DISSERTATION

UNDERSTANDING THE RELATIONSHIPS BETWEEN SWINE BEHAVIOR, PHYSIOLOGY AND MANAGEMENT TO IMPROVE ANIMAL WELFARE, REDUCE IN-TRANSIT LOSSES AND IMPROVE MEAT QUALITY IN SWINE

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY LILY NOWELL EDWARDS ENTITLED "UNDERSTANDING THE RELATIONSHIPS BETWEEN SWINE BEHAVIOR, PHYSIOLOGY AND MANAGEMENT TO IMPROVE ANIMAL WELFARE, REDUCE IN-TRANSIT LOSSES AND IMPROVE MEAT QUALITY IN SWINE" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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ABSTRACT OF DISSERTATION

UNDERSTANDING THE RELATIONSHIPS BETWEEN SWINE BEHAVIOR, PHYSIOLOGY AND MANAGEMENT TO IMPROVE ANIMAL WELFARE, REDUCE IN-TRANSIT LOSSES AND IMPROVE MEAT QUALITY IN SWINE

The objective of these studies was to determine the relationship between pre-slaughter management, physiological measures, and meat quality using commercial swine marketing practices. Experiments 1 & 2 were conducted to determine the effects of distance moved at the farm, time of lairage in holding pens prior to slaughter and distance moved from lairage pens to the stunning area on blood lactate concentration ([LAC]), rectal temperature and meat quality. Both [LAC] and temperature changed during the marketing process ($P< 0.0001$). The highest [LAC]s were observed at loading and exsanguination. Longer distance resulted in higher [LAC] during loading (Exp. 2). Unexpectedly, longer rest resulted in higher [LAC]s at exsanguination (Exp 1 & 2) and at the end of lairage (Exp. 2). An increase in [LAC] during loading reduced glycolytic potential (Exp. 2) and had a positive impact on meat quality measures, i.e. decreased drip loss, increased 24 hr pH and trends for lower L* and higher visual muscle color score (Exp 1 &2). Specific measurements of pre-stun handling, jamming, rearing, vocalization, electric prod use, backing up, were found to be correlated with exsanguination [LAC] ($P<0.05$).

Experiment 3 evaluated the persistence of blood changes in swine during the marketing process following alteration of dietary electrolyte balance (dEB). Experimental pigs were assigned to a Low (121 meq/kg) or High (375 meq/kg) dEB diet *ad libitum* for 3 days prior to slaughter. Prior to transport, animals were fasted in the barn for 10 h, then shipped to the plant, rested for 8 h and slaughtered. Blood was sampled four times, initial and final weights were obtained and meat quality parameters were measured. Increasing dEB had no adverse effects on growth performance, meat quality, carcass yield or incidence of pars esophageal ulcer scores. Exsanguination [LAC] was negatively correlated to 60 min pH ($r = -0.32$, $P = 0.0004$) and positively correlated ($r = 0.21$, $P = 0.02$) to drip loss. These results suggest that exsanguination [LAC] is predictive of the rate of early post-mortem metabolism. This study demonstrated that the effect of dEB on blood parameters was not maintained following a 10 h feed withdrawal ($P > 0.22$). Therefore, it is likely that the animal's ability to withstand any increased metabolic acid load associated with the stress of transport was lost following feed withdrawal.

This research supports the use of LAC as a sensitive tool to determine changes in handling and management. These studies emphasized the impact that pre-slaughter management has on physiological parameters of swine throughout the entire marketing process.

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OBJECTIVES OF DISSERTATION

I. To establish the relationship between specific pre-slaughter animal handling parameters such as electric prod use, jamming and vocalization and post-slaughter blood lactate concentration in a commercial swine slaughter facility.

II. To determine if a relationship existed between pre-slaughter animal management, (i.e. distance moved during loading at the farm, lairage time prior to slaughter at the packing plant and distance moved from the lairage pen to the stunning area), and physiological measures, blood lactate concentration and rectal temperature.

III. To determine the effect of pre-slaughter animal management, from farm to packing plant, on meat quality.

IV. To determine the persistence of dietary electrolyte balance (dEB) treatment on blood pH, bicarbonate and base excess, physiological indicators of buffering capacity, in addition to determining if there are any effects of dEB treatment on growth performance, meat quality, carcass yield and incidence of gastric ulcers.

CHAPTER ONE

Introduction

The overall objective of this body of research is to understand how blood lactate concentration, a physiological indicator of handling stress in swine, can be used to develop commercial management procedures that complement swine physiology to minimize stress and maximize animal welfare and meat quality. Blood lactate in swine is a quick and sensitive responder to marketing stressors such as fighting and aggressive handling. The ability for researchers to rapidly and easily obtain a blood lactate concentration measurement, from both live and dead animals, makes blood lactate concentration a useful tool for monitoring animal welfare in swine slaughter plants. A blood lactate concentration value also provides a quantitative objective measurement of stress providing an alternative to subjective measurements of animal welfare.

Additionally, in a broad sense, this body of research was designed to ultimately look at methods of reducing animal losses during the marketing process in the swine industry. Discussed at length in subsequent sections, the swine industry suffers from losses due to dead and non-ambulatory animals as they are transported from the production facility to the slaughter plant and subsequently harvested. It is believed that these animals die or become non-ambulatory due to the development of metabolic acidosis. It is hypothesized that feeding pigs a high electrolyte diet prior to slaughter may provide them with the

buffering capacity needed to combat the developing acidosis. In order for this type of feeding regimen to work over various feed withdrawal protocols, transportation times and lairage lengths used in the swine industry, it is necessary to determine how long the blood changes associated with a change in dietary electrolyte balance (dEB) would persist in the blood. Additionally, it was necessary to determine if electrolyte supplementation would have any negative effects on certain production variables important to swine producers.

It is hoped that the data gathered from these studies will provide recommendations for the swine industry regarding optimal pre-slaughter management techniques, from farm to slaughter, to both maintain and improve animal welfare, by reducing losses and stress level, and to improve meat quality.

CHAPTER TWO

Literature Review

2.1 Animal Welfare and Livestock Production

Farm animal welfare and well-being are important components of the livestock production industry. The majority of citizens are not involved in the production of food and have little knowledge regarding the management processes used to raise animals. Approximately one hundred years ago, 30% of the American workforce was involved in agriculture and 50% of citizens lived in rural areas (USDA, 2008). Currently, less than 2% of the American population work in agriculture and only 10% live in rural areas, a significant change from early agricultural tradition (USDA, 2008). Urbanization has drawn many people away from the family ranches and dairies and they have moved into the cities. Additionally, the agricultural industry has made great advances in production efficiency in both plant and animal production that enable fewer people to produce significantly more food. In approximately 50 years, the agricultural industry had gone from using two acres of land to produce 100 bushels of corn to producing the same 100 bushels on less than one acre (USDA, 2008). This improvement in efficiency has played a role in the reduction of the population involved in producing food. If has also made it possible to feed the growing population in the United States. Approximately 100 years ago, the population of the United States was 90 million; this value has more than tripled and is currently approximated at 304 million people (USCB, 2009). Despite this large

increase in population, agriculture has been able to still provide adequate food sources to the United States population (USDA, 2009b, d).

The development of Concentrated Animal Feeding Operations (CAFOs) has also contributed to this reduction in agricultural employment for citizens. Similar to plant crops, less space and human labor are needed to raise the same number of animals. Between 1988 and 2008 the number of both cattle and hog operations decreased by approximately 250,000 operations and the number of dairy operations decreased by approximately 155,000 operations (USDA, 2009a). While the number of livestock facilities has decreased, the actual number of animals being raised has increased demonstrating the industries vast improvements in production efficiency (USDA, 2009c). These larger concentrated livestock feeding operations were created to both improve production efficiency and improve aspects of animal welfare (i.e. protection from disease and inclement weather, individualized feeding for adequate nutrition and reduction of losses using farrowing crates as an example). Despite these improvements, certain aspects of animal welfare have been compromised with the creation of CAFOs, i.e. diminished space, inability to perform natural behaviors, production diseases). Currently, these aspects of today's agricultural system are drawing substantial attention from the public causing a growth in societal concern for livestock animal welfare and demanding a change in certain agricultural management practices.

The industrialization of agriculture has prompted a change from the tradition of animal husbandry to a newly developed concept of animal science as seen in the departmental name change at land-grant institutions from Animal Husbandry to Animal Science (Rollin, 2002), and even Animal Science and Industry at some universities

emphasizing the focus on improving production. Animal husbandry is understood as the raising and caring of livestock and while those concepts are included in the realm of animal science, animal science primarily focuses on the biology of animals. Husbandry focuses on the animal as a whole and animal science looks at separate biological mechanisms to see how they can be altered to improve efficiency of that particular system. Although the agricultural industry has vastly improved their level of production efficiency with this change to animal science, some aspects of animal welfare have been compromised as the livestock production systems have moved away from the traditional agricultural views of husbandry. It is necessary for the agricultural community and animal scientists to synthesize these concepts of animal husbandry and animal science to develop a productive agricultural system that focuses on both efficiency and welfare and that is ultimately consistent with the current societal ethic regarding animal welfare.

The livestock industry is now faced with a dilemma: continue to maximize production efficiency by maintaining large numbers of animals in smaller spaces, or provide more space and possibly lower animal production per unit of space to improve animal welfare. Currently, society, producers and scientists are struggling, in different ways, with this concept, i.e. maximizing production while maintaining animal welfare. For example, confinement hog operations must find a way to produce the same number of animals, in order to make the same profit, while providing more space, which means potentially lower animal production per unit of space, to the sows in gestation crates, increased space being one of the desired improvements in animal welfare status for gestating sows.

The agricultural industry is changing. People, i.e. producers, veterinarians, consumers, processors, scientists, are realizing that animal welfare has become more and more of an integral part of efficient and productive livestock animal production systems as these systems are becoming more industrialized and the amount of food needed to feed the country is ever increasing. Legislation has been and is still being developed to determine welfare standards for some livestock species. Niche markets have developed animal-friendly food labels. Animal welfare is playing a role in state referendums and consumers are beginning to demand what they deem to be animal friendly and humanely treated products. Animal scientists are working to find a balance between the improvement of animal welfare and the maintenance of production efficiency and practicality, a daunting task considering the ambiguity surrounding the measurement of an animal's welfare and the multitude of different species, management systems and processing systems found in our nation's agricultural system.

2.2 What is Animal Welfare?

Animal welfare is an important concept in today's society yet it is a term that is difficult for animal scientists, producers, veterinarians and consumers to adequately and completely define. Scientists have provided definitions of good animal welfare (Broom, 1986; Dawkins, 2006; Duncan and Petherick, 1991; Duncan and Dawkins, 1983; Fraser, 1995; Mason and Mendl, 1993; Mendl, 1991; Moberg, 1985; Ng, 1995; Sandoe, 1996), yet there is no consensus as to how to precisely define animal welfare. The inability of specialists to agree on a complete definition of welfare is in part due to the fact that there are so many different factors that could be used to determine an animal's welfare.

Each welfare specialist has his own definition of what "good" welfare means. Some specialists maintain that an animal's welfare is primarily based on health (Moberg, 1985). Can the animal reproduce? Is it free from disease? Looking deeper at specific physiological mechanisms, levels of various hormones (e.g. cortisol, lactate, epinephrine, etc.) are measured to assess welfare status (Moberg, 1985). Scientists use their knowledge of stress physiology and immune response to help identify an animal's welfare status. For example, calves respond to dehorning with physiological and immunological responses that indicate pain and stress (Petrie et al., 1996; Stafford and Mellor, 2005; Sylvester et al., 1998; Wohlt et al., 1994). Most people would agree that health, prevention of injuries, reducing death losses and lameness are primary components of good animal welfare.

Many welfare specialists extend the concept of animal welfare beyond what may be considered the more objective physiological stress data and health information. They say that animal welfare must also include emotional (affective) states, feelings and wants (Dawkins, 1988; Duncan and Petherick, 1991; Duncan and Dawkins, 1983; Sandoe, 1996). Are the cows "happy"? Are all their needs being met? Are they distressed? In discussion of mental states, some scientists state that the performance of natural behaviors is a necessary component for good welfare. Is the sow in the farrowing crate able to perform nesting behavior prior to parturition? If she cannot nest build, how is this affecting her well-being?

The plethora of definitions regarding animal well-being not only sets the platform for extreme debate, but additionally, it calls attention to the enormous task at hand for all those involved with assessing, determining, ensuring and simply just understanding

animal welfare. Despite debates on what animal welfare is, many guidelines and regulations have been created to protect an animal's well-being. In the United States, the Animal Welfare Act (AWA, 1989) is the federal law, first initiated in 1960, that provides minimum protection to certain species of animals, primarily companion animals and those used in research, for handling, care, housing and treatment. Unfortunately, the law's definition of an animal does not include livestock species raised for food or fiber. Farm animals were exempt from this law due to political pressure from livestock groups. The provisions set forth in the Animal Welfare Act apply mostly to the housing of each animal species. There is one amendment, officially made in 1985, that requires facilities housing nonhuman primates to provide an "environment adequate to promote the psychological well-being of the animals." Although clearly stated, it is unclear exactly what the definition of psychological well-being is and the amendment provides no suggestion as to how to satisfy this requirement. In this law the mental component of animal well-being applies only to nonhuman primates.

In the United Kingdom, the Brambell Commission (1965), a governmental committee, was created to assess the welfare of animals raised in intensive husbandry systems. They wrote a report proposing the conditions that farm animals should experience. Since the revision by the United Kingdom Farm Animal Welfare Council in 1979, these animal conditions are now universally referred to as the 'five freedoms' and read as follows: 1) freedom from thirst, hunger and malnutrition, 2) freedom from discomfort, 3) freedom from pain, injury and disease, 4) freedom to express normal behavior and 5) freedom from fear and distress (FAWC, 1979). The 'five freedoms' are

more inclusive of all aspects of animal welfare and have been the basis for welfare assessment in United Kingdom since their creation.

Examination of all the statements made by the various committees and lawmakers reveals a common theme: the emphasis on the emotional (affective) component of the animal's life in the consideration of animal well-being and welfare. The difficult question arising from these terms is: what is psychological well-being? How do you determine that an animal has good psychological well-being and more importantly how do we measure it?

2.3 Animal behavior

When humans want to express how they feel, they can communicate both through actions and verbal expression. Humans cannot communicate with animals verbally and therefore, must rely heavily on an animal's behavior to determine how it may be perceiving its environment. Behavior is significant in human-animal communication. A veterinarian, whose job it is to cure disease, cannot simply ask an animal how it is feeling. He must use clues, such as alterations in behavior, e.g. decreased eating, listlessness, increased water consumption, to determine what is wrong with the animal. Animal handlers use behavior to move livestock through various handling facilities, noticing the animals' responses to their environment to make handling more effective. If an animal balks at the opening to a single-file chute, the handler can respond to the animal's hesitation by determining if there is anything in the environment that the animal may fear. Animal behaviors are also used in slaughter plant audits. Behaviors, ones that are influenced by human interaction during animal handling, are used to determine adequacy of animal handling systems. Parameters such as falling, vocalization and

electric prod use are recorded to assess how the animals are responding and interacting with their environment thus judging the adequacy of handling. Animal behavior is the key to understanding an animal's experience in the environments in which we raise and handle them (Dantzer and Mormede, 1983; Darwin, 1872).

2.3.1Types of animal behavior

All species have an ethogram which is a detailed behavioral repertoire describing all the behaviors that that animal species performs. Among these behaviors are either learned or instinctual hard-wired behaviors. Dairy cows learn to line-up at milking time. Many zoos have taught primates and various other species to present limbs or stand still for husbandry and veterinary procedures (Grandin, 2000; Savastano et al., 2003). Dogs learn to do a multitude of behaviors during obedience training. Animals learn without the help of humans as well, an example being that of cheetah cubs learning how to efficiently and effectively kill prey. Young cheetahs can kill at a young age but take some time and lessons from their mother to develop their skills (Caro, 1994).

Hard-wired behaviors are different. These are innate behaviors that the animal does not have to learn. There are many examples of these hard-wired behaviors. Display behaviors performed by birds during courtship and mating are instinctual hard-wired behaviors. The nesting behavior we see sows perform before farrowing is hard-wired (Jensen, 1986; Stolba and Wood-Gush, 1984). The prey catching behavior observed when dogs chase squirrels in the park is an example of an instinctual behavior. Livestock exhibit many hard-wired behaviors: the point of balance and flight zone responses in cattle during handling, rooting behavior in pigs, dust-bathing behavior in chickens and using a nest box in hens (Appleby and McRae, 1986; Newberry and Wood-Gush, 1988;

Stolba and Wood-Gush, 1989; Studnitz et al., 2007; Vestergaard, 1982). These behaviors are referred to as fixed action patterns (FAPs) because they are behavioral sequences that are always performed in the same manner. A classic example of an FAP is egg-rolling behavior in Greylag geese (Lorenz and Tinbergen, 1938). When an egg rolls out of a goose's nest, it performs a highly predictable pattern of movement to bring the egg back towards the nest. Fixed-action patterns are hardwired but the particular sign stimulus that triggers the behavioral response can be dependent upon learning and emotional experiences.

Both animal instincts and learned behavior are driven by animal emotions. A cow's instinct to move away from a predator entering its flight zone is driven by fear; a pig's instinct to root for food is driven by its seeking emotion; a dog's instinct to do a play bow to a puppy is driven by its motivation to play. Behavior can be examined as patterns of actions performed to achieve a specific goal. Positive goal-directed behaviors can be dissected into three phases: 1) searching for the goal; 2) behavior directed at the goal and 3) quiescence following achievement of the goal (Manning, 1979). These goal-directed behaviors are usually highly-motivated as there is a reward (the goal) once the behaviors are performed.

When an animal's ability to perform highly-motivated behaviors, such as dust-bathing, foraging or nesting, is prohibited, they can begin to develop displacement behaviors, i.e. bar biting, pacing, rocking, self-narcotizing behavior, increased aggression, etc. Some of these behaviors are stereotypies. A stereotypy is defined as a repetitive behavior that repeats itself in a pattern that seldom changes and it serves no obvious purpose. The development of these repetitive, non-goal oriented behaviors are a

mechanism for the animal to cope with the frustration of not being able to perform certain innate behaviors. Sows in gestation crates often exhibit bar-biting shortly before being fed, perhaps a reaction to the inability to food search and root, innate motivated behaviors (Lawrence and Terlouw, 1993). These animals are self-medicating; filling a behavioral need with a maladaptive behavior. It has been demonstrated that the performance of stereotypical behaviors is related to the release of endorphins providing the animal with some relief from the stressful environment (Cronin et al., 1986; Dantzer, 1986). Supporting this concept of stereotypical behavior as a medication, when anti-addictive pharmacological agents such as nalmefene are given to horses, pigs and mice who perform stereotypies, the stereotypies stop (Cabib et al., 1984; Cronin et al., 1985; Dodman et al., 1988). Animals develop these stereotypical behaviors as a means of coping with a barren environment, an indication that their environment needs to be improved. Animal behavior can be used as an indicator of an animal's state of well-being (Duncan, 1998).

2.3.2 Using Animal Behavior to Understand Animal Welfare

An animal's need to express certain behaviors, e.g. dust-bathing, nesting, foraging, locomotion, social interaction, seeking, etc. is blocked in some confinement livestock production systems. This causes potential problems with animal welfare. The agricultural industry has created some housing systems in which animals are not able to behave naturally. Their drive to perform natural behaviors is thwarted and as a result they develop a higher level of frustration in their housing, manifested in various ways but particularly in their expression of behavior. Producers can economically raise livestock and poultry in intensive confinement systems. The question is, should we raise them this

way (B. Rollins, personal communication). The animals are never going to be able to "tell" people how they feel and therefore it is necessary to try to understand them in a different manner, i.e. through their behavior. Some people fear making assumptions about an animal's well-being through its actions. But, as one researcher profoundly stated, it is important to be "roughly right on something important than to be accurate but wrong or irrelevant" (Ng, 1995). Animal well-being is that "something important."

Just as humans, animals are driven to perform by their feelings and emotions (Manning, 1979). Temple Grandin emphasizes the concept of designing animal environments and putting animals in situations that reinforce the positive emotional circuits and avoid the negative emotional circuits to improve an animal's well-being (Grandin and Johnson, 2009). Animals will stimulate these positive emotional circuits through their behavior because these behaviors are highly motivated. When an animal can no longer express these highly motivated behaviors, some of them seemingly basic behaviors that most of us would expect to be able to do on a daily basis, their environment is no longer ideal.

Behaviors that are not highly-motivated can also be used to assess animal welfare. As mentioned, veterinarians observe animals for behaviors and affective states that may be indicative of illness or injury such as listlessness, reduced feed consumption and limping. Additionally, the American Meat Institute (AMI) slaughter plant audit guidelines (2007) have core criteria that are behaviorally based. When plant auditors perform an audit they score things such as the number of animals that fall, the number of animals that vocalize and the number of animals that are electrically prodded. The presence of these behaviors in the particular situation are negative to the animal's well-

being. An animal that is balking in the handling area will be electrically prodded. The presence of balking is indicating that something is making the animal uncomfortable in its environment. Vocalization in animals can be an indication of distress (Grandin, 2001, 1998; Haley et al., 2005; Noonan et al., 1996; Underwood, 2002; Watts and Stookey, 1999; White et al., 1995), therefore the number of animals that vocalize is monitored in order to minimize the amount of distress being caused just prior to slaughter.

