeostatic balance. Cell oxygen is reduced as soon as blood circulation is interrupted by animal death. Postmortem muscle cells continue to function by anaerobic glycogen depletion, making ATP through NAD+. Postmortem, NAD+ is reduced to NADH through glycolysis, resulting in the lactate and H+ production as end products. After intense exercise and muscle fatigue onset, living organisms can shift from anaerobic to aerobic metabolisms where NADH is oxidized through electron transport chain. In the current study, increased NADH and decrease NAD+ levels were observed, because under anaerobic metabolisms NADH can be oxidized and reused to continue ATP production. Lactate and free hydrogen measurements are required to confirm the previous theory; unfortunately, lactate concentration and pH were not analyzed.

CONCLUSION

Overall, EMS is a good methodology to replicate physical activity in harvested pigs by following RMS patterns of muscle fatigue of increased amplitude and decreased spectral frequency observed in living organism. Additionally, the used of EMS allows the assessment of signal interferences and poor electrode attachment issues with EMG during performance test in pigs. Metabolites patterns confirm the onset of muscle fatigue; however further analysis is required to verify theories in harvested pigs. In general the objective of this study was accomplish; however, this methodology requires different methods of data analysis decreasing accuracy and reliability.

CONFLICT OF INTEREST

In the present study, there were no real or perceived conflict of interest.

AUTHOR CONTRIBUTION

BM, JG, KT, and RD contributed to the conception and design of the study. EG, DA, JG, and KT contributed with the data collection for the study. DA and JG organized the data base. JG performed statistical analysis. DA wrote the first draft of the manuscript. JG, KT, and RD contributed with manuscript edits. All authors contributed to manuscript revision, read, and approved the submission.

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DATA AVAILABLE STATEMENT

The data presented in this study are available on fair request from the respective author.