2.4 The Swine Industry and the Incidence of Losses

In 2008, the swine industry in the United States consisted of approximately 68 million hogs at one time (the hog slaughter number for 2008 was 111 million) (USDA, 2009c). Of these hogs, approximately 0.22% (value from 2006) died during the marketing process (Ellis et al., 2003b; FSIS, 2007), a 2.5 fold increase from 1990. This value only represents the pigs that died either during transport to the plant or those that died during the time they spent in the plant prior to slaughter as this is a mandatory governmental reporting parameter. Other types of losses are not reported. Some have estimated that all industry losses represent between 0.8 and 1.0% of slaughter animals in United States swine packing plants (Ritter and Ellis, 2006, personal communication). These losses include non-ambulatory, injured animals (NAI) and non-ambulatory, non-injured animals (NANI). Although these animals may not die prior to slaughter they do pose both an economic loss and an animal welfare problem for the industry. The non-ambulatory, non-injured animals are commonly referred to as fatigued, slows or refusals as they are unable to move through the handling system. These animals require more time and special handling. Not only do the processors spend money in NANI animal management, the carcasses of these animals often produce lower quality meat, thus

incurring a discounted market price for the producers. Having non-ambulatory animals is also an animal welfare concern. The animals who are refusing to move and become injured during the marketing process are suffering. The reduction of non-ambulatory animals is a crucial matter for the swine industry.

2.4.1 Stress-susceptibility in swine and the incidence of losses

There are many reasons why an animal may become non-ambulatory during the marketing process. Animals can get injured, they can respond negatively to different weather conditions during transport or they may be transported in poor health. Pigs can also become non-ambulatory due to metabolic acidosis caused by a reaction to various marketing stressors. Stress-susceptiblity of swine has been a problem in the past and remains a current problem in the swine industry. Several syndromes have been identified to characterize some of the animals that become non-injured and non-ambulatory.

2.4.1.1 The Porcine Stress Syndrome

The porcine stress syndrome (PSS) is a stress-induced disease that has affected swine production in the United States in the past. The porcine stress syndrome was formerly identified around the 1960s at a time when commercial pigs were being bred for leanness and heavy muscling (Topel et al., 1968). This syndrome is characterized by specific physiological and behavioral changes that occur in response to various pre-slaughter stresses such as mixing and fighting with conspecifics, transport, and physical exertion (Anderson et al., 2002; Christian and Kuhlers, 1981). During the 1960's and 70's the industry suffered economic loss from the consequences of the porcine stress syndrome. When exposed to various pre-slaughter stressors, animals suffering from this syndrome often died, thus an obvious loss for the producers and processors.

Additionally, the animals that survived the symptoms of PSS often produced pale, soft, and exudative (PSE) pork, pork of lower quality that did not provide as high a profit to packers, estimated to have cost the swine industry 230 to 320 million dollars each year (Hall, 1972). In addition to being an economic loss, the prevalence of PSS in the industry was an issue of animal well-being.

The porcine stress syndrome reaction is characterized by extreme muscle rigidity, skin blotchiness, abnormal vocalization and open-mouth breathing, occurring upon immediate exposure to marketing stresses (Mitchell and Heffron, 1982; Topel et al., 1968). These animals are unable to move, have a low survival rate and are usually not able to recover from the stress-induced reaction. It was recognized that these symptoms were similar to symptoms of human malignant hyperthermia (MH). Malignant hyperthermia was discovered to affect certain individuals while undergoing anesthetic treatment with halothane causing muscle rigidity, cyanosis (skin blotchiness) and an extreme rise in core body temperature.

In the early 1990's, PSS was identified as a genetic syndrome caused by a point mutation of the ryanodine receptor channel, the calcium release channel on the sarcoplasmic reticulum of skeletal muscle, one of the critical components of muscular excitation-contraction coupling (Fujii et al., 1991; Otsu et al., 1991). Researchers identified this mutation in a location paralleling one of the genetic mutation locations identified in human malignant hyperthermia patients (Fujii et al., 1991). From this similarity with malignant hyperthermia, the PSS gene was named the halothane gene as both syndromes can be induced by halothane anesthetic.

At the onset of the porcine stress syndrome, animals enter an increased metabolic state with large increases in oxygen consumption, carbon dioxide production and increased heart rate. In the stress-susceptible pigs, the abnormal muscle contraction causes an increase in metabolism rate to provide more energy, initiated by a multitude of changes in hormone regulation (Mitchell and Heffron, 1982). The sarcoplasmic calcium concentration plays a role in the altered metabolism by activating glycogen phosphorylase, an enzyme that breaks down glycogen to glucose phosphate. This source of glucose phosphate is metabolized to lactate as a quick source of energy for the cell to sustain the abnormal muscle contraction. During a PSS reaction, animals have increased blood lactate, increased muscle glucose-6-phosphate levels, decreased muscle creatine phosphate concentration and decreased muscle pH (Ahern et al., 1980; Gronert et al., 1976; Mitchell et al., 1980; Schmidt et al., 1972).

Webb and Simpson (1986) have suggested that the leanness and muscle hypertrophy associated with the PSS mutation account for the addition of 2-3% to the lean dressed carcass weight. The potential for constant contractile activity by sedentary stress-susceptible animals (the abnormal ryanodine receptor function causing prolonged contraction and abnormal excitation patterns) perhaps adds to the muscle hypertrophy and limited fat deposition (resulting from an increased need and use of energy) in these PSS animals, making them desirable for breeding (MacLennan and Phillips, 1992). Although the heavily muscled features are desirable, other pork characteristics often associated with PSS are not. Carcasses from animals exhibiting PSS are often associated with pale, soft, exudative pork. As discussed, the PSS pigs' reactions to pre-slaughter stresses greatly increase their metabolic activity. Therefore, immediately post-slaughter,

the high metabolic rate and body temperature of the animals result in rapid muscle energy depletion and thus a rapid pH decline causing the pork to be pale, soft and exudative (Topel et al., 1968).

The porcine stress syndrome has been almost completely eradicated from the current commercial pig genetics. A recent study determined that the frequency of the halothane mutation in commercial pig genetics is low (Ritter et al., 2008a). Additionally, the industry has made efforts to minimize and control pre-slaughter stressors to reduce the number of losses.

2.4.1.2 The Fatigued Pig Syndrome

While loss due to PSS has been minimized through a better understanding of the cause of the syndrome and subsequent eradication from breeding stock, a new similar syndrome has come into existence beginning in the late 1990's, the fatigued pig syndrome. The fatigued pig syndrome is another stress-induced syndrome affecting pigs in the swine industry. From 1990 to 2006 the number of dead pigs upon arrival at the plant and those that died during lairage at the plant increased from 0.08% to 0.22% (Ellis et al., 2003b; FSIS, 2007). Currently, similar information on loss due to the fatigued pig syndrome has not been published as it is not a mandatory reporting parameter, therefore the information available on the widespread impact of this syndrome is merely an estimate. A review of several large field studies estimated that all dead, injured and fatigued animals represent approximately 0.62% of slaughter animals in United States swine packing plants (Ritter, 2008). Processing plants do spend a large amount of time and money dealing with the animals that succumb to this syndrome, e.g. moving them to a rest pen, removing them from the trucks upon arrival at the plant, and transporting to

the stunner. Transport losses have been estimated to cost the United States swine industry between $50 and $100 million each year (Ellis et al., 2003a).

The fatigued pig syndrome is characterized by a similar onset of observable events as found in PSS and MH. Fatigued pigs exhibit signs of open mouth-breathing, muscle tremors and blotchy skin (Anderson et al., 2002; Benjamin et al., 2001; Ellis and Ritter, 2006). As the severity of the reaction progresses, afflicted pigs will refuse to move, vocalize abnormally, collapse and potentially die if not given time to rest and recover. While the physical manifestations of these syndromes are similar, there are some distinguishing factors between PSS and the fatigued pig syndrome. The onset of PSS has been observed to be more immediate than that of the fatigued pig syndrome (G.G. Goodner, personal communication). The PSS animals have a quick onset of labored breathing, high-pitched squealing and intense muscle tremors compared to the fatigued pigs which have similar symptoms but gradually become worse as the stressors continue to accumulate. The fatigued pig syndrome occurs along a continuum and animals are able to recover and sometimes still ambulate. In the standard PSS reaction, once initiated, the subsequent changes represent a worsening of symptoms towards a probable death.

Unlike the porcine stress syndrome, the genetic aspect of the fatigued pig syndrome has not been largely explored. It seems likely that there may be more than the one point mutation in the swine genome affecting normal muscle contraction. Heavy muscling is still a strong selection parameter in the swine industry making it feasible that there could be a link between heavily muscled fatigued pigs and a different mutation of the the ryanodine receptor. Genetic studies with the human receptor have shown more

than 20 mutations of the ryanodine receptor resulting in malignant hyperthermia (see review (McCarthy et al., 2000)). Examining the physiological description of the PSS muscle abnormality and comparing it to the observations of the fatigued pigs, it seems possible that these animals may be suffering from an alteration in excitation-contraction coupling. The fatigued animals begin to have tremors in the highly developed muscles of the hindquarters eventually causing the animal to collapse. Recent research exploring the presence of halothane sensitivity in swine genetic lines free of the known halothane gene provides more evidence for another unidentified genetic mutation (Allison et al., 2005; Allison et al., 2006). This observed halothane sensitivity may be associated with greater susceptibility to the fatigued pig syndrome (Marr et al., 2004).

Whereas there is a paucity of research exploring the genetic and physiological changes in the muscle of fatigued animals, there is a large body of research investigating the metabolic changes associated with the syndrome (Allison et al., 2006; Anderson et al., 2002; Benjamin et al., 2001; Ivers et al., 2002b). Fatigued pigs have been characterized as exhibiting glycogen depletion, metabolic acidosis and physical exhaustion (Anderson et al., 2002; Ivers et al., 2002b; Ritter et al., 2005). Pigs that become fatigued have been observed to possess hypermetabolic properties prior to initiation of any stressor (Allison et al., 2006) suggesting they are predisposed to the stress reaction. Similar results have been reported in halothane sensitive animals alluding to a chronic up-regulated metabolism (Heinze and Mitchell, 1989; Jones et al., 1972; Veum et al., 1979). Several studies have reported altered values of certain metabolites, increased lactate, ammonia, CPK, glycerol, NEFA, BUN and decreased pH, bicarbonate and base excess, in fatigued animals suggesting a state of an upregulated

metabolism and a metabolic acidosis (Allison et al., 2006; Anderson et al., 2002; Ivers et al., 2002b; Sutherland et al., 2008). The body responds to a need for more energy by mobilizing triglycerides from the fat thus increasing circulation of glycerol and NEFAs. It has been observed that fatigued animals have a higher level of creatinine, a product of muscle breakdown, potentially indicating some muscle damage in the fatigued animals occurring during the pre-slaughter process (Sutherland et al., 2008).

The fatigued pigs also enter the immediate pre-slaughter phase at a high metabolic rate and temperature making their carcasses at risk for producing pale, soft and exudative meat. The physiological changes associated with pre-slaughter stresses have been shown to have detrimental effects on pork quality (Hambrecht et al., 2005a; Hambrecht et al., 2005b; Hambrecht et al., 2004b; Warriss et al., 1994). These studies explored the effect of stress on meat quality, they did not compare the meat quality of fatigued and non-fatigued animals. However, if the fatigued animals are allowed to rest, meat quality is superior to control animals, presumably due to reductions in muscle glycogen concentration (Carr et al., 2005).

The future direction for the industry will be to treat the fatigued pig syndrome as it did the porcine stress syndrome and fund efforts to determine what is causing these animals to react so extremely to pre-slaughter stressors. Because the syndrome is triggered by a multitude of factors (Anderson et al., 2002), it makes it extremely difficult to control. Methodologies used in the exploration of the porcine stress syndrome can be used as a framework to further study the intricacies of the fatigued pig syndrome. By understanding small components of the syndrome, i.e. the genetics, muscle physiology

and metabolism, it may make it easier to develop pre-slaughter management and/or breeding practices to accommodate these alterations in physiology.

2.4.2 Preventing Losses Due to the Fatigued Pig Syndrome Using Electrolyte Supplementation

There are many factors contributing to the fatigued pig syndrome potentially leading to in-transit death loss. The fatigued pig syndrome is a multi-factorial syndrome, being influenced by many management factors such as stocking density, handling, human exposure and genetics (Anderson et al., 2002). Several studies have indicated that pigs are susceptible to developing metabolic acidosis with aggressive handling and fatigued pigs are likely a result of excessive acidosis (Benjamin et al., 2001; Ivers et al., 2002b). The effects of metabolic acidosis (lower blood ph, lower base excess, etc.) can alter the pig's physiology potentially resulting in the incidence of fatigued pigs. Hyyppa and Poso (1998) proposed the following mechanisms to explain acidosis induced muscle fatigue: 1) hydrogen ions can have a direct effect on the myofibrillar apparatus; 2) calcium metabolism is affected in the muscle cell – higher calcium concentration is needed for contraction, the release of calcium from the sacroplasmic reticulum is inhibited and the activity of the sarcoplasmic reticulum calcium ATPase is inhibited at low pH; 3) acidosis may compromise ATP re-synthesis from intramuscular glycogen and from blood glucose since critical enzymes in the process are markedly inactivated with a small decrease in pH. Comparing these potential effects of acidosis with the behavioral symptoms of the fatigued pig syndrome, one can begin to make connections between the physiology of the pigs and the resulting behaviors. Pigs with acidosis are having trouble with muscular

mechanisms and obtaining enough energy to function; the fatigued pigs exhibit an inability to move and abnormal muscle tremors.

The fatigued pig syndrome can be influenced by so many factors that it becomes a difficult syndrome to study and prevent. Research has explored various methods of reducing incidence of fatigued pigs. One nutritional tactic for reducing the number of fatigued pigs is manipulating dietary electrolyte balance to alter the acid-base status of the pigs. It has been hypothesized that providing finisher weight pigs with a cation-rich diet just prior to slaughter may provide the animals with some buffering capacity against the development of acidosis and subsequently reduce the incidence of fatigued pigs (Ivers et al., 2002a). The acidogencity or the alkalogencity of a diet is determined by the dietary electrolyte balance (dEB). The dietary electrolyte balance of a diet is calculated by subtracting the fixed anions in the diet from the fixed cations using the following equation: $(Na^+ + K^+) - Cl^-$ meq/kg of feed, ultimately determining the net acid or alkaline load of the diet (Mongin, 1981). The balance of cations and anions have been shown to influence the acid-base status in a variety of species (Halperin and Jungas, 1983; Mongin, 1981; Ogilvie et al., 1983; Patience and Chaplin, 1997; Yen et al., 1981). A diet with a low dEB is acidogenic and a diet with a high dEB is alkalogenic. Therefore, diets that have a higher dEB could help to prevent the stress-induced acidosis experienced by some pigs during the marketing process by buffering the effects of low pH and low bicarbonate.

A study was conducted investigating the effects of dEB (dietary electrolyte balance) on the incidence of fatigued pigs in animals exposed to aggressive handling (Anderson et al., 2002; Ivers et al., 2002a). The pigs used in this study were fed either a

high dEB (+481 meq/kg) diet or a low dEB (81 meq/kg) diet four days prior to slaughter. After diet treatment, the pigs were handled through a model stress system. The stress model handling system required the pigs to walk approximately 300 m in an aisle (2.1 m wide narrowing to 0.6m for approximately 20m), an electric prod and a sorting panel. Blood and rectal temperature were sampled pre-handling, immediate post-handling and 2 hr post-handling. Results indicated that fewer ($P < 0.05$) fatigued pigs were identified in the high dEB diet group compared to the low dEB diet group. Additionally, fewer animals had to be euthanized for humane reasons in the high dEB diet group than the low dEB diet group. The pigs in the high electrolyte treatment also exhibited improved blood buffering capacity with higher blood pH, bicarbonate and base excess ($P < 0.05$) values.

Two unpublished studies evaluated the effect of electrolyte balance and the use of the electric prod on the incidence of fatigued animals (Gonyou, 2002 unpublished). They found no significant difference between the incidence of fatigued animals between the two treatment diet groups but numerically the total number of fatigued pigs was approximately halved in the high dEB diet group compared ot the low dEB group.

2.5 The Stress Response and Lactate Production in Swine

Blood lactate has been the focus of several studies exploring the stress reactions of swine (Benjamin et al., 2001; Hambrecht et al., 2005a; Hambrecht et al., 2005b; Hambrecht et al., 2004b; Warriss et al., 1994). Lactate is produced in the body by skeletal muscles, erythrocytes, the brain and the viscera. During normal aerobic metabolism, pyruvate, the end product of glycolysis, enters the citric acid cycle and produces electron carriers used to generate ATP during the electron transport chain reactions. When the body is in an energy-deficient state, which can often be an oxygen-

deficient state, the pyruvate is converted into lactate rather than entering the citric acid cycle. The production of lactate from pyruvate produces two ATPs and recycles two hydrogen ions, thus reducing the acidity of the cell. The production of lactate does not require oxygen, and is therefore often referred to as anaerobic metabolism. Lactate can, however, be produced in the presence of oxygen. Lactate is produced constantly within the body but is produced in higher quantities when the body is in need of a quick source of energy. Lactate levels do not rise in the body until the rate of production begins to exceed the rate of clearance. Glycolytic skeletal muscles that are engaged in strenuous activity will begin to produce lactate. Eventually, the build-up of lactate within the cell will produce a concentration gradient which will force lactate out of the cell and into the blood stream. If the amount of lactate entering the blood exceeds the amount being cleared by the body, the blood lactate concentration of the animal will rise. During a stressful event, the blood lactate level of fatigued pigs can increase from baseline (approximately 4 mM) to as high as 32 mM (Benjamin et al., 2001; Ivers et al., 2002b). Even non-fatigued animals respond to stress with increases in lactate, however their blood lactate concentrations do not tend to reach the extremes of those animals that are fatigued.

In humans, blood lactate concentration is commonly used in training and endurance studies of athletes (Davis et al., 1983; Heck et al., 1985; Ivy et al., 1980; Karlsson, 1986). These studies are conducted to determine the effects of various factors such as pedaling speed and training experience on the lactate threshold. The lactate threshold, often referred to as the anaerobic threshold, is the exercise intensity level at which the rate of lactate production and accumulation in the bloodstream exceeds the

circulatory system's ability to clear it. In swine research, lactate kinetics, i.e. determination of lactation thresholds, lactate production over time, etc., have not been studied. Some studies have examined how lactate changes with varied levels of exercise intensity in controlled laboratory settings (Kallweit, 1982; Zhang et al., 1989) but this area of research has not reached the extent found in human health and exercise science. Studies focused on understanding changes in blood lactate concentration in swine explore the impact that management and handling practices have on the production of lactate. Several studies have been conducted exploring the response of lactate production to handling stressors (Benjamin et al., 2001; Hambrecht et al., 2005b; Hambrecht et al., 2004b).

There are other physiological indicators of stress that could be used in the swine industry to evaluate management practices. Several studies conducted in commercial swine packing facilities have evaluated changes in standard serum stress parameters associated with pre-slaughter handling stresses (Averos et al., 2007; Guise and Penny, 1989; Hambrecht et al., 2005a; Hambrecht et al., 2004b; Hemsworth et al., 2002; Moss, 1984; Warriss et al., 1994; Weeding et al., 1993). Although other physiological indicators such as cortisol and epinephrine have been shown to indicate differences between stressed and non-stressed animals, blood lactate concentration is preferable due to its ease of analysis, small amount of blood needed to perform the analysis and high sensitivity to differences in stress level. Warriss et al. (1994) were able to demonstrate a correlation between the subjective assessment of stress level and the objective measures of stress in a survey study of 13 swine slaughter plants (Warriss et al., 1994). High stress was associated with high exsanguination blood lactate, CPK and cortisol. Although all

results were significant, the difference in lactate values between stressed groups was considerably larger than seen in the comparison of the other measured blood parameters.

2.5.1. Animal Handling and Lactate

Blood lactate concentration has been demonstrated to be a quick responder to exercise and handling induced stresses in swine (Allison et al., 2006; Anderson et al., 2002; Benjamin et al., 2001; Kallweit, 1982; Zhang et al., 1989). Benjamin et al. (2001) developed a laboratory model in which the incidence of stress response in pigs in reaction to various animal handling methods could be studied. In the aggressive handling treatment, the handler moved the animals through a 300 m course, including a high loading ramp, with frequent use of an electric prod. In the gentle handling treatment, the handler proceeded at a moderate pace through the same course, except that the loading ramp was lower and a plastic cane was used in place of the electric prod. Benjamin et al. (2001) found that gentle handling did not affect blood lactate concentration (4.0 mM), animals maintained a lactate concentration near baseline. However, aggressive handling quickly increased lactate (25.2 mM) indicating the impact handling can have on pig stress. Another significant finding by Benjamin's lab, is that with rest, lactate returned to normal baseline levels within 2 hours, indicating that lactate is a measure of acute handling stress.