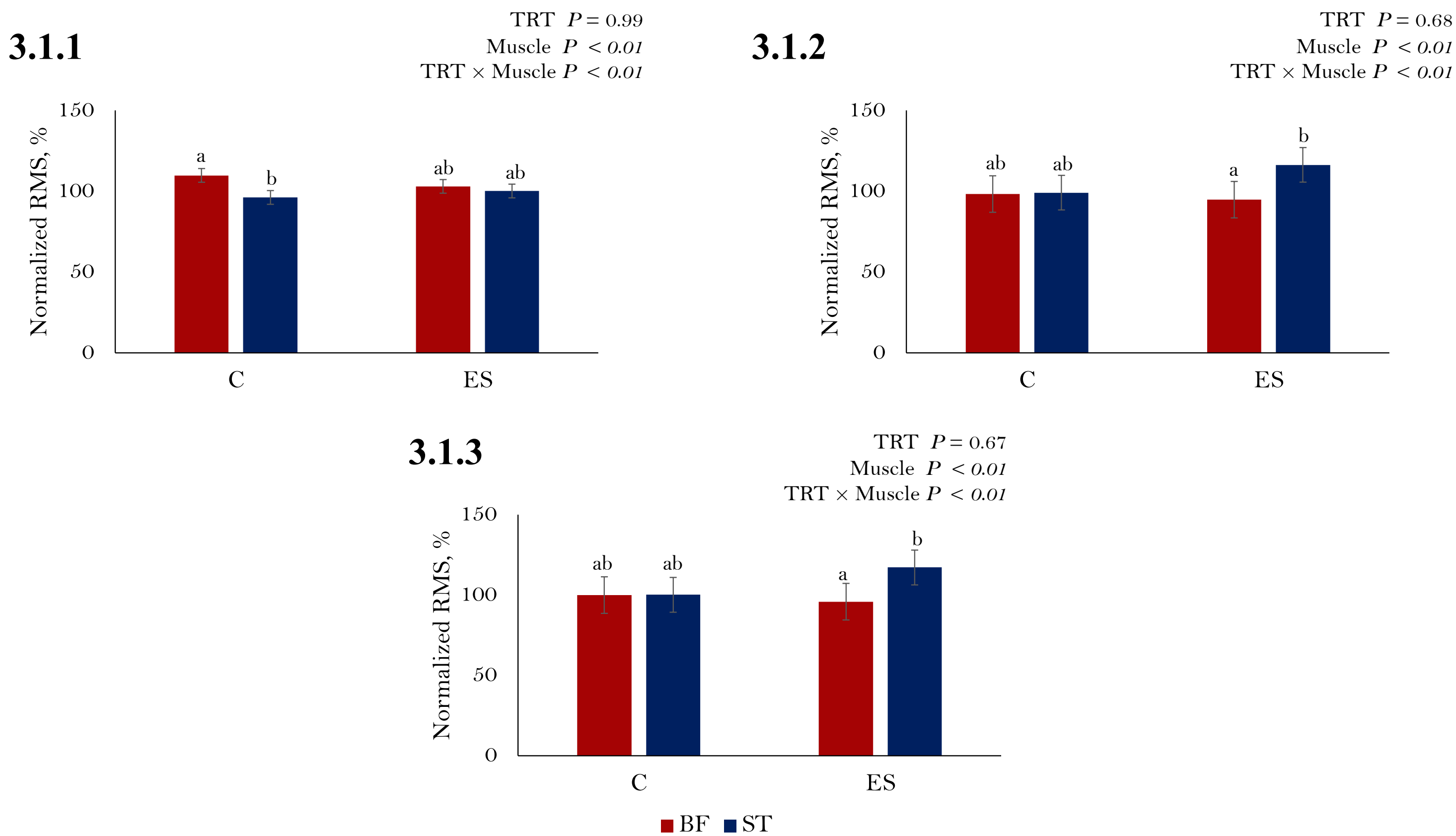

Figure 3.1. Root mean square data from electrical muscle stimulated muscle of pigs subjected to two super-contraction protocols normalized to (3.1.1) individual pig first 30 sec of stimulation (3.1.2) all control pig's first 30 sec of stimulation average (3.1.3) all control pig's 20 min of stimulation average of bicep femoris (**BF**) and semitendinosus (**ST**).