Blood lactate concentration has been reported to increase during immediate pre-slaughter animal handling (Hambrecht et al., 2005a; Hambrecht et al., 2004b; Warriss et al., 1994; Warriss et al., 1998). These studies have indicated that exsanguination blood lactate concentration can range from 4.4 mM to 31 mM dependent upon the categorization of the slaughter plant as a low or high stress handling system. The blood

lactate concentration found in the Warriss et al. (1994) study, 4.4 mM, is not significantly higher than baseline measurements of swine blood lactate concentration using a snare as the restraint method for sampling (Benjamin et al., 2001). The large range of exsanguination blood lactate measurements could be due to the definition of the stress level of the handling (high vs. low), the sampling technique (snare vs. other low-stress methods), the preservation of blood samples (the type of blood tube) and other overall facility design factors. Additionally, this large variation in exsanguination lactate could be indicating the sensitivity of lactate to differences in animal handling.

One study in commercial packing plants used two experimental stressor treatments to assess the impact of handling stress immediately prior to slaughter on swine (Hambrecht et al., 2005a). Experimental animals were subjected to one of two handling treatments: minimal or high stress. The minimal stress group was moved to the stunning area without electric prods in a calm manner. The high stress group was moved to the stunning area with the use of electric prods and yelling, moving four times back and forth in a corridor leading up to the stunning area. Hambrecht et al. (2005a) found that animals in the high stress handling group had higher exsanguination lactate than the minimal stress group, 17.9 and 30.1 mM, respectively. These values are greater than those reported in the Warriss et al. (1994) study. It is possible that despite the difference in experimental treatment protocols the pigs in the low stress treatment in the Hambrecht study may have experienced more aggressive handling (D. Newman, personal communication, 2008). Another study by Hambrecht et al. (2004) that implemented the same handling stress treatments reported similarly high blood lactate concentrations, 15.6 and 27.7 mM for the minimal and high stress treatment groups.

2.5.2. Pre-slaughter Management and Lactate

During the marketing process, from farm to stun, there are many factors that could act as potential stressors for pigs. The animals are sorted, loaded onto a truck, mixed with conspecifics, transported, exposed to weather and handled at the slaughtering facility. Not only is each one of these events stressful, the combination of multiple events could cause a cumulative effect of stress on blood lactate concentration in particular (Ritter et al., 2008a). The effect of stocking density, transport length, lairage duration and many other factors have been studied at length in swine and other species (Hambrecht et al., 2005a; Krawczel et al., 2007; Perez et al., 2002; Ritter et al., 2008b; Ritter et al., 2006; Sutherland et al., 2009; Warriss et al., 1992), but not many of these studies have focused on the response of lactate to pre-slaughter management factors in swine (Hambrecht et al., 2005a; Perez et al., 2002; Warriss et al., 1992).

Several studies have been conducted exploring the effects of lairage length on various physiological parameters in market swine (Perez et al., 2002; Warriss et al., 1992). Benjamin et al. (2001) demonstrated in their study that with at least two hours of rest, blood lactate concentrations return to baseline in market-weight swine. Slaughter facilities allow pigs varying durations of rest when they arrive at the packing plant in order to allow them time to recover from the stress of handling and transport. Additionally, pigs identified as fatigued animals at slaughter plants will be moved to a rest pen and allowed time to rest before they are moved through the pre-slaughter handling area and slaughtered. The optimal duration of lairage for pigs at packing plants has not been defined. Warriss et al. (1992) explored the effects of 1 hr or 21 hr lairage lengths on physiological parameters in market swine. Results indicated that there was no

difference in blood lactate concentration between pigs rested for l hr and pigs rested for 21 hrs. Another study explored the effects of different lairage lengths, specifically 0 h, 3 h, and 9 h, on physiological and behavioral measures (Perez et al., 2002). Once again, no significant differences in lactate at exsanguination was found between the various lairage treatment groups.

2.5.3. Meat Quality and Lactate

The physiological changes associated with pre-slaughter stresses, i.e. increased exsanguination blood lactate concentration, have been shown to have detrimental effects on pork quality (Hambrecht et al., 2005a; Hambrecht et al., 2005b; Hambrecht et al., 2004b; Warriss et al., 1998). Hambrecht et al. (2004) determined that swine with greater blood lactate at slaughter produced pork with greater drip loss. This effect was increased by elevated muscle energy values (glycolytic potential) at slaughter. Warriss et al. (1994) were able to demonstrate a correlation between the subjective assessment of stress level and the objective measures of stress and meat quality in a survey study of swine slaughter plants. High stress was associated with high exsanguination [LAC] and lower meat quality, i.e. decreased water holding capacity and lighter color. Additionally, Warriss (1994) has demonstrated that pork from pigs stressed immediately before slaughter had less acceptable eating quality than that from pigs handled carefully. This was despite there being no difference in the predictors of quality that were measured (early pH and temperature, L*, and drip loss). There were, however, highly significant differences in the low and high stressed groups for both blood lactate and cortisol (Warriss, 1994). Hill and Schultze-Kaster (2006) reported immediate improvements in meat quality with

improved animal handling. With improved animal handling, the levels of exsanguination lactate could have decreased thus improving meat quality.

Immediate pre-slaughter stress and the increased production of lactate in the muscle prior to slaughter cause the animal to enter post-mortem processing with an increased rate of metabolism. This metabolic state will cause the carcass to enter post-mortem processing at a high temperature and the high metabolic rate will cause a rapid decline in carcass pH. This combination is more likely to produce a lower quality pork product with increased drip loss and decreased ultimate pH. As determined in the Hambrecht et al. (2004) study, these negative impacts on pork quality are impacted by high glycolytic potential. Glycolytic potential is a measure of the amount of glycogen in the muscle that can be metabolized to lactate, a by-product of anaerobic metabolism. Monin and Sellier (1985) determined that the glycoltyic potential is calculated by combining the amount of lactate in the muscle with two times the amount of glycogen, glucose and glucose-6-phosphate. Ultimately, this value represents the amount of substrate in the muscle that will add to the rate of metabolism and impact on this metabolism will have on ultimate pork quality, i.e. with more substrate available, more ATP will be created and degraded thus causing an increase in hydrogen ion production and a decrease in pH which will negatively impact pork quality.

Being able to manipulate glycogen stores in the muscle at slaughter would be beneficial to the improvement of pork quality; it would help to raise ultimate pH and decrease drip loss by reducing the amount of substrate to be degraded in the muscle at slaughter. Glycogen can be depleted during multiple locations during the marketing process of swine, e.g. any instance of handling and significant physical exertion will use

some of the muscle glycogen stores. If this happens early on in the marketing process it is unknown how long it will take for the depleted glycogen to be repleted, i.e. if glycogen was depleted in an animal during loading onto the transport truck, it is unclear whether the animal will be able to resynthesize those glycogen stores during transport and lairage prior to slaughter at the plant. There is a lack of research regarding glycogen repletion in pigs. Studies in humans have demonstrated that after glycogen has been depleted due to extreme exercise, glycogen stores are partially restored after 5-10 hours on a high carbohydrate diet and fully restored after 1-2 days (Piehl, 1974). A study performed with young bulls indicated that after stress with subsequent feeding, glycogen was restored approximately 50% after 1 day and up to 70% after two days (McVeigh et al., 1982). Pigs are not provided with food at the slaughter plant and therefore the rate of glycogen repletion may vary. If there were any remaining feedstuffs in the gut during transport and lairage, there is potential for this to aid in glycogen repletion. One practice that is common in the swine industry is feed withdrawal prior to transport thus making it unlikely that there would be enough gut contents remaining upon arrival at the plant to aid in glycogen repletion. It has also been shown in humans and pigs that glycogen is depleted at different rates dependent upon the muscle fiber type, exercise intensity and time of feed withdrawal (Gollnick et al., 1973; Gollnick et al., 1974; Karlsson et al., 1994; Vollestad and Blom, 1985; Wittmann et al., 1994). Animals can have the same level of glycogen in the muscle, but exhibit either poor or good pork quality dependent upon the muscle fiber types that contain the majority of the glycogen (Karlsson et al., 1994). This is an area of study that could have a large impact on pork quality and should

be studied further to understand the system of glycogen storage, breakdown and repletion in the pig.

CHAPTER THREE

THE EFFECTS OF DISTANCE MOVED DURING LOADING, LAIRAGE TIME PRIOR TO SLAUGHTER AND DISTANCE MOVED TO THE STUNNING AREA ON BLOOD LACTATE CONCENTRATION AND RECTAL TEMPERATUER OF PIGS IN A COMMERCIAL PACKING PLANT

ABSTRACT

Two studies were conducted to assess the relationship between swine management and physiology during the marketing process. Both studies were repeated measures 2 x 2 x 2 factorial designs (Exp. 1, n = 64; Exp. 2, n = 144) evaluating the effects of distance moved at the farm (DIS), lairage duration (REST) and distance moved to stun (STUN) on blood lactate concentration ([LAC]) and rectal temperature (TEMP) of swine in a commercial slaughter plant. For the DIS treatment, pigs were moved a short (15 m) or a long (46 m) distance during loading in the barn. Pigs were transported for approximately 2.5 h to the packing facility where they were unloaded and rested for a short (30 min) or long (4.5 hrs) duration. After lairage, the pigs were moved a short (20 m) or long (300 m) distance to stun. All pigs were electrically stunned and exsanguinated. Temperature and [LAC] were sampled on each experimental animal using a low-stress sampling method. Parameters were measured at seven times points during the marketing process: baseline, post-load, pre-unload, post-unload, post-lairage, post-

movement to stun and at exsanguination. In both studies [LAC] and TEMP changed ($P<$ 0.0001) during the marketing process; the highest [LAC] were observed at truck loading and exsanguination. Longer distance moved in the barn resulted in higher ($P = 0.0001$) [LAC] during loading (Exp. 2). Unexpectedly, longer REST resulted in higher [LAC] at exsanguination ($P = 0.0001$, Exp. 2) and greater increases in [LAC] during pre-slaughter handling than those rested a shorter time ($P = 0.003$, Exp. 1; P=0.001, Exp. 2). Animals rested for a longer period also had higher TEMP at exsanguination ($P = 0.02$, Exp. 2). Also unexpectedly, [LAC] was greater in animals moving a short distance to stun ($P =$ 0.04, Exp. 1; P=0.02, Exp. 2). Post-movement and exsanguination TEMPs were greater in animals that moved a longer distance to STUN in both Exp 1 & 2 ($P < 0.02$) resulting in increased 45 min post-mortem muscle temperature (P=0.006, Exp. 1). In conclusion, [LAC] and TEMP are sensitive tools to determine changes in animal handling and management. Both [LAC] and TEMP were highest during loading at the farm and movement through the stunning chute, indicating areas of focus to improve animal handling during marketing.

Key Words: lactate, lairage, marketing, pre-slaughter, rectal temperature

INTRODUCTION

Recently, there has been increased interest in the reduction of in-transit losses in the swine industry both for economic and animal welfare reasons (Ellis and Ritter, 2005). These losses include a combination of deaths and non-ambulatory pigs. Studies in laboratory and commercial settings have identified high blood lactate concentration ([LAC]) as a characteristic of fatigued pigs (Anderson et al., 2002; Benjamin et al., 2001; Ivers et al., 2002b). Additionally, [LAC] has been identified as an easily measurable,

quick responder to exercise and handling stresses in swine (Allison et al., 2006; Anderson et al., 2002; Benjamin et al., 2001; Kallweit, 1982; van der Wal et al., 1985; Zhang et al., 1989). Pre-stun handling stress has been the focus of research exploring the relationship between stressors and physiological responses of swine (Hambrecht et al., 2005a; Hambrecht et al., 2004b; Warriss et al., 1994). Other studies evaluating lairage and transport conditions have emphasized cortisol and other stress responses rather than [LAC] (Moss, 1984; Warriss and Bevis, 1986; Weeding et al., 1993). Although minimizing [LAC] during immediate pre-slaughter handling can improve animal welfare and meat quality (Edwards et al., 2009c; Hambrecht et al., 2005a; Hambrecht et al., 2004b; Warriss et al., 1994) maintaining low [LAC] throughout the marketing process may impact these factors in addition to reducing of the incidence of fatigued pigs.

The objective of this study was to characterized the change in [LAC] and TEMP throughout the marketing process, and to determine the relationship between pre-slaughter animal management, (i.e. distance moved during loading, lairage time, and distance moved to stunning), and the physiological measures, [LAC] and rectal temperature (TEMP). The ultimate goal is to establish recommendations for the swine industry regarding optimal pre-slaughter management, i.e. those practices that result in the lowest [LAC] and TEMP.

MATERIALS AND METHODS

Two experiments were conducted: Experiment 1 in July 2007 and Experiment 2 in January 2008 in the Midwestern United States. Study replications were analyzed as separate experiments due to difference in season, packing plant facility design and experimental protocol. Prior to the initiation of these experiments, all animal use,

handling, and sampling techniques described herein were approved by the Colorado State University Animal Care and Use Committee.

Experimental Design and Treatments.

These studies were designed as repeated measures utilizing a 2 x 2 x 2 factorial arrangements. Factors included: 1) distance moved during truck loading [DIS (15 m vs. 46 m)], lairage duration [REST (30 min vs. 4.5 h)], and distance moved from the lairage pen to the stunning area [STUN (20 m vs. 300 m)]. In Experiment 1, a total of 64 (32 barrows, 32 gilts) commercial crossbred pigs with an average live weight of 125 ± 9 kg were used. In Experiment 2, 144 crossbred pigs (120 barrows, 24 gilts) with an average market weight of 128 ± 3 kg were used. The mean live weights were back calculated using the hot carcass weights using an estimated 75% dressing percentage.

Distance moved during loading (DIS). Test pigs were moved in small groups (4 – 6 animals) from home pens on the farm to the loading chute. In Exp. 1, there were 4 home pens per finisher barn, 2 located approximately 15 m (short distance) from the barn load-out chute on each side of a middle alley and 2 located approximately 46 m (long distance) from the load-out chute on each side of the middle alley. In Exp. 2, two adjacent home pens were used at each of the aforementioned barn locations (i.e. total of 8 test pens per barn). This change was made in order to select the appropriate number of test pigs from the animals previously identified as ready for slaughter.

Lairage time at the plant (REST). After unloading at the plant, all test animals were moved to a lairage pen and rested for either a short duration or a long duration. Due to plant schedule and antemortem inspections lairage times varied slightly. In Exp. 1, the lairage lengths were as follows, not including blood and rectal temperature sampling

times (to be discussed later): short lairage was 29 ± 3 min (mean ± SD) and long lairage was 3 h 41 ± 38 min (mean ± SD). In Exp. 2, short lairage was 32 ± 23 min (mean ± SD) and long lairage was 4 h 17 ± 26 min (mean ± SD), not including sampling times.

In Exp. 1, test animals were rested together in a lairage pen with no other pigs. Initially, the stocking density in the pen was approximately 3.2 m^2 per pig. After the short rested group was removed the stocking density was approximately 6.3 m^2 per pig. In Exp. 2, test animals were rested with a group of approximately 30 contemporary animals from the same truck load. Initially, the stocking density in the lairage pen was approximately 1.9 m^2 per pig. After the short rested group was moved to the stunner, the stocking density became approximately 3.7 m^2 per pig.

Distance moved from the lairage pen to the stunner (STUN). After lairage, the test animals were either moved a short or a long distance to the pre-slaughter handling area. Animals in the short distance group were moved approximately 20 m directly to the pre-slaughter handling area. The animals in the long distance group were gently moved approximately 280 m back and forth in rest pens and then 20 m to the pre-slaughter area. In Exp. 1, test animals were moved with other test animals. In Exp. 2, long distance animals were moved in a small group with some non-test pigs to more appropriately simulate standard plant practice. After exercise, as mentioned, pigs were moved to the pre-slaughter area. In Exp. 1, pigs were brought to the area and had several minutes rest due to the amount of time it took to sample all the animals in the group. In Exp. 2, this procedure was altered so that the animals were constantly in motion, never having an opportunity for rest.

Animals, Housing and Feeding.

Finishing facilities from a single production company were used in this study to standardize production and management factors (i.e. genetics, nutrition, slaughter weights and health) across experimental animals. The buildings were mechanically ventilated with slatted floors. The majority of pigs in this study were fed Paylean 9®[1] at a dosage of 4.5 g/ton for approximately 21 days prior to market. During Exp. 2, two truckloads of pigs were not fed Paylean® as they represented the first sort from the barn and Paylean® had not yet been included in the diets.

Experiment 1. In Exp. 1, pigs were of similar genetic background, i.e. a Fast 536 sow and a Duroc boar produced by the production company. The Halothane status of these animals was unknown. There were three animals that became non-ambulatory during this experiment but none exhibited classical Porcine Stress Syndrome symptoms (Topel et al., 1968). The mean blood lactate concentration of these three animals was at the time they became non-ambulatory during pre-stun movement and handling was 8.7 mM ± 3.6 (Mean ± SD).

The 64 animals used in this experiment were transported to the packing facility in 4 truckloads across 2 days in mid-July, 2007. The approximate maximum and minimum ambient temperatures were 25ºC and 15ºC, respectively. The trucks used to transport the pigs were split axle cattle pot trailers. Each of the truckloads included 16 experimental animals with 4 pigs per treatment subclass per truck. Gilts and barrows were evenly distributed among treatments. *Experiment 2*. These animals were progeny of a Fast 536 sow-line and a boar based off of the 337 PIC line. The Halothane status of these animals was unknown. There was only one animal that became non-ambulatory during this

[1] Ractopamine hydrochloride sold by Elanco Animal Health, Greenfield, IN.

experiment and it did not exhibit classical Porcine Stress Syndrome symptoms (Topel et al., 1968). The blood lactate concentration of this animal at the time it became non-ambulatory, during pre-stun handling, was 6.2 mM.

Experimental animals were transported in 6 truckloads across 3 days in January, 2008. The approximate maximum and minimum temperatures were -5ºC and -12ºC, respectively. The trucks used to transport the pigs were split axle cattle pot trailers. Each of the truckloads included 24 experimental pigs with 6 pigs per treatment subclass on each truckload. Treatments were balanced by gender within each truckload. There were 5 truckloads of barrows and 1 truckload of gilts due to availability of slaughter weight animals.

Animal selection. Researchers arrived at the finishing facilities the day prior to slaughter to identify experimental animals. Prior to project initiation, barn personnel marked all pigs in the barn that were to be sent to slaughter. In Exp. 1, slaughter-identified pigs were marked with marking spray and sorted from the rest of their pen into smaller holding pens. The smaller holding pens were constructed by partitioning off part of the larger pen. By doing this prior to transport day, the slaughter pigs did not have to be sorted from non-slaughter pigs just prior to loading; the entire pen of pigs was loaded onto the truck. During Exp. 2, pigs were identified prior to transport day but they were not sorted out of their home pens. Therefore, upon loading, slaughter pigs needed to be sorted out of the group; the whole pen was not loaded onto the truck.

Test pigs were selected based on the following criteria: structurally sound, healthy, subjective average live weight, and gender. Pigs selected for sampling were restrained using sorting boards, randomly assigned to a treatment subclass (a REST and

STUN treatment, their DIS treatment determined by which pen they were housed in) given an ear tag identification number and slap tattooed with the ID number. Baseline blood sample and temperature (discussed in a subsequent section) were taken prior to animal identification so as not to affect the physiological measurements taken. The same test pigs were sampled at all of sampling points from loading to the stunner.

Marketing Process.

Loading and transport. Several hours after the baseline sample (blood and temperature) were obtained (Table 3.1), the pigs were loaded for transport to the packing facility. In Exp. 1, the portable loading chute was approximately 0.76 m wide and the ramp was at a 14º incline. The barn personnel loaded all pigs. During Exp.2, the portable loading chute was approximately 0.76 m wide and the ramp was at an 11 º incline. The researchers aided in loading. Electric prods were used during loading, primarily as animals were entering and moving up the loading chute; there was minimal prod use in the barn. Experimental pigs in a short distance and a long distance pen from the same side of the barn (either left or right) were loaded onto a truck. During Exp. 2, on the final shipment day, the barn pens were loaded in a cross-over manner (i.e. the long distance pens from the right side were loaded with the short distance pens from the left side). This was done in order to keep animal gender balanced across treatment subclasses.

Pens containing experimental animals were loaded onto the top deck of the truck. Once the test pigs were loaded, researchers went onto the top deck of the trailer and sampled all the test pigs. After sampling the test pigs, those animals, in addition to some non-test pigs from the same pens, were segregated

into compartments. To control for differences in truck compartment, all test pigs were supposed to be transported in similar compartments. Due to a miscommunication between plant and farm personnel during Exp. 1, some pigs were transported on the bottom deck in the nose and others on the top deck. This alteration only occurred on the first slaughter day (2 truckloads). On Truck 1, 11 pigs (out of 16) were transported in the bottom nose and 5 pigs were transported on the top deck. On Truck 2, 9 pigs (out of 16) were transported in the bottom nose and 8 pigs were transported on the top deck. The other 2 truckloads in this experiment were loaded correctly, all pigs remaining in the two top middle compartments. During Exp. 2 all truckloads were loaded with the experimental animals in the top two middle compartments. The compartments that contained the test pigs had approximately 18 pigs per compartment (approx. 0.42 m^2/pig). All test pigs could not be placed in the same compartment due to the trailer stocking density guidelines. The top two middle compartments were chosen to provide the most similar transport environment possible. After dividing the test pigs into their respective compartments, the remainder of the slaughter weight pigs in the barn were loaded onto the trailer. Loading and sampling took approximately 1.5 hrs for each truckload. The truckloads departed approximately one hour apart to stagger protocol implementation at the packing plant.