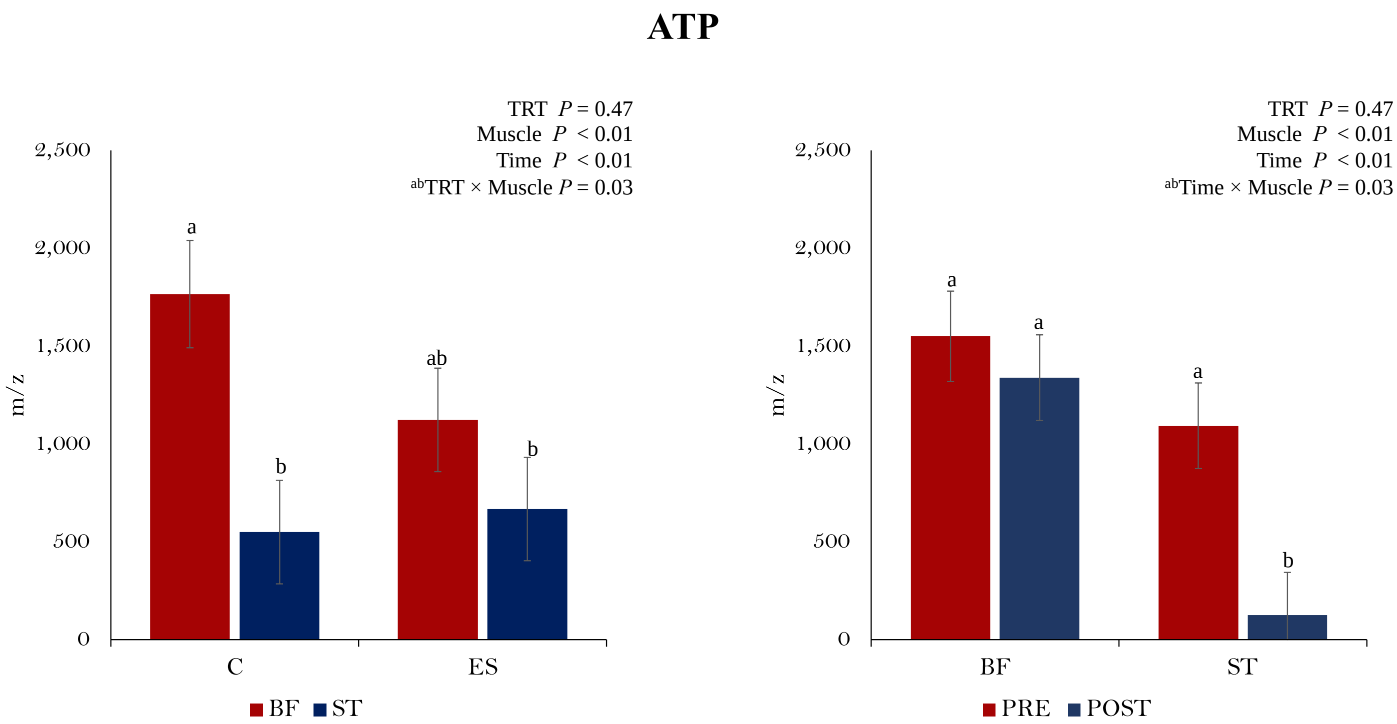

Figure 3.2. Adenosine triphosphate (**ATP**) LC-MS analysis of bicep femoris (**BF**) and semitendinosus (**ST**) samples by time before (**PRE**) and after (**POST**) electronic muscle stimulation (**EMS**) and by treatment of barrows subjected to electric stunner (**ES**) or not (**C**).