Facilities were selected to provide a transport time to the packing plant of approximately 2.5 hrs. In Exp.1, the farms were located closer than this distance from the plant and truck drivers were instructed to extend their transport time to

the requested amount by taking alternate routes. In Exp. 2, the location of the farms was approximately a 2.5 hr direct drive to the packing plant.

Unloading at the plant. Upon arrival at the packing plant, trucks were directed to a designated unloading dock. Non-test pigs that were not in the same compartment as the test pigs were unloaded from the truck by the driver and given a lot tattoo on the right shoulder upon exiting the truck by a plant worker. After non-test pigs had been unloaded, researchers entered the truck to sample (blood and temperature) the experimental animals, as described below. After sampling was completed the remaining animals, including experimental animals, were unloaded. One observer made behavioral and handling observations on the test pigs as they were unloaded (described below). All test pigs were moved by plant workers and researchers to a designated lairage pen and no electric prods were used.

Pigs were re-sampled (blood and temperature) in lairage pens and after the designated rest period pigs were again sampled and moved to the pre-stun handling area. After sampling in this location, the animals were put into the normal flow of pigs through the plant. They were electrically stunned and exsanguinated entering the standard post-mortem processing at the facility. The average line speed at this plant was 630 animals per hour. Animals were slaughtered at two different times due to the difference in rest times (Table 3.1).

Timeline. On each experimental, day two truckloads arrived at the plant approximately one hour apart and the animals were moved via the plant protocol. The time between truck arrivals varied depending on on-time departure from the farm and

road conditions. Table 3.1 provides an approximate timeline of events from baseline sample at the farm through slaughter.

During Exp. 1, there were two slaughter days separated by one non-slaughter day. Two experimental trucks arrived at the plant each day and 32 experimental animals were slaughtered on each of the kill days. In Exp. 2, there were three consecutive slaughter days. Similar to Exp. 1, two experimental trucks arrived at the plant each day. Forty-eight experimental animals were slaughtered on each of the experimental days.

Sampling Points. Each of the experimental animals was sampled at seven different points throughout the marketing process: (1) Baseline, (2) Post-load, (3) Pre-unload, (4) Post-unload, (5) Post-lairage, (6) Post-movement and (7) Exsanguination. The baseline sample point (1) was obtained in the barn pens prior to transport. The post-loading sample point (2) was taken on the truck after the experimental animals had been loaded. The test pigs were sampled on the trailer before the remainder of the trailer was loaded starting approximately 15 minutes after the first pig was loaded. The pre-unload sample point (3) was taken on the truck upon arrival at the plant prior to unloading of the experimental animals. Driver/plant personnel unloaded all pigs on the trailer except the test pigs prior to the third sampling. The post-unload sample point (4) was collected from the test animals after they had been unloaded and moved to their respective lairage pens. The post-lairage sample point (5) was collected in the lairage pen at the end of the rest periods. This sampling point occurred at different times within the slaughter day determined by the REST treatment group. The post-movement sampling point (6) was obtained immediately after the test pigs had been moved the assigned treatment distance to stun (STUN). This sample was obtained just outside of the pre-slaughter handling area

(Edwards et al., 2009f). The final sampling point (7) was obtained at approximately 15 sec following exsanguination after pigs had moved through the pre-slaughter handling area and been electrically stunned.

Sampling Protocol. At each sampling point, pigs were restrained to obtain a blood sample for lactate concentration ([LAC]) and a rectal temperature (TEMP). Pigs were restrained using a low-stress sampling technique, i.e. a snare was not used to restrain the animals, to help reduce any effect of handling stress on the parameters measured. One or two researchers gently restrained each pig using sorting boards and most pigs did not squeal during sampling. A third person pricked one of the pig's distal ear veins with a retractable 20 gauge needle. A sample strip was inserted into a hand-held lactate analyzer (Lactate Scout, EKF Diagnostic GmbH, Magdeburg, Germany) and a drop of blood was immediately administered to the sample strip. In Exp. 1, this was accomplished by collected blood from the ear prick with a transfer pipet and placing a droplet into the sample strip. In Exp. 2, it was determined that sampling was more efficient if the person pricking the ear held the lactate analyzer with the sample strip to the drop of blood on the pig's ear. The analyzer provided [LAC] in approximately 15 s and the information was recorded. After the blood sample was obtained, the ear vein was pressed for several seconds to induce clotting. Simultaneously, the researcher restraining the pig at the rear of the animal obtained a rectal temperature using a Vick's SpeedRead digital thermometer (Model #: V911F, Kaz, Inc., Southborough, MA) providing a measurement within 8 s. Lactate analyzer and thermometer models were selected to obtain readings within 15 s to minimize sample time accommodating slaughter plant line-speed. A fourth researcher recorded the data for each pig. The time from which the

sampling session began to which it ended was recorded. A temperament score to classify the pig's behavior during sampling was also recorded using the following scale: (1) no vocalization, no movement during sampling; (2) initial vocalization upon boarding, no movement during sampling; (3) intermittent vocalizations, some movement during sampling; and (4) constant vocalization, rearing, struggling, escape attempts. The mean sampling time was 69 ± 37 sec (mean ± SD) in Exp. 1 and was 46 ± 23 sec (mean ± SD) in Exp. 2.

Equipment Standardization. During both experiments, prior to data collection on each slaughter day, both the hand-held lactate analyzers and the thermometers were standardized. Thermometers were tested in two samples of water with different temperatures (approximately 35° C and 40.5° C). Only the thermometers that gave the similar readings ($\pm$ 0.2° C) were used in the experiment. Lactate analyzers were also tested with a standard solution to ensure accuracy. The coefficient of variation reported by the analyzer manufacturer is 3-8% dependent on the concentration being measured (Lactate Scout, EKF Diagnostic GmbH, Magdeburg, Germany).

Behavioral and Handling Observations. Behavioral observations and handling measurements were obtained on the experimental animals during loading, unloading and pre-slaughter handling. During loading one observer stood in the barn and one remained on the truck to make handling measurements. Observations were made on a group of animals with individual animal numbers being recorded when possible. During unloading, one observer stood to the side of the holding pen and observations were made on the group of animals as they exited the truck. During loading and unloading, handling scores were tabulated as the number of times a particular event occurred. In the pre-

slaughter handling area, observations were made on individual animals (Edwards et al., 2009d). During Exp.1, in the pre-slaughter handling area, one observer followed each animal as it moved through the crowd pen and single-file chute. During Exp. 2, this area was divided into two sub-areas; one observer followed each individual animal as it moved through the crowd pen and one observer followed each animal upon entrance into the single-file chute to moved to the v-restrainer. During pre-slaughter handling, scores were tabulated as the number of times each animal experienced each of the observed events. The following behavior and handling measurements were taken: electric prod use, vocalization in response to prod use, jamming, rearing, falling, rooting, being rooted, backing up, turning back and presence of vocalization (only in Exp. 2).

Statistical Analysis. Animals that did not complete treatments were removed from the analyses. There were four animals, three from Exp. 1 and one from Exp. 2 that became non-ambulatory during the experiment. These animals were removed from the analyses for all sampling points following identification of their non-ambulatory status. For [LAC] measurements, data were removed if the values were ± 5 standard deviations from the sampling point mean. For TEMP measurements, data were removed if the values were ± 3 standard deviations from the sampling point mean.

Statistical Analysis: DIS, REST and STUN. Data were analyzed using a restricted maximum likelihood-based, mixed-effects model with repeated measures analysis and a spatial power covariance structure in SAS 9.1 (SAS Inst., Inc., Cary, NC). Individual animal was the experimental unit. To normalize the data, [LAC] were logarithmically transformed for all analyses. For both the [LAC] and TEMP ANOVA models, all fixed effects and possible interactions were included and non-significant effects were removed

in a step-wise manner. These data were analyzed to account for the behavior score at time of sampling as well as duration of sampling. Behavior score at time of sampling and duration of sampling did not influence the parameters measured.

Experiment 1. The ANOVA model for [LAC] included the following independent fixed effects: distance moved at the farm during loading (DIS), lairage time (REST), distance moved at the plant from the lairage pen to the stunning area (STUN), time (sampling point), day, the day*time interaction and the STUN*time interaction. The ANOVA model for TEMP included the following independent fixed effects: DIS, REST, STUN, time, gender and the STUN*time interaction. Intercept was included as a random effect and the Satterwaith approximation was used to calculate denominator degrees of freedom. Means were separated using the PDIFF option at a significance level of $P < 0.05$. An analysis was done using the measured handling parameters as covariates at sample point 7 (exsanguination) in order to determine if they had any significant effect on [LAC] and TEMP. The covariates were not used in the final model as they did not improve the estimate of the effect of treatment.

Experiment 2. The ANOVA model for [LAC] included the following independent fixed effects: DIS, REST, STUN, time, the DIS*time interaction, the REST*STUN interaction, the REST*time interaction, the STUN*time interaction, the REST*DIS interaction, the REST*STUN*time interaction, and the REST*DIS*time interaction. The ANOVA model for TEMP included the following independent fixed effects: DIS, REST, STUN, time, the REST*time interaction and the STUN*time interaction. Similar to Exp. 1, an analysis was done using the handling parameters as covariates at sample point 7 to

determine if they had any significant effect on [LAC] and TEMP. The covariates were not used in the final model as they did not improve the estimate of the effect of treatment.

Difference in [LAC] and TEMP between Sampling Points. The changes in [LAC] and TEMP between sample points were individually analyzed to explore the effects of REST, STUN and DIS on the magnitude of differences in physiological measures between sampling points. The changes in physiological parameters were defined as follows: Loading (sample point 2 – sample point 1) = effects of distance moved during loading; Transport (sample point 3 – sample point 2) = effects of transport; Unloading (sample point 4 – sample point 3) = effects of unloading; Lairage (sample point 5 – sample point 4) = effects of lairage time; Movement to stun (sample point 6 – sample point 5) = effects of distance moved from lairage to stun area; and Pre-slaughter handling (sample point 7 – sample point 6) = effects of single file run from crowd pen to stunner. Data were analyzed using a restricted maximum likelihood-based, mixed-effects model in SAS 9.1 (SAS Inst., Inc., Cary, NC). The ANOVA model for each parameter change only included the main effects and interactions that were relevant to the particular changes between two given sample points (i.e. DIS was the only factor used when analyzing differences in the change associated with loading as the other treatment factors had not yet been applied).

Experiment 1. The ANOVA model for the change in [LAC] during: loading included DIS, transport and unloading included DIS and kill day, lairage included REST, movement to the stunner included REST and STUN, pre-slaughter handling included DIS, REST and STUN and the interaction effects of STUN*DIS, STUN*REST, DIS*REST and REST*STUN*DIS.

The ANOVA model for the change in TEMP during: loading included DIS and kill day, transport included DIS, unloading included DIS and gender, lairage included REST and kill day, movement to stun included DIS, REST and STUN and pre-slaughter handling included DIS, REST, STUN, kill day, STUN*DIS, STUN*REST, DIS*REST and REST*STUN*DIS.

Experiment 2. The ANOVA model for the change in [LAC] during loading included DIS and kill day, transport included DIS and gender, unloading included DIS, lairage included DIS, REST and kill day, movement to stun included DIS, REST, STUN, STUN*REST, DIS*REST and gender, and pre-slaughter handling included DIS, REST, STUN, STUN*REST and gender.

The ANOVA model for the change in TEMP during: loading included DIS, transport included DIS and gender, unloading included DIS, kill day and gender, lairage included DIS, REST, DIS*REST and gender, movement to stun included DIS, REST, STUN, kill day and gender, and pre-slaughter handling included DIS, REST, STUN, STUN*REST, kill day and gender.

RESULTS

Effects of sampling point on [LAC] and TEMP.

Experiment 1 & 2. Blood lactate concentration ($P < 0.001$) and rectal temperature ($P < 0.001$) changed between sampling points (Figures 3.1a, 3.1b, 3.1c, 3.1d). The highest [LAC] was recorded after loading at the farm and after stunning. Similar to [LAC] results, the highest TEMPs were observed during the times of movement, i.e. loading, unloading and movement to the stunner.

Effect of DIS, REST and STUN on [LAC].

Experiment 1 (Table 3.2). Analysis of the main effects across all sampling points indicates that there was no impact of DIS, REST or STUN on [LAC] (P = 0.96, 0.50 and 0.65, respectively; data not shown). There tended to be a time*STUN interaction (P = 0.10) indicating that the distance moved to the stunner had an effect on [LAC] at specific sampling points; the animals moving a shorter distance had a higher (P = 0.04) [LAC] than those moving the long distance to the stunning area at exsanguination. Kill day tended (P = 0.08) to have an effect on [LAC] (data not shown). There was also a time*day interaction for [LAC] showing higher (P = 0.001) [LAC] in pigs slaughtered on the first kill day at sample point 3, pre-unloading at the plant (Table 3.2).

Experiment 2 (Table 3.3). There tended (P = 0.08) to be a main effect of DIS on [LAC] (data not shown). There was a time*DIS interaction (P = 0.001) for [LAC] (data not shown). Post-loading, animals traveling a long DIS during loading had greater [LAC] (P = 0.0001). The main effect of REST (P = 0.002) indicated that animals rested for a longer period had greater [LAC] than animals rested a short period (data not shown). The effect of REST was also impacted by sampling point (P = 0.003). Animals in the long REST treatment had higher [LAC] after lairage (P = 0.02), after movement to stun (P = 0.01) and at exsanguination (P = 0.0001; Table 3.3). There was no main effect of STUN (P = 0.66) on [LAC] (data not shown). There was a relationship between STUN treatment and sampling point. Similar to Exp. 1, the animals moving the shorter distance to the stunner had higher (P = 0.02) [LAC] than the animals moving the long distance. Additionally, there was an interaction of REST, STUN and time (P = 0.004, data not shown). At exsanguination, experimental animals in the long REST, short STUN group had a higher mean [LAC] than all other treatment subclasses.

Effects of DIS, REST and STUN on changes in [LAC] between sampling points.

Experiment 1 (Table 3.4). Table 3.4 describes the main effects of DIS, REST and STUN in relation to changes in [LAC]. The change in [LAC] was affected by REST (P = 0.003); animals that rested longer had a larger increase in [LAC] than animals rested for a shorter period at exsanguination. The change in [LAC] during pre-slaughter handling was affected (P = 0.01) by the distance moved to the stunner. Animals that moved the longer distance to stun had smaller increases in [LAC] than animals that moved a short distance. Kill day also had an impact in the changes in [LAC] during transport and loading (P = 0.0001).

Experiment 2 (Table 3.5). The change in [LAC] during loading was affected (P = 0.0002) by DIS. Animals traveling the longer distance in the barn exhibited greater increases in [LAC] than animals that moved a short distance. Animals that moved the long DIS (P = 0.002) also had larger decreases in [LAC] during transport. Animals that rested for a longer duration had smaller decreases (P = 0.001) in [LAC] as measured at the end of lairage than animals rested for a short period of time. A similar effect of long REST was also observed when examining the change in [LAC] associated with pre-slaughter handling and stunning. Animals that rested for a longer period had a greater (P = 0.001) increase in [LAC] during this portion of the marketing process. The change in [LAC] associated with pre-slaughter handling and stunning was larger in animals traveling the short distance to stun (P = 0.05). Gender had an effect on the change in [LAC] due to transport, movement to the stunner and pre-slaughter handling (P < 0.02). Barrows had a larger (P = 0.01) decrease in [LAC] during transport but larger increases in [LAC] during movement to stun (P = 0.02) and exsanguination (P = 0.01). Kill day also had an effect

on the change in [LAC]. Animals slaughtered on the second kill day (Day 2) had the largest increases in [LAC] during loading ($P = 0.05$) and had the largest decreases in [LAC] during lairage ($P = 0.04$).

Effects of DIS, REST, STUN on TEMP.

Experiment 1 (Table 3.6). The main effects of DIS, REST and STUN did not affect TEMP ($P = 0.23$, 0.12 and 0.33, respectively, data not shown). The time*STUN interaction ($P = 0.04$) indicated a difference in TEMP between STUN treatments immediately after movement to the stunner ($P = 0.002$) and at exsanguination ($P = 0.02$); pigs that moved the longer distance to the stunning area had higher TEMP than those that moved the shorter distance. The 45 min post-mortem muscle temperature of pigs moved short and long distance was 103.2 and 104.5 ^{o}F respectively (P=0.006).

Experiment 2 (Table 3.7). The main effect of DIS had no effect ($P = 0.31$) on TEMP. Despite [LAC] being higher in the longer DIS animals, TEMP was not different (data not shown in table). Although there was no main effect of REST ($P = 0.35$) there tended to be an interaction of REST treatment with time ($P = 0.06$; value not shown). Unexpectedly, animals in the long REST group had greater ($P = 0.02$) TEMP at exsanguination than animals that rested for a shorter duration. This was consistent with changes in [LAC] (Table 3.3). Distance moved to the stunner tended ($P = 0.10$) to influence TEMP (value not shown) but the STUN*time interaction was significant (P=0.001). Animals that moved a longer distance to stun had greater TEMP post movement ($P = 0.001$) and at exsanguination ($P = 0.001$). The 45 min post-mortem muscle temperature was not measured in Experiment 2.

Effects of DIS, REST and STUN on changes in TEMP between sampling points.

Experiment 1 (Table 3.8). The distance moved to the stunning area affected the change ($P = 0.02$) in TEMP associated with movement to stun. Kill day had an impact on the change in TEMP during lairage ($P = 0.03$) and tended to have an impact during loading ($P = 0.08$), and pre-slaughter handling ($P = 0.09$). Gender affected the change in TEMP during unloading ($P = 0.04$); barrows had a larger increase in TEMP than gilts.

Experiment 2 (Table 3.9). Animals moving the longer distance to stun had a greater increase in rectal temperature ($P = 0.02$). Consistent with the effects of REST on [LAC[, the duration of REST also had an effect ($P = 0.0001$) on the change in rectal temperature. Gender impacted the change in TEMP during transport, unloading, lairage, movement to stun and pre-slaughter handling ($P < 0.0003$). In general, gilts had larger increases or smaller decreases in TEMP than the barrows. Kill day had an effect on change in TEMP during pre-slaughter handling ($P = 0.0002$) and tended to have an effect on change in TEMP during unloading ($P = 0.09$) and movement to stun ($P = 0.10$). The differences between days were not consistent, i.e. there was not one day that resulted in larger increases in TEMP.

DISCUSSION

Blood Lactate

Blood lactate concentration was highest during loading prior to transport and during pre-slaughter handling prior to exsanguination. Although loading impacted [LAC] in both experiments, the mean [LAC] was greater in Exp. 2. In Exp.1, [LAC] did increase during loading but there was no significant difference between animals moved the varying distances to the barn exit. In Exp. 2 however, the long DIS animals did have significantly greater [LAC] ($P = 0.0001$) than the short DIS animals. Additionally, the

analyses using the changes in [LAC] between sampling points, indicated that the long DIS animals had greater increases in [LAC] ($P = 0.002$) during loading, in Exp. 2.

There are several possible explanations for this observed difference. In Exp. 2 animals were not pre-sorted prior to transport. Therefore, just prior to loading, the slaughter animals had to be sorted out of their group and then moved onto the truck. This would have created a longer period of physical exertion. Perhaps the distance an animal moves while being loaded becomes more important when combined with the stresses of additional handling, i.e. a cumulative effect of stresses (Ritter et al., 2008a) . Additionally, the pigs in Exp. 2 were handled by less experienced swine handlers which may have decreased the quality of handling causing more unfavorable handling events such as increased electric prod use, jamming and vocalization. This is only speculation as observers were not able to record individual handling information on the experimental animals thus making it difficult to correlate high [LAC] during loading with handling. Additionally, it is difficult to compare the occurrence of each handling event, i.e. the percentage of animals prodded in one experiment versus the other, as there were different numbers of experimental animals per truckload. Handling scores recorded during loading did not necessarily indicate the number of animals experiencing a handling event but more accurately the number of times a handling event occurred, i.e. 12 electric prods could mean twelve animals were prodded or 4 animals were prodded three times each. Lastly, the two experiments were conducted in different seasons and perhaps the differences in outdoor temperature and humidity added to the observed differences between experiments.

The length of rest at the packing plant impacted changes in [LAC]. In Exp. 2, the longer rested animals had greater [LAC] after lairage (P=0.02), after movement to stun (P=0.01) and at exsanguination (P = 0.001). Similarly in Exp. 1, the change in [LAC] did indicate that animals rested for a longer period had a larger increase in [LAC] at stun (P=0.003) than animals rested a shorter period. These same results on the change in [LAC] at stun were seen in Exp. 2 (P=0.001) as well as a smaller decrease in [LAC] during rest (P=0.001).

Even though the effect was consistent in this experiment, it is unclear why the differences between rest periods were observed. A previously published study explored the effects of lairage length on various physiological parameters in market swine (Warriss et al., 1992). Results from this study indicated that there was no difference in exsanguination [LAC] between pigs rested from less than 1 hr to 21 hrs. One possible explanation for our results is that over time the experimental animals may have begun to fight or alter their behavior. Although animal behavior was not recorded during the two different lairage periods, anecdotal observations imply that there was no increase in agonistic behavior during the longer rest period. When researchers returned to the laraige pen to take samples at the end of the designated laraige period animals appeared to be resting with no indication of prior fighting or aggression. Another published study explored the effects of different lairage lengths, specifically 0 h, 3 h and 9 h, on various physiological and behavioral measures (Perez et al., 2002). In this study, there was no significant difference in [LAC] at exsanguination between the various lairage treatment groups. Additionally, no differences in behavior patterns during the first three hours of rest were reported between lairage treatments. Researchers reported that during the first

three hours the incidence of resting behaviors, i.e. sitting and lying down, actually increased; the animals did not become more active. Although no differences in behavior patterns were identified between lairage treatments, animals were not observed during the entire rest period. To make assumptions about how animal behavior may be affecting [LAC] it would be necessary to conduct a study in which swine behavior was observed throughout the entire lairage duration.

Another study was conducted to understand how length of lairage affected agonistic encounters in swine (Geverink et al., 1996). This study observed animals in lairage for 1.5 hrs, not long enough to make assumptions about the 4.5 hr lairage utilized in this study. Geverink et al. (1996) did report that agonistic behavior increased over time with a peak at approximately 70 min of rest. Researchers in the study made it clear that there were distinct individual differences in the incidence of aggression. An additional study investigating the changes in agonistic behavior in swine associated with both time in lairage and mixing with contemporary pigs found similarly that the initial half hour of the rest period contained the highest instance of agonistic encounters (Moss, 1978). Once again, this study did not explore the lairage length that was used in the current study.

One other potential explanation for our observation of increased [LAC] with increased REST is that animals were able to replenish liver and muscle glycogen with a longer rest period and when they were subsequently handled they responded by increased glycogen breakdown raising blood [LAC]. During this experiment it was observed that gut motility may have decreased on the truck during transport (minimal manure present in the truck following transport). The long rested animals may have had time to begin

digestion of the remaining feedstuffs beginning to replenish glycogen while the short rested animals did not have enough time to resume digestive processes. These observations were merely anecdotal and further research would need to be conducted to determine gut contents, changes in liver and muscle glycogen during rest, and other metabolic parameters.

Another consideration for understanding the effect of rest length on exsanguination [LAC] as that the longer rested animals were handled at the same general time of day in both Exp. 1 & 2, as plant workers were approaching their lunch break. Although covariate analyses were performed to account for any change in handling, there may still be some relevance to the results that were observed.

The distance moved to STUN also had an impact on [LAC] in both experiments. The animals moving the shorter distance to the stunner had greater exsanguination [LAC] than the animals moving the longer distance (Exp 1, $P = 0.04$; Exp 2, $P=0.02$). Additionally, analyses indicated that animals moving the shorter distance to the stunner had larger increases in [LAC] (Exp 1, $P < 0.01$; Exp 2, $P=0.05$). All animals in all treatments were gently handled prior to mixing in the crowd pen. A laboratory study of the effects of handling indicated that gentle handling did not increase [LAC] in pigs during a 300 m course (Benjamin et al., 2001). Although the work by Benjamin et al. (2001) may help explain why [LAC] did not increase with gentle handling over a longer distance, it does not provide clear information as to the reason for the increase in [LAC] in the short STUN animals. There is no previously documented research that can provide an explanation for this result. It is hypothesized that perhaps the pigs moving a short STUN responded to the movement with a quick burst of energy; they may have

responded to the handling rapidly and intensely causing an immediate increase in [LAC]. The long STUN animals had a longer period to adjust to the movement as they were being moved gently in the lairage pen for several minutes prior to being moved the final 20 m to the stunning area. Perhaps this additional low-intensity exercise gave the experimental animals time to adjust to any increase in muscle lactate production thus obtaining a better clearance rate than the animal moving the shorter distance.

In Exp. 1, kill day also had an impact on [LAC] during the pre-unloading sampling point. As mentioned, on the first day of the trial some pigs were loaded in the incorrect compartment, the bottom deck nose. Prior to taking the third sample, the truck driver had to move pigs out of the nose (so that researchers could sample blood and temperature on the top deck) which involved moving up a ramp and this could have influenced the [LAC] of those animals. In Exp. 1, kill day also had an impact on changes in [LAC] during transport and unloading ($P < 0.0001$), but these differences were not consistent, i.e. the first kill day did not always have greater increases in [LAC] than the second. Similar to these results, Exp. 2 analyses on the change in [LAC] between sampling points indicated that kill day had some impact on these changes at certain sampling points but that the direction of changes were not consistent. Similar findings were observed in the analysis of rectal temperatures; kill day effects were not consistent.

In Exp. 2, there was a gender effect on the changes in [LAC] during transport, movement to slaughter and pre-slaughter handling ($P < 0.02$). Similar to the effect of kill day, the differences seen between genders was not consistent, i.e. barrows did not always have greater increases in [LAC] as compared to gilts. One possible reason for this result in Exp. 2 is the difference in the number of truckloads of gilts and barrows. There was

only one truckload of gilts which could have impacted the results. Additionally in Exp. 1, there were both barrows and gilts on each truckload, whereas in Exp. 2 all pigs on a truck were the same gender. The gender effect is confounded by truck effect in Exp. 2.

During pre-slaughter handling, blood lactate concentrations observed in this study were lower than what has been previously reported in the literature. Some studies have reported exsanguination [LAC] ranging from 12 to 31 mM (Hambrecht et al., 2005a; Hambrecht et al., 2004b), substantially higher than the average 6.6 mM found in this study. The difference between studies could be reflective of differences in animal handling, facility design, exposure of experimental animals to humans, sample collection or sample analysis. All of these factors probably account for some of the variation between these studies. Our values are more similar to data reported by Warriss and coworkers (1994) where packing plants were classified by low and high stress (4.4 mM and 12 mM [LAC], respectively). To determine any potential effects of prior sampling (experimental animals had been handled/sampled more than pigs in the regular plant flow) on [LAC] some randomly chosen animals from the regular flow of pigs were selected and exsanguination [LAC] measured. These animals had slightly higher [LAC], approximately 10.5 mM, a value within the range of values reported at the packing plants in the Warriss et al. (1994) study.

Rectal Temperature

During both experiments, rectal temperatures increased during instances of handling and movement (i.e. loading, movement to the stunner, pre-slaughter handling) indicating the impact of physical exertion on body temperature. Although temperatures increased during loading, there was no difference between the short and long distance

treatment groups in either experiment. The additional distance was not substantial enough to increase body temperature.

Rest period had no impact on temperature in Exp. 1 but there were some effects of REST observed in Exp. 2. Similar to the [LAC] results, animals that rested longer had greater TEMPs at exsanguination (P=0.02) and greater increases in TEMP during pre-slaughter handling (P = 0.0001). The reason for this observation is unclear. It seems that more rest is not necessarily better in terms of maintaining baseline [LAC] and TEMP. Examining durations in-between 30 min and 5 hours would help to clarify exactly what is influencing these parameters.

Animals moving a longer distance to STUN had higher TEMPs post-movement (Exp 1, P=0.002; Exp 2, P=0.001) and at exsanguination (Exp 1, P=0.02; Exp 2, P=0.001) and greater increases in TEMP during movement to the stunner in both experiments (P=0.02). This may be due to the higher level of physical exertion required to walk a longer distance therefore yielding more body heat and thus raising body temperature. Animals moving the longer distance not only moved farther but they also were exercised for a longer period of time to move that distance adding to the increase in body temperature. This increase in TEMP resulted in a higher 45 min post-mortem muscle temperature in Exp 1 (P=0.006) potentially affecting early rate of post-mortem metabolism. The 45 min post-mortem temperture was not measured in Exp 2. In a previous study, gentle movement for a long distance resulted in higher TEMP even when animals had no change in [LAC] (Benjamin et al., 2001).

IMPLICATIONS

This study reported the impact that pre-slaughter management has on physiological parameters of swine throughout the entire marketing process, from farm to slaughter. Blood lactate concentration and rectal temperature change throughout the marketing process, increasing substantially during times of intense handling, i.e. loading and pre-slaughter handling, and returned to baseline levels during periods of rest, i.e. transport and lairage. There are several management recommendations that can be made from the results of these studies. The highest [LAC]s were observed at loading and exsanguination, emphasizing that these are focal points for improved animal handling. During loading, the observed increase in [LAC] was caused by an accumulation of stressors, e.g. handling, physical exertion, sorting. Sorting at the time of loading greatly increased [LAC] on the truck post-loading; the combination of a long distance traveled to the barn entrance and sorting immediately prior to loading caused a significant increase in [LAC] during Exp. 2. To minimize increases in [LAC] during this time, slaughter-weight animals should be pre-sorted prior to loading, providing a rest period in which animals can return to baseline [LAC]. This study also provided evidence that a lairage length of approximately 4.5 hours may not be the optimum amount of rest to maintain exsanguination [LAC] at a minimum. Further research needed to determine the cause of this increase in [LAC] with longer rest. In addition, more research is needed to explore the interaction between rest and physical exertion following lairage to determine if there is an optimal combination of management factors. These experiments indicate that [LAC] does respond to alterations in pre-slaughter swine management and may be used as a tool to help develop effect systems of pre-slaughter management practices.

CHAPTER FOUR

USE OF EXSANGUINATION BLOOD LACTATE TO ASSESS THE QUALITY OF PRE-SLAUGHTER PIG HANDLING

ABSTRACT

The objective of this research was to characterize the relationship of pre-slaughter animal handling to exsanguination blood lactate concentration ([LAC]) in a commercial pork processing plant. The two studies, Exp. 1 (n=76) and Exp. 2 (n=140), differed in facility design. Animal behavior and handling were scored in the crowd pen and single-file chute up to the v-restrainer. Measurements included jamming, rearing, turning back, backing up, rooting behavior, vocalization, electric prod use and duration of time spent in the handling area. Scores were tabulated as the number of times each animal experienced a handling event. Exsanguination [LAC] was measured following electric stunning. Data were analyzed using Pearson correlations to determine the relationship between behavior and [LAC]. In Exp.1, results indicated positive correlations between [LAC] and jamming ($r = 0.23$, $P < 0.05$), backing up ($r = 0.27$, $P < 0.05$) and rearing ($r = 0.25$, $P < 0.05$). In Exp. 2, data were collected in two sections: the crowd pen and the single-file chute. In the crowd pen, [LAC] was positively correlated to electric prod use ($r = 0.18$, $P < 0.05$) and vocalization in response to prod use ($r = 0.28$, $P < 0.05$). In the single-file chute, [LAC] was positively related to jamming ($r = 0.24$, $P < 0.05$). Single degree of freedom

contrasts were used to compare [LAC] based on the presence or absence of handling events. In Exp. 1, animals that experienced one or more of the following events: electric prod use, jamming, rearing and/or backing up, had a greater [LAC] than animals that did not experience these events ($P < 0.05$). The same relationships were observed between [LAC] and the listed handling events in Exp. 2 in the single-file chute ($P<0.05$). In the crowd pen, animals prodded or vocalizing with prodding had greater [LAC] ($P = 0.03$ and $P = 0.07$, respectively) than animals that did not. This is the first time that specific animal handling events have been related to [LAC] in a commercial setting. Data support the on-line measurement of [LAC] to monitor the quality of animal handling during the period immediately prior to stunning.

Key Words: handling, lactate, pig, slaughter

INTRODUCTION

Blood lactate concentration ([LAC]) and body temperature have been demonstrated to be quick responders to exercise and handling induced stresses in swine (Allison et al., 2006; Anderson et al., 2002; Benjamin et al., 2001; Kallweit, 1982; Zhang et al., 1989). Research using a laboratory model of animal handling has demonstrated aggressive handling using an electric prod rapidly increases [LAC] (Benjamin et al., 2001). With rest following controlled exercise and handling sessions, [LAC] and temperature return to normal levels in 2 hrs or less (Benjamin et al., 2001; Kallweit, 1982). These studies demonstrate that [LAC] is a sensitive tool that could be used as a measure of acute handling stress in swine.

Several studies conducted in commercial swine packing facilities have evaluated changes in standard serum stress parameters associated with pre-slaughter handling

stresses (Averos et al., 2007; Guise and Penny, 1989; Hambrecht et al., 2005a; Hambrecht et al., 2004b; Hemsworth et al., 2002; Moss, 1984; Warriss et al., 1994; Weeding et al., 1993) but few of these studies also evaluated the effects of animal handling on [LAC] (Hambrecht et al., 2005a; Hambrecht et al., 2004b; Hemsworth et al., 2002; Warriss et al., 1994). Previous work has demonstrated a relationship between the subjective assessment of stress and the objective measures of stress, particularly exsanguination [LAC], in commercial swine slaughter facilities (Hambrecht et al., 2005a; Hambrecht et al., 2004b; Warriss et al., 1994). Warriss et al. (1994) demonstrated that the difference in [LAC] between pigs handled in low versus high stress systems was more distinct than with either one of the other two blood parameters measured, cortisol and CPK, emphasizing the sensitivity and responsiveness of [LAC] to pre-slaughter stress.

By monitoring pre-stun animal handling, discouraging aggressive handling procedures and ultimately reducing stress, packing plants can reduce exsanguination [LAC] and improve animal well-being. The objective of this study was to establish the relationship between specific pre-slaughter animal handling parameters and post-slaughter [LAC] in a commercial swine slaughter facility.

MATERIALS AND METHODS

Prior to the initiation of these experiments, all animal use, handling, and sampling techniques described herein were approved by the Colorado State University Institutional Animal Care and Use Committee.

Experiment 1

Experimental Animals. Commercial crossbred pigs (n=76, 36 barrows and 40 gilts) of average estimated live weight (125 ± 9 kg, mean ± SD) were used in this study. The

mean live weight was back calculated using the hot carcass weights and a dressing percentage of seventy-five. Animals were slaughtered at a commercial swine packing facility where the research was conducted. All animals came from the same finishing facility and were of similar genetic background, i.e. Fast 536 sows and a Duroc boar line produced by the production company. Approximately twelve hours prior to arrival at the plant, experimental animals were selected in the finishing barn based on the following criteria: structural soundness, health and average live weight. Selected animals were identified with a unique tattoo and ear tag number.

Timeline. This study was conducted in July 2007. Experimental animals were shipped to the packing facility (average line speed of 630 animals per hour) and slaughtered over two non-consecutive days, approximately forty animals harvested each day. On the slaughter days, pigs were euthanized in four groups during a single shift prior to 1300 h.

Behavior and Handling Observations. All animals were observed by one observer on an individual basis as they were moved through the pre-slaughter handling area depicted in Figure 4.1. One experimental animal was moved into the crowd pen with approximately 15 random pigs from the packing facility. Behavioral observations were performed as the animal moved through a circular crowd pen (diameter = 4.57 m) and a single-file chute (length = 13.11 m, width = 0.40 m). The single-file chute was set at a 13% incline leading up to the v-restrainer. Observations were terminated upon entrance to the v-restrainer upon which time observations began on the next experimental animal. Animals were identified by their ear tag number. Animal behavior and handling parameters, defined in Table 4.1, were measured within the described handling area. The electric prod used by plant personnel was a live prod set at 13 V. Animals were also observed for

blotchy skin, muscle tremors and open-mouth breathing during handling. Scores were tabulated as the number of times each animal experienced a handling event. Upon entrance to the v-restrainer animals were electrically stunned and subsequently exsanguinated.

Experiment 2

Experimental Animals. One hundred forty crossbred pigs (n = 140, 23 gilts and 117 barrows) of average market weight (128 ± 3 kg, mean ± SD) were used in this study. These animals were progeny of Fast 536 sows and a PIC 337 boar line. Similar to Experiment 1, animals were destined for slaughter at the same commercial facility. The same procedure for animal selection and identification used in Experiment 1 was followed.

Timeline. This study was conducted in January 2008. Experimental animals were shipped to the packing facility (average line-speed of 630 animals per hour) and slaughtered in three consecutive days, approximately forty-eight animals harvested each day. On the slaughter days, pigs were euthanized in four groups during a single shift prior to 1300 h.

Behavior and Handling Observations. Behavior and handling observations were conducted in a similar manner to Experiment 2 with slight deviations. There were two observers making handling measurements in Experiment 2. One observer scored animals as they entered the crowd pen until their hindquarters were entirely in the single-file chute. The second observer scored the animals as their hindquarters entered the single-file chute until they entered the v-restrainer. Handling scores were analyzed as events taking place in specific locations: the crowd pen, the single-file chute and the two areas

combined (entire handling area). In Experiment 2, the single-file chute was no longer at a 13% incline; there was no incline. Additionally, the electric prod used by plant personnel had been adjusted to 17.5 V.

Exsanguination Measurements (Experiments 1 and 2). Blood lactate concentration ([LAC]) was measured using a hand-held lactate analyzer (Lactate Scout, EKF Diagnostic GmbH, Magdeburg, Germany). Exsanguination blood was collected from each pig in a plastic transfer pipet. A drop of blood was immediately administered to the sample strip in the hand-held lactate analyzer and a blood lactate measurement was obtained in approximately 15 seconds. Simultaneously, a rectal temperature was obtained from each animal using a Vick's SpeedRead digital thermometer (Model #: V911F, Kaz, Inc., Southborough, MA) providing a measurement within 8 s. This procedure was followed for each individual animal upon exsanguination.

Equipment Standardization (Experiments 1 and 2). Lactate analyzer and thermometer models were selected to obtain readings within 15 s to minimize sample time accommodating slaughter plant line-speed. Prior to data collection on each slaughter day, both the hand-held lactate analyzers and the thermometers were tested for reproducibility. Thermometers were tested in two samples of water with different temperatures (approximately 35° C/95° F and 40.5° C/104.9° F). Only the thermometers that gave the same reading ($\pm$ 0.2° C) were used in the experiment. Lactate analyzers were also tested with a standard solution to ensure accuracy. The coefficient of variation reported by the analyzer manufacturer is 3-8% dependent on the concentration being measured.

Statistical Analysis (Experiments 1 and 2). Animal was used as the experimental unit for all analyses. Pearson correlations were conducted relating the handling scores (i.e.

the number of times a particular event was experienced) and physiological measurements, [LAC] and rectal temperature. Differences among means of [LAC] and rectal temperature were determined using preplanned single degree of freedom contrasts. Contrasts were constructed to compare a group of animals that experienced a particular event (e.g. animals that were electrically prodded) to a group of animals that did not experience the particular event (e.g. animals that were not electrically prodded).

RESULTS AND DISCUSSION

Descriptive Information.

Experiment 1. The pigs in this trial were part of a larger study (Edwards et al., 2009e). The average exsanguination [LAC] of the pigs in this study was 7.4 ± 0.4 mM (mean ± SEM). A [LAC] baseline measurement was also taken on each of these individual pigs at the farm prior to transport. For this sample, pigs were restrained using a low-stress sampling technique, i.e. a snare was not used to restrain the animals. Animals were restrained using sorting boards and a blood sample was taken from a distal ear vein using a retractable needle and a blood lactate concentration was obtained. The baseline [LAC] was 4.3 ± 0.3 mM (mean ± SEM), approximately half the [LAC] at exsanguination. The pre-slaughter handling, movement and stunning doubled the [LAC] of the experimental animals indicating that this pre-stun phase was a source of stress for the experimental animals.

Although [LAC] increased during pre-slaughter handling, previously published studies in commercial facilities report exsanguination [LAC] between 9 and 31 mM (Hambrecht et al., 2005a; Hambrecht et al., 2004b; Warriss et al., 1998); the [LAC] in our study were on the low end of these reported values. Our values are more similar to

data reported by Warriss and coworkers (1994) where packing plants were classified by low and high stress (4.4mM and 12 mM [LAC] respectively). It is possible that the pigs in the low-stress treatment in the Hambrecht studies may have experienced more aggressive handling than the animals in the current study (D. Newman, personal communication, 2008). Also, in the current experiment, the experimental animals had been previously handled during additional experimental protocols implemented prior to pre-slaughter handling which may have altered their response to handling within the designated handling area and could explain the lower [LAC] (Edwards et al., 2009e). The large variation in exsanguination [LAC] values betweens studies may be indicative of the sensitivity of this measurement to differences in animal handling.

Additionally, the method of lactate analysis differed between this study and previously published work on blood lactate concentrations. The hand-held lactate analyzer provides readings slightly lower than other methods of lactate analysis, e.g. collection in potassium oxalate sodium fluoride tubes and subsequent spectrophotometric analysis with lactate dehydrogenase, but the values obtained between methods were highly correlated ($r = 0.97$, $P = 0.0001$) (Edwards et al., unpublished data). Thirty-nine samples were tested to assess similarities between methods. The mean [LAC] using the hand-held analyzer was 7.4 ± 3.2 (mean ± SD, mM) and the mean [LAC] using the potassium oxalate sodium fluoride tubes was 9.5 ± 4.1(mean ± SD, mM).

The average rectal temperature at exsanguination in July was 103.6 ± 0.1 ºF (mean ± SEM). Other commercial studies have not determined rectal temperatures at exsanguination. The exsanguination temperature increased minimally from the baseline measurement (103.0 ± 0.10 ºF, mean ± SEM), taken as described previously.