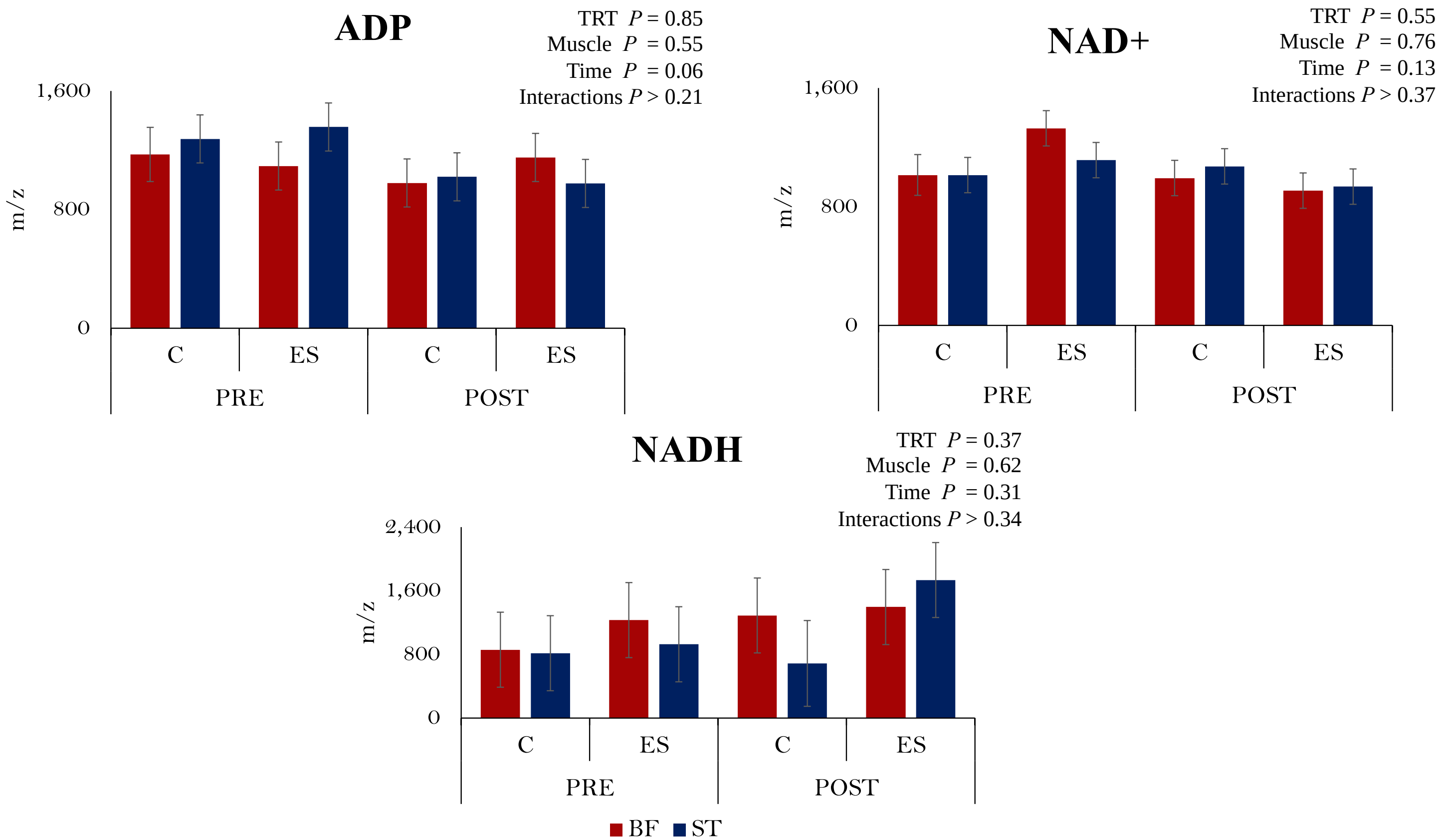

Figure 3.2. Adenosine diphosphate (**ADP**), nicotinamide adenine dinucleotide (**NAD+**), and nicotinamide adenine dinucleotide + hydrogen (**NADH**) LC-MS analysis by time before (**PRE**) and after (**POST**) electronic muscle stimulation (**EMS**) of bicep femoris (**BF**) and semitendinosus (**ST**).

Table 3.1. Root mean square values from electrical muscle stimulated muscle of pigs subjected to two super-contraction protocols and normalized to individual pig's first 30 sec of stimulation

	Treatment[1]			*P*-value[2]						
Normalized value, %	Control	Stimulus	SEM	TRT	Muscle	Period	TRT × Muscle	TRT × Period	Muscle × Period	TRT × Muscle × Period
Individual pig			5.57	0.99	<0.01	0.08	<0.01	0.62	0.73	0.92
Bicep femoris										
Period 1	103.26	100.63								
Period 2	112.23	104.16								
Period 3	111.25	103.74								
Period 4	110.44	103.52								
Period 5	111.80	103.17								
Semitendinosus										
Period 1	96.55	101.72								
Period 2	96.53	101.77								
Period 3	96.21	99.51								
Period 4	95.84	98.34								
Period 5	95.57	99.44								

[1]Barrows either being subjected to a hog electric stunner postmortem (**ES**) or not (**C**).
[2]TRT, treatment.

Table 3.2. Root mean square values from electrical muscle stimulated muscle of pigs subjected to two super-contraction protocols and normalized to all control pig's first 30 sec of stimulation average

Normalized value, %	Treatment[1] Control	Stimulus	SEM	P-value[2] TRT	Muscle	Period	TRT × Muscle	TRT × Period	Muscle × Period	TRT × Muscle × Period
Individual pig			12.76	0.68	<0.01	0.88	<0.01	0.96	0.99	0.99
Bicep femoris										
Period 1	100.80	92.65								
Period 2	102.51	96.50								
Period 3	97.94	95.33								
Period 4	96.81	94.90								
Period 5	96.07	94.48								
Semitendinosus										
Period 1	102.84	117.02								
Period 2	101.02	119.10								
Period 3	98.64	116.17								
Period 4	96.90	114.21								
Period 5	96.14	114.97								

[1]Barrows either being subjected to a hog electric stunner postmortem (**ES**) or not (**C**)
[2]TRT, treatment.