Experiment 2. Similar to Experiment 1 results, the average exsanguination [LAC] of the pigs in Exp. 2 (7.4 ± 0.3 mM, mean ± SEM) was almost double the baseline measurement taken prior to transport (4.1 ± 0.2 mM, mean ± SEM). Despite the difference in season and facility design, the pre-slaughter handling area was still a source of stress for the experimental animals. Once again, the mean [LAC] in this study was found to be lower than blood lactate concentrations established in some published studies (Hambrecht et al., 2005a; Hambrecht et al., 2004b) and intermediate to the [LAC] found in the high and low stress facilities reported by Warriss et al. (1994).

The average rectal temperature at exsanguination in January was (104.1 ± 0.1 ºF, mean ± SEM). The baseline temperature was 104.0 ± 0.1 ºF, only slightly lower than the measurement taken at exsanguination. The baseline measurement in the barn was taken in a climate-controlled environment, different from the packing plant environment. Additionally, this experiment was conducted in January perhaps altering the pig's temperature maintenance resulting in a smaller increase in temperature with movement.

Correlations between [LAC] and handling parameters.

Experiment 1. Pearson correlations between behavior and handling parameters and [LAC] are shown in Table 4.2. A significant relationship was found between the incidences of jamming, backing up and rearing with [LAC] ($r = 0.23$, $P = 0.05$; $r = 0.27$, $P = 0.02$; $r = 0.25$, $P = 0.03$, respectively). Therefore, as the incidence of these handling events increased, exsanguination [LAC] also increased. Although these r-values do not explain a large proportion of the variability in exsanguination [LAC], they do emphasize the importance of pre-slaughter handling demonstrating the direct effect of these events

on lactate levels. These events are related to increased [LAC] indicating the role that handling plays as one pre-slaughter stressor.

Experiment 2. As previously indicated, the handling parameters recorded in this experiment were analyzed as three different "locations" within the pre-slaughter area: the entire handling area, the crowd pen and the single-file chute. Pearson correlations between behavior and handling parameters and [LAC] are shown in Table 4.3. Within the entire handling area, electric prod use tended ($r = 0.16$, $P = 0.06$) to be correlated to [LAC] and vocalization in response to prod use was significantly correlated to [LAC] ($r = 0.23$, $P = 0.006$); as incidences of these events increased, [LAC] increased. Similarly, in the crowd pen, as the incidence of electric prod use and vocalization in response to prod use increased, [LAC] increased ($r= 0.18$, $P = 0.04$; $r = 0.28$, $P = 0.0009$). In the single-file chute results were different from those in the crowd pen but were similar to those found in Experiment 1. There was a significant positive relationship between jamming and [LAC] ($r = 0.24$, $P = 0.005$) and a tendency for a positive relationship between rearing and [LAC] ($r = 0.15$, $P = 0.08$).

These results indicate that location can have an influence on the effects of particular handling parameters on [LAC]. In the crowd pen, use of the electric prod increased [LAC], a similar finding to that of Benjamin et al. (2001) in the laboratory handling model. The prod was used in this area on animals that continuously hesitated to move into the entrance of the single-file chute. Animals were scored as remaining in the crowd pen until their entire hindquarters had entered the single-file chute. Therefore, there were several animals, indicated in Table 4.5, that were reluctant to move forward requiring greater use of the electric prod, which ultimately resulted in higher [LAC] at

exsanguination. This effect of the electric prod was not seen in Exp. 1 and was perhaps due to the difference in prod voltage setting between the two experiments. Additionally, despite the reconstruction of the single-file chute to eliminate the incline to the V-restrainer, the act of jamming still affected [LAC]. The effect of jamming found in this study was similar to that found by Benjamin et al. (2001) in the laboratory handling model. Benjamin et al. (2001) found that an increase in jamming resulted in an increase in fatigued animals, which demonstrates the significance of this behavioral parameter in lactate accumulation.

Correlations between rectal temperature and handling parameters.

Experiment 1. Pearson correlations between behavior and handling parameters and rectal temperature are shown in Table 4.2. Only one relationship was found between rectal temperature and the observed handling parameters. As animals spent a longer time in the pre-slaughter handling area, the rectal temperatures significantly decreased ($r = -0.26$, $P = 0.03$). From anecdotal observations of the observer, there were several instances at which time the animals were not being moved through the handling system due to a line-stoppage. Animal activity tended to stop during these periods. Thus, with no physical exertion to increase body temperature, temperatures began to drop the longer the animals remained inactive.

Experiment 2. Pearson correlations between behavior and handling parameters and rectal temperature are shown in Table 4.3. Analysis of the entire handling area indicated that there was no significant relationship between any of the animal handling measurements and rectal temperature. In the crowd pen, a positive relationship between prodding and rectal temperature was also observed ($r = 0.17$, $P = 0.05$). As indicated, the electric prod

was set at a higher voltage as compared with Exp. 1 perhaps explaining this difference. Additionally, as mentioned, the animals receiving prods in the crowd pen were those refusing to move into the single-file chute. Perhaps the increased avoidance activity led to increased rectal temperature. There were no significant relationships between measured handling parameters and rectal temperature in the single-file chute.

Contrasts between mean [LAC] of various handling groups.

Experiment 1. The group means from the single-degree of freedom contrasts are shown in Table 4.4. Comparisons were made between groups of animals that experienced events and those that did not. Additional comparisons were made to look at the cumulative effects of certain handling events as well. In agreement with correlation data, animals that jammed (+, 8.5 ± 0.68; -, 6.9 ± 0.43; P = 0.05) and backed up (+, 9.0 ± 0.76; -, 6.9 ± 0.41; P = 0.01) had higher [LAC] than those that did not. There was a tendency for animals that reared to have a higher [LAC] (+, 8.7 ± 0.89; -, 7.1 ± 0.40; P = 0.11). Animals that experienced one or more of particular handling events (i.e. animals that were prodded, jammed, reared or backed up; animals that jammed, reared or backed up and animals that reared or jammed) had higher [LAC] demonstrating the cumulative effect of handling parameters on blood lactate concentration (Ritter et al., 2008a).

Experiment 2. The group means from the single-degree of freedom contrasts are shown in Table 4.5 subdivided by handling "location". Similar to Experiment 1, contrasts were performed to compare [LAC] means between groups of animals experiencing handling events and those that did not, in addition to looking at the cumulative effects of handling events. In the analysis of the entire handling area, animals that were electrically prodded

tended to have a higher mean [LAC] than those that were not prodded (+, 7.9 ± 0.44; -, 6.9 ± 0.45; P = 0.13). Vocalization also tended to affect [LAC]. Animals that vocalized as they were moved through the handling area tended to have higher [LAC] (+, 7.9 ± 0.45; -, 6.9 ± 0.44; P = 0.09) than animals that did not vocalize. In the crowd pen, electric prod use affected [LAC]; animals prodded had higher [LAC] (+, 9.1 ± 1.11; -, 7.2 ± 0.33; P = 0.03) than those that did not. Vocalization in response to the prod also tended to have a positive effect on [LAC] (+, 9.9 ± 1.40; -, 7.3 ± 0.32; P = 0.07). From the previous discussion regarding correlation results in the crowd pen, Table 4.5 provides the number of animals (n=11) that were electrically prodded in the crowd pen. As discussed, this was a small group of animals that exhibited greater aversion to entering the single-file chute thus requiring more instances of prodding and thus higher [LAC]. In the single-file chute, animals that jammed (+, 8.7 ± 0.56; -, 6.8 ± 0.37; P = 0.005); animals that reared, jammed, backed up and/or were electrically prodded (+, 8.0 ± 0.35; -, 5.7 ± 0.61; P = 0.002); animals that reared, jammed and/or backed up (+, 8.1 ± 0.44; -, 6.7 ± 0.44; P = 0.03); animals that reared and/or jammed (+, 8.7 ± 0.54; -, 6.8 ± 0.38; P = 0.003) and animals that vocalized (+, 8.1 ± 0.47; -, 6.9 ± 0.42; P = 0.05) had higher [LAC] than those animals that did not experience those events. Similar to Experiment 1, the cumulative effect of particular handling events was demonstrated by the increase in [LAC] mean of animals experiencing those handling parameters.

Contrasts between mean rectal temperatures of various handling groups.

Experiment 1. The group means from the single-degree of freedom contrasts are shown in Table 4.4. The presence or absence of handling parameters did not have any significant

effects on rectal temperature. It seems that the experience of the handling parameters did not increase physical exertion at such a rate as to increase body temperature.

Experiment 2. The group means from the single-degree of freedom contrasts are shown in Table 4.5. Results indicate that in the entire handling area, animals that vocalized had higher rectal temperatures than animals that did not vocalize (+, 104.3 ± 0.015; -, 103.8 ± 0.15; P = 0.01). In the crowd pen, animals that were prodded tended to have higher rectal temperatures than animals not prodded (+, 104.7 ± 0.37; -, 104.0 ± 0.11; P = 0.08) and the animals that vocalized in response to prodding tended to have higher rectal temperatures than those that did not vocalize (+, 104.8 ± 0.47; -, 104.0 ± 0.11; P = 0.11). As described in the correlation results from Experiment 2, rectal temperatures could have been higher due to the electrical prodding and any additional sort of physical exertion of the pigs that may have caused. In the single-file chute, vocalizing animals also tended to have higher rectal temperatures than those that were not vocal in the single-file chute (+, 104.3 ± 0.16; -, 103.9 ± 014; P = 0.06).

These studies are the first to report that specific handling events of individual pigs within a pre-stun slaughter group are related to exsanguination [LAC]. Previously published studies have looked at pre-slaughter handling stress imposed on pigs just prior to stunning (Hambrecht et al., 2005a; Hambrecht et al., 2004b) or have compared slaughter facilities that were classified as “low” or “high” stress facilities (Warriss et al., 1994). This current study tried to identify specific aspects of the pre-slaughter handling that contributed to this increase in [LAC] at exsanguination and thus the increase in animal stress. By identifying specific handling events and behaviors that contribute to increases in exsanguination [LAC], this study was able to provide evidence that [LAC]

can be used as a quantitative tool to improve animal handling. By understanding that stress causes an increase in [LAC], and thus has a negative impact on well-being, by being able to associate particular events with an increase in [LAC] and by adjusting animal handling to avoid some of these adverse handling events, exsanguination [LAC] and thus animal stress can be kept at a minimum. Based on this work, the development of an exsanguination [LAC] on-line monitoring system is recommended. By having real-time measurement of [LAC], packing companies will be able to gain knowledge about which factors are affecting [LAC] and try to manage these factors. Using [LAC] as a quantitative tool could become an integral part of animal handler training, plant internal auditing and overall improvement of animal well-being in the swine industry.

IMPLICATIONS

In the current study, blood lactate concentration was doubled from baseline during the immediate pre-slaughter phase. Specific negative behaviors (jamming, rearing and backing-up) were correlated to an increase in exsanguination [LAC] explaining a portion of this increase. These experiments were the first to report that specific handling events of individual pigs within a pre-slaughter group are related to [LAC] in a commercial setting. By being able to identify certain aspects of animal behavior and pre-slaughter handling that affect blood lactate concentration, we can improve our animal handling and training protocols to minimize the occurrence of these handling events. This research provides evidence that [LAC] can be used as a quantitative tool to improve animal handling and enhance commercial packing plant auditing systems. Based on this work, the development of on-line monitoring of [LAC] is recommended to improve animal handling thus improving pig well-being in the packing plant. By understanding how an

animal is physiologically responding to movement, the environment and handling in the pre-slaughter area, there is an opportunity to manipulate the current system to better complement the animals' physiological system thus improving animal welfare.

CHAPTER FIVE

THE EFFECTS OF PRE-SLAUGHTER PIG MANAGEMENT FROM THE FARM TO THE PROCESSING PLANT ON PORK QUALITY

ABSTRACT

Two studies differing in facility design and season were conducted to determine the effects of pre-slaughter pig management on pork quality by monitoring blood lactate concentration ([LAC]) and rectal temperature (TEMP) through the marketing process, from loading at the farm to stunning. In Exp.1, 80 crossbred pigs (39 barrows, 41 gilts) were used. In Exp. 2, 144 crossbred pigs (120 barrows, 24 gilts) were used. Multiple sampling of [LAC] and TEMP were made for each animal at seven locations during the marketing process: (1) baseline at the farm, (2) post-loading on the truck, (3) pre-unloading after transport on the truck, (4) post-unloading at the plant, (5) post-lairage at the plant, (6) post-movement to the stunning area and (7) at exsanguination. A low-stress restraint method was used to obtain physiological measurements. Pearson correlations were used to determine relationships between [LAC] and TEMP at the seven sampling points and meat quality. Correlations were also used to relate the changes in [LAC] and TEMP between sampling points to meat quality. Increased [LAC] during loading at the farm resulted in improved meat quality, i.e. increased 24 hr pH ($P < 0.002$), decreased L* ($P < 0.03$) and decreased drip loss ($P < 0.02$) (Exp. 1 & 2). Similarly, as the change in

[LAC] between baseline and post-load measurements (i.e. effect of loading) increased, 24 hr pH increased ($P < 0.01$) and drip loss decreased ($P < 0.04$), in both Exp. 1 & 2. Exsanguination [LAC] was not related to ultimate meat quality (Exp. 1 & 2). It is hypothesized these results are due to calm handling in the stunning chute preventing excessive [LAC] production by the skeletal muscles at exsanguination. There was also a correlation between [LAC] at loading and [LAC] at exsanguination (P =0.003, Exp. 1 & 2) suggesting that animals with high [LAC] at loading tended to maintain a high [LAC] at exsanguination. The data suggest that high [LAC] during calm loading is associated with higher ultimate pH, darker color, and lower drip loss. Therefore, improving handling at the farm during loading will not necessarily translate to direct improvements in fresh pork quality traits.

Key Words: drip loss, glycolytic potential, handling, lactate, pre-slaughter

INTRODUCTION

The physiological changes associated with pre-slaughter handling stresses immediately prior to stunning have been shown to have detrimental effects on pork quality (Hambrecht et al., 2005a; Hambrecht et al., 2005b; Hambrecht et al., 2004b; Warriss et al., 1998). Hill and Schultz-Kaster (2006) reported immediate improvements in meat quality with improved pre-stun animal handling. One physiological change in swine associated with animal handling stress is an increase in blood lactate concentration ([LAC]) (Anderson et al., 2002; Benjamin et al., 2001). Hambrecht and coworkers (2004, 2005a) have explored the relationship between high exsanguination [LAC] and pork quality and determined that swine with higher [LAC] at slaughter resulted in pork with higher drip loss. This effect was compounded by high muscle glycolytic potential

indicating the complexity of determining ultimate pork quality. Warriss (1994) has demonstrated that pork from pigs stressed immediately before slaughter, with higher exsanguination blood lactate, had less acceptable eating quality than from pigs handled carefully, despite there being no difference in the predictors of quality that were measured.

Warriss et al. (1994) were able to demonstrate a correlation between the subjective assessment of stress level and the objective measures of stress and meat quality in a survey study of swine slaughter plants. High stress was associated with high exsanguination [LAC] and lower meat quality, i.e. decreased water holding capacity and lighter color.

Although several studies have been conducted exploring the effects of pre-slaughter stresses on pork quality (Hambrecht et al., 2005a; Hambrecht et al., 2004b; Warriss et al., 1994), these studies have specifically focused on animal management immediately before slaughter and have not explored how changes in physiological parameters throughout the marketing process may affect meat quality. The objective of this study was to determine if a relationship existed between pre-slaughter animal management, not only immediately prior to stunning but from the farm to the meat processing plant, and meat quality.

MATERIALS AND METHODS

Two studies were conducted, Experiment 1 in July 2007 and Experiment 2 in January 2008. Study replications were analyzed as separate experiments due to difference in season, packing plant facility design and experimental protocol. Prior to the initiation of these experiments, all animal use, handling, and sampling techniques

described herein were approved by the Colorado State University Animal Care and Use Committee.

Experimental Design. Blood lactate concentration and TEMP were measured on each individual experimental animal at seven different sampling points throughout the marketing process. Meat quality measurements were obtained on each of the individual animals following slaughter and post-mortem processing. Data were analyzed to explore the relationship of physiological parameters, blood lactate concentration ([LAC]) and rectal temperature (TEMP), with pork quality. In Exp. 1, 80 commercial crossbred pigs (39 barrows and 41 gilts) of average estimated live weight (125 ± 9 kg, mean ± SD) were used. One hundred forty-four crossbred pigs (n = 144, 24 gilts and 120 barrows) of average market weight (128 ± 3 kg, mean ± SD) were used in Exp. 2. The estimated mean live weight was back calculated using the hot carcass weights and a dressing percentage of seventy-five percent.

Animals, Housing and Feeding.

In Exp. 1, pigs were of similar genetic background, i.e. a Fast 536 sow and a Duroc boar produced by the production company. The animals used in this experiment were transported to the slaughter facility in four truckloads. In Exp. 2, animals were progeny of a Fast 536 sow-line and a boar derived from the 337 PIC line. Experimental animals were transported to the slaughter facility in six truckloads.

The animals used in these studies were the same experimental animals used in a study exploring various management factors and the relationship with [LAC] and TEMP (Edwards et al., 2009b). Refer to this paper for a detailed description of animal selection and housing.

Transport, Lairage and Slaughter. The detailed marketing process and additional experimental protocols have been described (Edwards et al., 2009b). Briefly, animals in both experiments were transported approximately 2.5 hr to the same packing facility. At the packing facility, they were unloaded and rested. After the designated rest period (30 min or 4.5 hr) animals were moved to the pre-stun handling area. Animals were electrically stunned with a head-to-chest constant amperage, variable voltage stunning system and subsequently exsanguinated. At this point, all test animals entered normal post-mortem processing of the packing facility.

Sampling Points. Each of the experimental animals was sampled at seven different points throughout the marketing process: (1) Baseline, (2) Post-load, (3) Pre-unload, (4) Post-unload, (5) Post-lairage, (6) Post-movement and (7) Exsanguination. The baseline sample point (1) was conducted in the barn pen prior to transport. The post-loading time point (2) was obtained on the truck after the experimental animals had been loaded. The test pigs were sampled on the trailer before the remainder of the trailer was loaded. The pre-unloading time point (3) was obtained on the truck upon arrival at the plant prior to unloading of the experimental animals. Trucker/plant personnel unloaded all pigs on the trailer except the test pigs prior to the third sampling. The test pigs were in a separate compartment so unloading the rest of the truck did not impact the test animals. The post-unload time point (4) was collected from the test animals after they had been unloaded and housed in their respective lairage pens. The post-lairage time point (5) was collected in the lairage pen approximately 20 minutes prior to the end of the scheduled rest time. The post-movement time point (6) was obtained immediately after the test pigs had been moved to the pre-stunning/pre-slaughter handling area. The exsanguination time point

(7) was obtained after pigs had moved through the pre-slaughter handling area and been electrically stunned and exsanguinated.

Sampling Protocol. At each sampling point, pigs were restrained to obtain a blood sample for blood lactate concentration ([LAC]) and a rectal temperature (TEMP). The technique used was a low-stress restraint method that did not require use of a snare or excessive restraint, described in detail by Edwards and coworkers (2009b). Briefly, researchers restrained each pig using sorting boards. Another researcher lanced one of the pig's distal ear veins with a retractable 20 gauge needle. A sample strip was inserted into a hand-held lactate analyzer (Lactate Scout, EKF Diagnostic GmbH, Magdeburg, Germany) and a drop of blood from the pig's ear was immediately administered to the sample strip. The analyzer provided [LAC] in approximately 15 s and the information was recorded. After the blood sample was obtained, pressure was applied to the ear vein for several seconds to induce clotting. Simultaneously, a rectal temperature was obtained using a Vick's SpeedRead digital thermometer (Model #: V911F, Kaz, Inc., Southborough, MA) providing a measurement within 8 s.

Behavior and Handling Observations. All animals were observed by one observer on an individual basis as they were moved through the pre-slaughter handling area (Edwards et al., 2009d). One experimental animal was moved into the crowd pen with approximately 15 random pigs from the packing facility. Behavioral observations were performed as the animal moved through a circular crowd pen (diameter = 4.57 m) and a single-file chute (length = 13.11 m, width = 0.40 m). The single-file chute was set at a 13% incline leading up to the v-restrainer in Exp. 1 and no incline in Exp. 2. Observations were terminated upon entrance to the v-restrainer upon which time observations began on the

next experimental animal. Animals were identified by their ear tag number. Animal behavior and handling parameters, defined in Table 4.1, were measured within the described handling area. The electric prod used was a live prod set at 13 V in Exp. 1 and 17.5 V in Exp. 2. Scores were tabulated as the number of times each animal experienced a handling event. Upon entrance to the v-restrainer animals were electrically stunned and subsequently exsanguinated.

Meat Quality Analyses. The left side of the carcass was used for all meat quality measurements. Forty-five minute pH was measured at the 10th rib of the *longissimus dorsi* using a pH meter (Version 1.5, Meat Probes, Inc., Topeka, KS) with a glass tip probe. Forty-five minute temperature was measured in approximately the same location using an Extech Instruments Instant Read thermometer (Extech Instruments Corporation, Waltham, MA). The temperature measurement was only obtained during Exp. 1. At approximately twenty-four hours post-mortem, pH was measured again from the same location on the carcass using the same pH meter as listed above. An approximately 2.5 cm thick loin chop was removed from the *longissimus dorsi* at the 10th rib and used for all of the subsequent measurements and analyses. Blood splash was determined on the chop as a whole using the National Pork Producers' Council standards, a 0-3 scale based off of the square inches of blood splash within the pork sample. Blood splash was only measured in Exp. 1. After blood splash was determined and all chops were collected, they were placed on ice until analyzed for color and sampled for drip loss. Subjective color of the chop was determined using the a 1-6 color score system (NPPC, 2000b) Drip loss was determined using the method of Rasmussen and Stouffer (1996). A 2.54 cm coring device was used to remove two meat samples from each loin chop. The remainder

of the chop was placed in a sealed plastic bag and frozen at -80ºC for future analyses. Each of the meat cores was assessed for Lab* color using a CR 400 Chroma meter (Konica Minolta Holdings, Inc., Tokyo, Japan) with D65 illuminant and a 2 degree observer. Each sample was then weighed and placed in a 3 cm x 3 cm plastic drip loss tube with an exudate collection funnel. Drip loss containers were stored for twenty-four hours at 4ºC. After 24 hrs, the meat samples were taken out of storage and reweighed. The percentage drip loss was calculated. Duplicate recordings for color score and drip loss were averaged for each individual chop.

Glycolytic Potential. Glycolytic potential was determined using the remainder of the loin chop used for the previously described analyses. Glycolytic potential was determined using the following equation as reported by Monin and Seller (1985): Lactate + [2 x (Glucose + Glucose-6-Phosphate + Glycogen). Loin chops were moved from -80ºC storage to 8ºC storage twelve hours prior to analysis. A one gram sample of unexposed tissue was obtained from each of the loin chop samples and used for glycolytic potential determination. The extraction protocol of Bergmeyer (1974) was followed to convert muscle glycogen to glucose. Briefly, samples were homogenized. Duplicate samples were taken from this original homogenate. Amyloglucosidase (Catalog #: A1602, Sigma Aldrich, St. Louis, MO) was added to the homogenate to convert the glycogen to glucose. The glucose and lactate content in this homogenate were determined using absorbency at specific wavelengths, discussed below, on a plate reader (BioTEK Synergy II, BioTECH Instruments Incorporated, Winooski, VT). A glucose kit (Catalog #: 10009582, Cayman Chemical Company, Detroit, MI) was used to determine the glucose concentration of the homogenate. This kit utilized a glucose oxidase linked assay. The concentration of

glucose in the homogenate was low and therefore to fit within the standard curve of the assay, five times the recommended volume of sample was used in the analysis. The absorbency setting for the analysis was 510 nm. Lactate content was determined using a lactate kit (Catalog #: K607-100, Biovision, Inc, Mountain View, CA). The absorbency for this analysis was 450 nm. Preliminary analyses assessed the abundance of glucose-6-phosphate in the meat samples. A glucose kit utilizing glucose-6-phosphate dehydrogenase was initially used to compare the accuracy of this particular assay with the cited glucose oxidase assay. Differences in assays were negligible, therefore the oxidase kit was utilized for all meat analyses. Glycolytic potential analyses were performed in duplicate. If there was a coefficient of variation between the duplicate samples that was greater than 10%, samples were re-analyzed. If this occurred for a second time, the sample values were not included in the statistical analysis.

Statistical Analyses. Pearson correlations were performed to determine the relationships between [LAC] and TEMP obtained at all sample points and all meat quality parameters in addition to all meat quality parameter intercorrelations. The changes in [LAC] and TEMP between sample points were also calculated. The changes in physiological parameters were defined as follows: loading (sample point 2 – sample point 1) = effects of distance moved during loading; transport (sample point 3 – sample point 2) = effects of transport; unloading (sample point 4 – sample point 3) = effects of unloading; lairage (sample point 5 – sample point 4) = effects of lairage time; movement to stun (sample point 6 – sample point 5) = effects of distance moved from lairage to stun area and pre-slaughter handling (sample point 7 – sample point 6) = effects of handling in crowd pen, single-file chute and stunning. Pearson correlations were conducted to relate the changes

in [LAC] and TEMP to the meat quality parameters. The same analyses were performed for both Exp. 1 and 2.

RESULTS AND DISCUSSION

Blood lactate concentration ([LAC]), TEMP and meat quality. Means for [LAC] and TEMP at all sampling points in both experiments have been reported in detail (Edwards et al., 2009b). Means for [LAC] and TEMP at each sampling point are shown in Tables 5.3 & 5.5 for Exp. 1 and Tables 5.4 & 5.6 for Exp. 2. Previously published studies in commercial facilities report exsanguination [LAC] between 9 and 31 mM (Hambrecht et al., 2005a; Hambrecht et al., 2004b; Warriss et al., 1998). In the present experiment, [LAC] were on the low end of reported values, more similar to data reported by Warriss and coworkers (1994; 4.4mM and 12 mM [LAC] respectively). Low blood lactate in exsanguination blood reflects gentle handling techniques prior to stunning. Monitoring exsanguination lactate would provide an excellent tool for animal handling training and for auditing animal handling adequacy prior to slaughter (Edwards et al., 2009d).

Interrelationships between meat quality parameters. *Experiment 1.*Table 5.1 provides Pearson correlations illustrating the interrelationships between all meat quality parameters. Significant correlations confirmed expected relationships between the various meat quality characteristics. For example, as 45 min pH and 24 hr pH decreased, drip loss increased ($P = 0.0001$), a relationship that would be expected. As the pH of meat approached the isoelectric point of main muscle proteins (i.e., myosin), the water holding capacity decreases. Additionally, as glycolytic potential increased, drip loss values simultaneously increased ($P = 0.05$). Slaughter animals with higher muscle glycogen had decreased ultimate pH (P=0.07) and increased drip loss ($P = 0.05$).

Experiment 2. Table 5.2 provides Pearson correlations between meat quality characteristics for Exp. 2. The various correlations indicate, once again, the extensive and expected interrelationships between the various meat quality characteristics. A relationship similar to that observed in Exp. 1 was observed between drip loss and glycolytic potential; as glycolytic potential increased, drip loss increased ($P = 0.008$). There are several expected correlations between meat quality characteristics indicating expected physiological relationships between the various measurements, e.g. twenty-four hour pH was negatively correlated with L* ($P = 0.001$), drip loss ($P = 0.001$) and glycolytic potential ($P = 0.0007$), and it was also positively correlated with 45 min pH ($P = 0.01$).

Blood lactate concentration ([LAC]) at the seven sample points. *Experiment 1*. Table 5.3 provides the Pearson correlations for all meat quality parameters and the [LAC] values of experimental animals at the seven described sampling locations. There were several significant relationships between meat quality parameters and [LAC] throughout the marketing process. The sampling point that exhibited the greatest number of relationships with meat quality was just after loading at the farm. Results indicate that as [LAC] increased during loading, meat quality improved; increases in [LAC] were related to an increase in 24 hr pH ($P = 0.002$), a decrease in L* ($P = 0.03$), a decrease in drip loss ($P = 0.02$) and a tendency towards increased visual color score ($P = 0.07$). There was also a tendency for [LAC] at loading to be positively correlated to muscle glucose content ($P = 0.11$) and glycolytic potential ($P = 0.14$) at 24 h post-mortem. It is likely that physical exertion during loading reduced muscle glycogen. If muscle glycogen was not

replenished during transportation and lairage, the result would be improvement of pork quality (higher 24 h pH).

Another area of interest is the relationship between exsanguination [LAC] and meat quality. In this experiment, exsanguination [LAC] was not related to meat quality parameters as in previously reported research (Hambrecht et al., 2005a; Hambrecht et al., 2004b) as well as some unpublished results (Edwards et al., 2009a). Previously published studies report that as the increase in exsanguination [LAC] was indicative of accelerated early post-mortem metabolism (higher 45 min temperature, lower 45 min pH), resulting in greater drip loss. This contradiction may be due to the high correlation between [LAC] at loading and [LAC] at exsanguination (Exp. 1, $P = 0.001$; Exp. 2, $P = 0.03$; Combined experiments, $P = 0.003$; data not shown) indicating that animals with high [LAC] at loading tend to be the same animals with high [LAC] at exsanguination. It is likely that animals that were difficult to load (high [LAC] at loading) were also difficult to move to the stunner (high [LAC] at exsanguination). It is postulated that the effects of loading on [LAC] and meat quality (glycolytic potential, 24h pH, L*, visual color score, and drip loss) override any effects on early post-mortem metabolism reflected in exsanguination [LAC]. Perhaps changes in muscle glycogen (with resultant changes in 24 h pH) have a greater influence on pork quality and override changes in early post-mortem metabolism (with resultant changes in drip loss). Therefore, if a pig experiences both high [LAC] at loading (reduced muscle glycogen) and high [LAC] at exsanguination (rapid post-mortem metabolism), it can be speculated that effects at loading (improved meat quality) may predominate. As handling during loading at the farm improves, thus decreasing lactate levels, exsanguination lactate may become a better

predictor of meat quality. If there is no substantial reduction in muscle glycogen during loading at the farm, maintaining low lactate during immediate pre-stun handling could become more important. This effect may have been difficult to detect in the other studies because high stress handling treatment was imposed on all "high stress" treatment animals prior to stunning (Hambrecht et al., 2005a; Hambrecht et al., 2004b).

Additionally, the pigs in this study had generally lower [LAC] values than those observed in previous reports (Hambrecht et al., 2005a; Hambrecht et al., 2004b). Perhaps the impact of exsanguination [LAC] on pork quality is most apparent in high stress handling systems. In the current project, animals were handled gently, exhibited low [LAC] and had experienced extensive handling prior to slaughter, all potentially adding to a lack of exsanguination [LAC] effects on meat quality. In the unpublished study by and co-workers (2009a), the mean exsanguination [LAC] values were similar to this study but the effect of high [LAC] on meat quality was observed; as exsanguination [LAC] increased, drip loss increased ($r = 0.22$; $P = 0.02$). Differences with the current study are as follows: (1) In unpublished experiment, animals were handled very gently at loading. Pigs were loaded onto the lower level of the truck. The loading chute had no incline angle. The upper deck of the truck was raised hydraulically. (2) Carbon dioxide stunning was used rather than electrical stunning as in the current study.

There were relationships between [LAC] and meat quality at other locations throughout the marketing process such as [LAC] just after transport prior to unloading and following unloading. These relationships are more difficult to evaluate. Not only are they perhaps more difficult to manipulate, in terms of being able to change management

to accommodate the physiological changes at those specific times, but there many factors, that influence these sampling points.

Experiment 2. Table 5.4 shows Pearson correlations for Exp. 2. This experiment provided similar results to those found in Exp. 1. As [LAC] at loading increased, meat quality improved, i.e. 24 hr pH increased ($P = 0.0001$), L* decreased ($P = 0.001$) and drip loss decreased ($P = 0.002$). Additionally, the same relationship of lower muscle glucose content ($P = 0.03$) with higher loading [LAC] was observed indicating a potential reduction in glycogen content present at slaughter due to the potential increased glycogen use at the farm.

In this experiment, exsanguination [LAC] was negatively correlated with 45 min pH but unexpectedly was also negatively correlated to L* ($P < 0.01$) and drip loss ($P < 0.03$). Similar explanations to those provided in the Exp. 1 discussion can be applied to the results seen in this experiment as the results were comparable. Perhaps this improvement in drip loss was related to the reduction in muscle glycogen. The low levels of muscle glycogen going into the pre-slaughter handling area may have negated any potential negative effects of high exsanguination [LAC], i.e. perhaps the low glycogen had a greater impact in determining ultimate meat quality than the high exsanguination [LAC].

Change in [LAC] between sample points. *Experiment 1.* The Pearson correlation results for the changes in [LAC] related to all meat quality parameters are shown in Table 5.5. There are several significant relationships present between the change in [LAC] due to loading and pork quality. As the change in [LAC] during loading increased, 24 hr pH increased ($P = 0.0006$), drip loss decreased ($P = 0.04$), 45 min pH tended ($P = 0.07$) to

increase and visual color tended ($P = 0.09$) to increase, i.e. improvements in meat quality. Additionally, as the change in [LAC] during loading increased, glycolytic potential decreased ($P < 0.05$). The other correlations between changes in [LAC] during the marketing process and meat quality tended to be sporadic and were not consistent between Exp. 1 and 2.

Experiment 2. Again, change in [LAC] at loading was correlated with improved meat quality. i.e. increased 24 hr pH (P = 0.01), decreased drip loss (P = 0.01), decreased L* (P = 0.01) and a tendency for increased visual color (P = 0.06). Consistent with previous work (Hambrecht et al., 2005b; Hambrecht et al., 2004b), 45 min pH decreased as the change in [LAC] during pre-slaughter handling increased (P < 0.02); indicating that the animals may have entered post-mortem processing at a higher metabolic rate resulting in a more rapid drop in pH.

Rectal temperature (TEMP) at the seven sample points. *Experiment 1 and 2.* Results from Pearson correlations between rectal temperature (TEMP) and the various meat quality characteristics are shown in Table 5.7 and 5.8. As would be expected from the results of the loading [LAC] relationship to meat quality, an increase in loading TEMP was related to increased 24 h pH (Exp. 1, $P = 0.07$; Exp. 2, $P = 0.0001$) and decreased drip loss (Exp. 2, $P = 0.003$). Unexpectedly in Exp. 1, higher body temperature at two sample points (pre-unloading and post-unloading) correlated with better pork quality, i.e. higher 24 hr pH ($P < 0.03$), higher color score ($P = 0.01$), lower L* and b* ($P < 0.01$) and trend ($P < 0.11$) for lower drip loss. There was no tendency for this relationship in Exp. 2 nor with changes in [LAC] in either Exp. 1 or Exp. 2. Data from both experiments seem

to indicate that the more exertion of the pigs during the loading process results in improved pork quality.

Change in TEMP between sample points. *Experiment 1.* Changes in TEMP did not appear to greatly affect the various meat quality measurements as shown in Table 5.9. The change in TEMP during movement to the crowd pen was positively correlated to TEMP at exsanguination, i.e. the pigs with larger increases in TEMP post-movement had higher exsanguination TEMPs ($P = 0.001$). Additionally, pigs with larger increases in TEMP during pre-slaughter handling from the crowd pen to stun had greater exsanguination TEMPs. These pigs most likely entered post-mortem metabolism at a higher rate. This could be related to the trend seen in the relationship of drip loss to the change in TEMP when moving to stun; there was a trend ($P = 0.10$) for animals that had greater increases in TEMP to have higher drip loss values.

Experiment 2. In this experiment, more relationships were demonstrated between changes in temperature and meat quality measurements as shown in Table 5.10. A greater number of carcasses were evaluated in this study perhaps influencing the number of relationships detected. Greater body temperature changes during the marketing process were related to increased 24 hr pH (Loading, $P = 0.02$; Transportation, $P = 0.002$; Unloading, $P = 0.04$; Lairage, $P = 0.09$; Movement to circle corral, $P = 0.01$). Additionally, there may have been seasonal effects on meat quality as temperature regulation may vary between seasons; larger changes in temperature having larger ultimate impacts on quality. This experiment was conducted in January and Exp. 1 was conducted in July thus environmental temperatures were quite different.

IMPLICATIONS

In both experiments, the expected inter-relationships between meat quality characteristics were observed strengthening the confidence in the accuracy of the analyses. Although results indicated that ultimate meat quality was related to physiological measures throughout the marketing process, [LAC] and TEMP as a result of loading in the farm had the strongest relationship to meat quality. The large increase in [LAC] during loading due to physical exertion up a steep ramp and handling, had a positive impact on meat quality. Exsanguination [LAC] did not have the same strong relationship to meat quality that has been published in previous studies conducted in commercial swine slaughter facilities. It is postulated that this relationship was not as evident due to the substantial relationship between ultimate meat quality and changes in [LAC] during loading. Another possibility is that the processing plant where the experiments were conducted had careful calm handling in the stunning chute and an electric prod was only used on pigs that refused to move. With a substantial increase in [LAC] early during the marketing process and the resultant increase in 24 h muscle pH, the effects of exsanguination [LAC] on meat quality were not seen. Since it is unacceptable to roughly handle pigs during loading to reduce glycogen content and increase 24 h pH, more focused emphasis must be given to on-farm handling and loading. As handling improves at the barn, the importance of maintaining low [LAC] at exsanguination through careful animal handling will very likely have a greater impact on meat quality.

CHAPTER SIX

PERSISTENCE OF BLOOD CHANGES ASSOCIATED WITH ALTERATION OF THE DIETARY ELECTROLYTE BALANCE (dEB) FOLLOWING FEED WITHRDRAWAL, TRANSPORTATION AND LARIAGE IN COMMERICAL PIGS AND THE EFFECTS ON PERFORMANCE AND CARCASS QUALITY

ABSTRACT

Increasing dietary electrolyte balance (dEB) has previously been shown to reduce the incidence of non-ambulatory, non-injured swine, improve meat quality and reduce the incidence of gastric ulcers. The objective of this study was to evaluate the effect of dEB under commercial conditions. Due to the variability in feed withdrawal, transport and lairage conditions in the swine industry, it was necessary to determine first the persistence of blood changes during the marketing process following alteration of dEB. Sixteen pens of eight cross-bred barrows were assigned to either a Low (121 meq/kg) or High (375 meq/kg) dEB diet, calculated as $Na^+ + K^+ - Cl^-$, to determine the persistence of blood changes associated with the alteration of dEB. Diets were formulated to meet or exceed NRC (1996) requirements for energy, protein, vitamins, and minerals. Treatment feed was provided *ad libitum* for 3 days prior to slaughter. Prior to transport, animals were fasted in the barn for approximately 10 h. Following fasting, animals were shipped to the packing plant, rested for 8 h and subsequently slaughtered. Initial and final weights

of the animals were obtained. Blood was sampled at baseline (5 days before administration of diets), at the time of feed withdrawal (0 h), following feed withdrawal (10 h) and at exsanguination (20 h). Administration of the High dEB diet for 3 days resulted in an increase in blood TCO_2 ($P = 0.001$), bicarbonate ($P = 0.001$) and base excess ($P = 0.0003$) and a decrease in blood chloride ($P = 0.0002$) and anion gap ($P = 0.01$). These differences, however, were not maintained for any of the parameters after the 10 h feed withdrawal ($P > 0.22$). Increasing dEB had no adverse effects on growth performance, meat quality or carcass yield and did not decrease pars esophageal ulcer scores. This study demonstrated that the effect of dEB on blood parameters was not maintained following a 10 h feed withdrawal. Therefore, it is likely that the animal's ability to withstand any increased metabolic acid load associated with the stress of transport was lost following feed withdrawal. Further research is needed to determine the effects of dEB alteration in animals that have not been fasted prior to shipment and using diets with a larger difference in dEB.

KEY WORDS: acidosis, cation-anion balance, dietary electrolyte balance, swine

INTRODUCTION

During the 1990's intransit losses of market hogs quadrupled (FSIS, 2007 2008).While the number of in-transit losses (i.e. dead and non-ambulatory animals) in the swine industry has decreased in the past several years (FSIS, 2007 2008), there is still potential to reduce the number of animals lost. Previous work has shown that fatigued pigs (i.e. non-ambulatory, non-injured animals) were characterized with metabolic acidosis (Anderson et al., 2002; Ivers et al., 2002b). By providing pigs with a high dietary electrolyte balance (dEB) prior to transportation, it is hypothesized that pigs will

have the ability to more successfully buffer the metabolic acidosis associated with periods of high stress and subsequently prevent the onset of the fatigued pig syndrome and in-transit losses. Animal handling studies in a laboratory environment showed that increasing dietary electrolyte balance (dEB) significantly reduced the incidence of fatigued pigs (Ivers et al., 2002a).

When evaluating the practical commercial application of increasing the dEB of swine diets to reduce the incidence of fatigued pigs, it is also important to understand any negative effects the treatment may have on growth performance and meat quality. It has been demonstrated that increasing the dEB, in the feed or water, can improve meat quality while lowering dEB can produce negative effects (Ahn et al., 1992; Boles et al., 1994; Patience and Beaulieu, 2003; Shand et al., 1995). Additionally, increasing the electrolyte concentration of water has been shown to reduce the incidence of gastric ulcers in pigs, another potential benefit to cation-rich swine diets (Ange et al., 2000).

Due to the variation in feed withdrawal protocols, transportation times and lairage lengths used in the swine industry during the marketing process, the current trial was designed to test the persistence of dEB treatment on blood pH, bicarbonate and base excess, physiological indicators of buffering capacity. In addition, the current trial was designed to determine if there were any effects of dEB treatment on growth performance, carcass yield, meat quality and incidence of gastric ulcers.

MATERIALS AND METHODS

Prior to the initiation of these experiments, all animal use, handling, and sampling techniques described herein were approved by the Colorado State University Animal Care and Use Committee.