Table 3.3. Root mean square values from electrical muscle stimulated muscle of pigs subjected to two super-contraction protocols and normalized to individual pig's 20 min of stimulation

Normalized value, %	Treatment[1]		SEM	P-value[2]						
	Control	Stimulus		TRT	Muscle	Period	TRT × Muscle	TRT × Period	Muscle × Period	TRT × Muscle × Period
Individual pig			2.01	0.81	0.88	0.86	0.88	0.97	0.68	0.99
Bicep femoris										
Period 1	101.05	98.28								
Period 2	103.31	101.17								
Period 3	99.16	100.39								
Period 4	98.28	100.27								
Period 5	95.45	99.88								
Semitendinosus										
Period 1	102.89	101.68								
Period 2	101.77	101.55								
Period 3	99.97	99.37								
Period 4	98.47	98.14								
Period 5	97.48	99.25								

[1]Barrows either being subjected to a hog electric stunner postmortem (**ES**) or not (**C**)
[2]TRT, treatment.

Table 3.4. Root mean square values from electrical muscle stimulated muscle of pigs subjected to two super-contraction protocols and normalized to all control pig's 20 min of stimulation average

Normalized value, %	Treatment[1]		SEM	*P*-value[2]						
	Control	Stimulus		TRT	Muscle	Period	TRT × Muscle	TRT × Period	Muscle × Period	TRT × Muscle × Period
Individual pig			12.90	0.67	<0.01	0.86	<0.01	0.98	0.99	0.99
Bicep femoris										
Period 1	101.96	93.71								
Period 2	103.69	97.60								
Period 3	99.07	96.42								
Period 4	97.92	95.99								
Period 5	97.17	95.56								
Semitendinosus										
Period 1	103.63	116.21								
Period 2	102.13	120.40								
Period 3	99.72	117.44								
Period 4	97.96	115.45								
Period 5	97.20	116.22								

[1]Barrows either being subjected to a hog electric stunner postmortem (**ES**) or not (**C**)
[2]TRT, treatment.

Table 3.5. Root mean square values from electrical muscle stimulated muscle of pigs subjected to two super-contraction protocols and normalized to the last 30 sec of stimulation

Normalized value, %	Treatment[1]		SEM	*P*-value[2]		
	Control	Stimulus		TRT	Muscle	TRT × Muscle
Individual pig			2.91	0.48	0.96	0.55
Bicep femoris	95.99	99.83				
Semitendinosus	97.24	98.35				
Control pig[3]			10.89	0.53	0.18	0.22
Bicep femoris	95.83	95.41				
Semitendinosus	96.96	114.25				

[1]Barrows either being subjected to a hog electric stunner postmortem (**ES**) or not (**C**)
[2]TRT, treatment.

CONCLUSION

Transport losses are a concern in the swine industry; however, trailer vibrations during transportation were not considered a factor affecting the nonambulatory condition incidence before Morris et al. (2021). Collected data indicated pigs are exposed to dangerous environmental conditions during summer and to vibration magnitudes that may influence health risks. Although, muscle damage or exhaustion quantification after transportation is needed to accredit vibration exposure as a muscle fatigue catalyst. Unfortunately, there is still a need for improvement in models and technologies which estimate muscle fatigue in real time. Momentarily, the use of EMS and EMG are promising technologies which can be employed, but these tools must be refined. In future research, collected data from winter and summer pig transportation will be combined with muscle fatigue measurement techniques to allow the creation of comfort levels similar to the ones provided by ISO for humans.

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