Feeding. Sixteen pens of eight cross-bred barrows were assigned to either a Low (121 meq/kg) or High (375 meq/kg) dEB diet (8 pens per treatment, 64 animals per treatment). Dietary electrolyte balance was calculated as $Na^+ + K^+ - Cl^-$ (meq/kg of feed) in the diet. Diets were formulated to meet or exceed NRC (1996) requirements for energy, protein, vitamins, and minerals. Treatments were originally formulated to have an approximate 400 meq/kg of feed difference in dEB between the low and high electrolyte diets. A corn and soy based diet was supplemented with 0.7% calcium chloride to formulate the Low dEB diet. The High dEB diet was created via supplementation of 2.5% sodium bicarbonate. The Low dEB diet was on the lower end of the normal range of electrolyte balance that would be expected in practical swine diets. The High dEB diet was formulated to provide base excess buffering in the pig but to not adversely affect feed intake and growth performance. Due to mixing error the dEB difference was determined to be 254 meq/kg of feed (Low dEB = 121 meq/kg, High dEB = 375 meq/kg), approximately a 35% smaller difference than expected.

Additionally, the test diets were supposed to include 10 ppm of Paylean® following standard finisher phase feeding. The pelleted test feeds were formulated incorrectly, the resulting Paylean® levels being 9.8 ppm in the High dEB diet and 6.8 ppm in the Low dEB diet. Treatment diet compositions are shown in Table 6.1. All test feeds were manufactured on the same day. A red color dye (iron oxide) was added to the High dEB treatment to differentiate between dietary treatments and reduce the possibility of any feeding errors at the research barn.

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Treatment diets were weighed and hand delivered to each feeder three days prior to slaughter. During this time, pigs had *ad libitum* access to feed and water. At the completion of the three day feeding period, feeders were closed and weigh backs were recorded. Pigs remained in the barn without feed for 10 hours at which time they were loaded onto the trailer. Loading was low stress; the pigs were moved from the barn to the truck on a flat angle loading chute (width = 0.89 m; length = 3.35 m). The trailer was a straight deck with two decks. The stocking density was approximately 0.45 m^2 per pig. There were no internal ramps within the trailer between decks; a hydraulic lift raised the floor of the trailer to create the second deck. After loading, pigs were transported approximately 1 hour to the packing facility. They remained in lairage at the facility for approximately 8 hours. Following lairage, they were stunned with carbon dioxide, exsanguinated and entered normal post-mortem processing. Water was provided until the pigs were loaded and shipped to the slaughter facility.

Animals and Housing. All animals used in the study were barrows originating from the same farrowing unit. The pigs used in the trial were a cross between a Topigs Tempo boar and a Topigs C40 sow (Topigs International, Netherlands). Barrows were housed in a finishing facility typical of the ones used in commercial practice in the local area (Quebec, Canada). Each pen was 8 m^2 with 8 test pigs in each pen (stocking density = 1 m^2 per pig). There was one feeder and one waterer per pen.

Pens of test pigs were selected based on the following criteria: structurally sound, healthy and subjective average live weight. Once pens were selected, treatments were randomly allocated to the 16 pens balancing treatment groups by pen weights, previous treatments received during previous feeding trial and location in the barn (accounting for

distance traveled at loading across treatments). Animals in each test pen were identified by a unique numbered plastic ear tag. On the day before slaughter, a unique carcass tattoo number was applied to each pig as well to facilitate identification at the packing plant.

Weights. All test animals were weighed two days prior to the initiation of the feeding trial. These weights were used to assign pens to the dietary treatments. Pigs were weighed again at the start of the feeding trial immediately before the treatment feeds were put into the feeders. They were weighed a third time to obtain a final weight just prior to feed withdrawal.

Blood Sampling Timeline. Four blood samples were taken throughout the trial: pre-treatment (Baseline), pre-feed withdrawal (0 h), pre-transport (10 h) and exsanguination (20 h). The baseline sample was taken two days prior to treatment initiation. Blood was sampled from 4 randomly selected pigs per pen in a total of twenty pens (only 16 of the pens were selected for the trial). The 0 h blood sample, taken at the beginning of the ten hour feed withdrawal, was obtained on the third day of diet treatment. Blood was sampled from 6 pigs in each of the sixteen test pens. The 10 h blood sample was taken approximately ten hours following feed withdrawal, just prior to loading. Blood was sampled from the same six pigs per pen that were sampled at the previous blood collection (0 h). The final sample was taken at exsanguination, approximately 20 h after feed withdrawal. Exsanguination blood was sampled from all trial pigs.

Sample Collection.

Feed Sampling. A representative composite sample was taken from the two basal diets used for the prior feeding study and for each of the two treatment diets used for this trial. The primary sample from each dietary treatment was then divided into four sub-samples

for: nutrient and electrolyte analysis, lysine analysis, Paylean® analysis and a retainer sample. The nutrient, electrolyte and lysine analyses were performed by Agri-Foods Laboratories (Guelph, Ontario). The Paylean® analysis was performed by Elanco Animal Health (Guelph, Ontario).

Growth Performance. Average daily feed intake was calculated as total feed intake per pen divided by the number of pigs per pen and days during the treatment period. Average daily gain was calculated on an individual animal basis as the total bodyweight gain per animal divided by the number of days during the treatment period. Individual animal average daily gains were averaged across pen. Feed efficiency was calculated as body weight increase divided by total feed consumption. Mean bodyweight was calculated on a pen basis for both initial and final weights.

Blood Sampling. Blood was obtained via jugular vena puncture into lithium heparin vacutainer tubes (Catalog #: 02-689-7, Fisher Scientific, Pittsburgh, PA). Blood samples were analyzed using a portable iSTAT blood analyzer with EG-8+ cartridges (i-STAT Handheld Clinical Analyzer, Heska Corporation, Loveland, CO 80538, www.heska.com) to provide the following measurements: pH, base excess, bicarbonate, sodium, potassium, chloride, glucose, urea nitrogen (BUN), total CO_2, anion gap, hematocrit, hemoglobin, and partial CO_2. Two teams of three researchers were used to obtain blood samples: one person snared the animal, one person obtained a blood sample and another person recorded the time it took to obtain the sample. Two additional researchers were responsible for running the iSTAT blood analyzers. Each sample

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required 2-3 min to load and run through the analyzer and blood sampling took approximately 2 min per pig. Three analysis devices were required for simultaneous analysis of blood samples from the two bleeding crews. Lactate was measured in blood samples collected at exsanguination using a hand held lactate analyzer (Lactate Scout, EKF Diagnostic, Magdeburg, Germany). For the lactate analysis, blood was transferred to potassium oxalate sodium fluoride tubes to inhibit further glycolytic metabolism (Catalog #: 02-688-48, Fisher Scientific, Pittsburgh, PA). For blood gas analysis, exsanguination blood was collected in heparin tubes (Catalog #: Catalog #: 02-689-7, Fisher Scientific, Pittsburgh, PA).

Stomach pars esophageal ulcer score. Stomachs were collected during slaughter and opened along the greater curvature. Stomachs were rinsed and assigned a score based on the following scoring system: 0 = normal, 1 = moderate hyperkeratosis, 2 = severe hyperkeratosis, 3 = light ulcer, 4 = moderate ulcer and 5 = severe ulcer.

Carcass measurements. The following measures were recorded on all test pigs by the packing facility: hot carcass weight, dressing percent (HWT), cold carcass weight, dressing percent (CWT) and percent lean. Cold carcass weight of each pig was recorded approximately 22 hrs after slaughter, then the head was removed and the carcass was split in two parts. The right side of the carcass was used for meat quality evaluation.

Meat quality measurements. Muscle temperature and pH were taken at the third/fourth last rib at 60 min post-mortem approximately 20 min after carcasses were moved into the chill cooler. Muscle pH and temperature were measured with a pH meter (Oakton Instruments Model pH 100 Series, Nilis, IL) with a spear type electrode (Cole Parmer Instrument Company, Vernon Hills, IL) and an automatic temperature compensation

probe. At approximately 22 hours post-mortem, chilled carcasses were transported to a carcass processing facility and fabricated into wholesale cuts. The loin from each test carcasss was removed from the fabrication line and a 2 cm loin chop was taken at the third/fourth last rib from each carcass for the following measurements: 24 hr pH and temperature , subjective muscle color (Japanese Color Standards; 1 = pale to 6 = dark color) (Nakai et al., 1975), objective color (Minolta L*, a*, b* values), 48 hr drip loss, muscle firmness (1 = firm, 3 = soft) and marbling (1 = devoid, 10 = abundant) (NPPC, 2000a). Minolta color was measured with a Minolta Chromameter CR 300 (Minolta Canada, Missisanga, Ontario, Canada) with a D65 light source with 0º viewing angle geometry. Drip loss was determined using a modified "juice container" protocol (Christensen, 2003). Drip loss was conducted in triplicate using three muscle cores taken with a 25 mm diameter cork borer. Each core was weighed and placed into a plastic container (Christensen Aps Industrivaengetand, Hilleroed, Denmark). The containers were stored at 4ºC and after forty-eight hours the samples were removed, carefully dabbed and weighed. Drip loss was calculated by determing the difference between the initial and final weight of each muscle core. A final drip loss value was determined by calculating the average drip loss of all three cores.

Based on the various quality measurements the chops were classified into the following categories: PSE – pale, soft, exudative; PSE trend; normal; PFN – pale, firm, normal; RSE – red, soft, exudative; DFD trend and DFD – dark, firm, dry (Table 6.2).

The remainder of the chops were packaged and frozen at -80ºC for glycolytic potential determination. Glycolytic potential was calculated using the following equation (Monin and Sellier, 1985): lactate concentration + 2(glucose + glucose-6-phosphate +

glycogen). The extraction protocol of (Bergmeyer, 1974) was followed. Briefly, samples were homogenized in buffer with amyloglucosidase (Catalog #: A1602, Sigma Aldrich, St. Louis, MO) to degrade glycogen to glucose. A lactate kit (Catalog #: K607-100, Biovision, Inc., Mountain View, CA) was used to determine lactate concentration in the homogenized samples and a glucose kit (Catalog #: 10009582, Cayman Chemical Company, Detroit, MI) was used to determine glucose concentration (glycogen plus free glucose). The amount of glucose-6-phosphate in the muscle homogenates was negligible and therefore was not included in the analysis. The samples were analyzed on a BioTEK Synergy II plate reader (BioTek Instruments Incorporated, Winooksi, VT).

Statistical Analysis. The significance and magnitude of treatment group differences for blood parameter outcomes were assessed with a restricted maximum likelihood-based, mixed effects model analysis via PROC MIXED in SAS. Analyses included the categorical fixed effects of dietary treatment, time and treatment*time interaction (time refers to sampling point). Pen was experimental unit for all parameter analyses. Due to the effects of carbon dioxide stunning on the parameters targeted to assess persistence of blood changes due to alteration of electrolyte balance, exsanguination samples (i.e. 20 h post feed withdrawal) were not used to assess persistence.

RESULTS AND DISCUSSION

Persistence of the dEB treatment following feed withdrawal. Pigs were fed a Low or High dEB diets for 3 days prior to slaughter. Blood was obtained prior to treatment initiation, prior to feed withdrawal and 10 hours following feed withdrawal and analyzed for glucose, BUN, sodium, potassium, chloride, total CO_2, anion gap, hematocrit, hemoglobin, pH, pCO_2, bicarbonate and base excess (Table 6.3). Treatment

diets did not generally have an effect when analyzed over three of the sampling points (baseline, 0h, and 10h). However, there was a significant treatment*time interaction for chloride ($P = 0.002$), anion gap ($P = 0.02$), total CO_2 ($P = 0.003$), bicarbonate ($P = 0.002$) and base excess ($P = 0.002$). The High dEB diet decreased blood chloride and anion gap before feed withdrawal ($P < 0.01$) but not after a 10 hr feed withdrawal ($P > 0.51$). Similarly, TCO_2, bicarbonate and base excess were increased ($P < 0.001$) before feed withdrawal but not after a 10 hr feed withdrawal ($P > 0.45$). Blood pH tended to be higher in pigs fed the high dEB diet following a 10 hr feed withdrawal ($P = 0.06$). The pigs fed the high electrolyte diet exhibited blood changes indicative of an altered acid-base status, i.e. more alkaline. These results are similar to other studies that have explored the ability of diets with a high dEB to alter acid-base indicators in swine (Haydon et al., 1990; Patience et al., 1987; Patience et al., 1986). The administration of a High dEB diet for 3 days (+250 meq/kg feed) was able to induce a more alkaline physiological state in the pigs but none of these differences persisted following a 10 hr feed withdrawal. Since there was not even a trend for a difference between treatments in blood bicarbonate and base excess following feed withdrawal, it is unlikely that a treatment dEB difference of 400 meq/kg, as was originally desired, would have given a different result.

Previously conducted studies have explored the effect persistence of blood changes with the administration of electrolyte supplementation in water but have not explored the effects of feed withdrawal on these changes (Ahn et al., 1992; Cole et al., 2004). One study determined that providing pigs with water containing sodium bicarbonate for twenty-four hours would alter the acid-base status of the animal, i.e.

greater blood bicarbonate, pCO_2 and urine pH ($P < 0.05$), making it more alkaline (Cole et al., 2004). This same study additionally tested the effect of providing buffered water for an extended period of time (10 days). At both days 5 and 10, pigs in the control and supplemented groups did not show differences in acid-base indicators, e.g. pH, bicarbonate, pCO_2. This is perhaps a result of adaptation to the excess base intake. It has been demonstrated that approximately three days of feeding a high dEB diet can produce alkaline changes in the blood, after which point the blood changes begin to fluctuate and eventually return to baseline (i.e. control diet animal profiles) (Ahn et al., 1992), perhaps due to renal adaptation (Scott, 1971). In the current study, differences in blood acid-base status between treatment groups were observed after 3 days of providing the dietary treatments, consistent with previous work (Ivers et al., 2002a) which showed that 1-4 days was the optimum duration of feed administration to get the greatest physiological response in blood.

Effect of dEB on blood parameters at exsanguination. The process of CO_2 stunning resulted in values for blood CO_2, bicarbonate and potassium that that were outside the range of the iSTAT blood analyzer and therefore were not useful in determining persistence of the dEB effect. The High dEB diet resulted in higher blood chloride ($P = 0.02$) and a trend for higher sodium ($P = 0.07$) and lower lactate ($P = 0.06$) (Table 6.4). There were 3 pigs in the Low dEB treatment that had blood CO_2 in the normal range, all the others had immeasurable CO_2 values due to stunning. When these outliers were removed from the analysis there was no difference in blood pH ($P = 0.25$). When included in the analysis, blood pH of the Low dEB treatment was higher than the High dEB ($P = 0.04$).

In general, CO_2 stunning resulted in substantial increases in blood bicarbonate, TCO_2, pCO_2, and base excess. It is possible that this change in electrolyte balance could have a positive influence on meat quality. Previously published studies have indicated that carbon dioxide stunning may produce better meat quality in pork than electric stunning (Channon et al., 2002; Channon et al., 2003; Channon et al., 2000; Velarde et al., 2000). These studies have demonstrated that carbon dioxide stunning results in lower drip loss and higher initial muscle pH as compared with electrical stunning (Channon et al., 2002; Channon et al., 2003; Channon et al., 2000; Velarde et al., 2000). Results documenting effects of stunning method on ultimate pH however are inconsistent, some indicating higher pH with carbon dioxide stunning (Velarde et al., 2000) and some indicating no difference (Channon et al., 2003). Perhaps the differences between stunning methods is partially due to changes in acid-base parameters.

The electrolyte diet treatments, however, did not influence exsanguination blood parameters. The higher chloride and sodium in the blood of pigs fed the High dEB diets can potentially be explained by looking at the baseline values for those parameters (Table 6.3). Prior to initiating the dietary treatments, the group of pigs to receive the High dEB treatment had a higher blood chloride ($P = 0.05$) and trend for higher sodium ($P = 0.08$) than the group of pigs receiving the Low dEB diet, despite randomized allocation to treatments. Since these differences existed pre-treatment, it is not surprising to see higher blood chloride ($P = 0.02$) and trend for higher sodium ($P = 0.07$) in the high dEB treatment at slaughter.

Effect of dietary electrolyte balance on growth performance. During the 3 day treatment period, live weight gain for all pigs was approximately 2 kg. Average daily

gain, average daily feed intake, and gain/feed are shown in Table 6.5. Due to the short feeding period, measured growth performance parameters on individual animals showed substantial variation. There was no significant effect of dEB on growth performance. Numerically, however, the growth performance of the pigs consuming the High dEB diet was below that of the pigs consuming the Low dEB diet. This may have been the result of a lower than expected Paylean assay in the high dEB diet.

One study exploring sodium bicarbonate supplementation (1-3% sodium bicarbonate; 238-399 meq/kg of feed) in swine feed indicated that as the amount of sodium bicarbonate in the feed increased, average daily gain decreased ($P = 0.005$) and average daily feed intake tended to decrease ($P = 0.12$) (Wondra et al., 1995). A similar trend was found in a study in which nursery pigs were fed a diet with a high dEB (Austic et al., 1982). A study looking at growth performance of pigs in the growing phase fed various dEB treatments (-85 to 341 meq/kg of feed) indicated that dEB and average daily gain and feed intake were related quadratically; as dEB increased, ADG and ADFI increased up to approximately 250 meq/kg at which point performance values began to plateau with an indication of a potential decrease at higher dEB levels (Patience et al., 1987).

One large difference between the current study and the others studies is the length of time the experimental animals were fed the dietary treatments, 3 days versus 28 in the Patience et al. (1987) study and an average of 66 days in the Wondra et al. (1995) study. Therefore, if a high dEB diet was fed for too long, it could be detrimental to animal performance. There were no negative effects on performance characteristics in the current study, perhaps due to the short feeding period.

Effect of dietary electrolyte balance on pars esophageal ulceration. There was no difference in the ulcer scores across diet treatments (Table 6.6). Researchers have indicated that delivery of electrolytes in swine feed does not provide a large enough buffering effect to show marked differences in stomach damage between supplemented and non-supplemented animals, due to the normal increase in stomach pH occurring after feeding bouts (Gamble et al., 1967; Wondra et al., 1995). One study did indicate a tendency ($P < 0.10$) for pigs fed a diet supplemented with sodium bicarbonate to decrease the incidence of stomach ulcers in swine (Wondra et al., 1995). Researchers have found that oral bicarbonate administered in water is more effective in reducing the incidence of pars esophageal ulcers in swine (Ange et al., 2000). They found that pigs provided with buffer-supplemented water had greater gastric pHs during the course of the experiment, suggesting less chance of gastric epithelial cell damage. No effect was seen in this study perhaps due to rapid diminution of the electrolyte effect during feed withdrawal.

Effect of dietary electrolyte balance on meat quality. Carcass data were collected on all animals at the termination of the trial. The measurements are reported in Table 6.6. The results indicated that dietary electrolyte balance in this study had little effect on meat quality parameters. Previous studies have explored the effects of electrolyte supplementation in the water on meat quality in swine (Ahn et al., 1992; Boles et al., 1994). The Ahn et al. (1992) study indicated improvements in 45 min pH in animals treated with the high electrolyte supplementation but there were no significant improvements in other meat quality factors such as color (*a, b, L*) and water holding capacity. Despite the lack of differences between supplemented groups and controls, the results of enzyme assays indicated that carcasses of pigs receiving bicarbonate may have

had a slower rate of post-mortem glycolyis, providing some indication that an increase in dEB could improve meat quality. The Boles et al. (1994) study found no significant differences in meat quality between control animals and the high electrolyte-supplemented group. It is important to indicate that all experimental animals in this study were halothane positive and in general, there was a greater incidence of PSE carcasses, perhaps affecting the meat quality results. The lack of effect in this current study may have been due again to the diminution of the effect during feed withdrawal or also a lower than expected difference in dEB between treatments (250 vs. 400 meq/kg).

The only parameter that was affected by treatment diets was glycolytic potential (Low dEB = 101.2 umol/g muscle, High dEB = 105.0 umol/g muscle; $P = 0.05$). The effect of dEB on glycolytic potential was unexpected and would be useful to follow in subsequent experiments. Perhaps the lower reduction in glycogen content in the High dEB pigs suggested that this diet may reduce some of the physiological responses of the animals to marketing stress.

Meat classification also numerically indicated a higher proportion of meat classified as Normal in the High dEB treatment and a higher proportion of meat classified as PFN in the Low dEB treatment (Table 7). These differences were not significant when data was analyzed with pen as the experimental unit. However, when analyzed with individual animal as the experimental unit the effect on PFN was significant ($P = 0.05$) (data not shown). It will be interesting in subsequent experiments to evaluate if High dEB diets are able to improve meat classification.

IMPLICATIONS

This study demonstrated that feeding a high electrolyte diet (375 meq/kg) for three days changed the acid-base status of pigs determined by blood parameters compared to pigs fed a low electrolyte diet (121 meq/kg). However, the blood changes associated with the electrolyte supplementation were not maintained following a 10 h feed withdrawal. It is therefore likely that any beneficial effect of feeding a high dEB diet on the pig's ability to withstand any increased metabolic acidosis associated with the stress of transport was lost following feed withdrawal. In the swine industry, some animals are shipped a substantial distance to slaughter and therefore the diet would need to maintain blood changes for a considerable period of time. The next step will be to determine if a larger dEB difference would achieve and maintain adequate buffering capacity for the pigs during the entire marketing process. If the beneficial effect of high dEB on transport losses is confirmed under commercial conditions, the most practical method of electrolyte delivery will be in water. The effects of a high electrolyte diet on growth performance seem negligible when fed for a three day period. Meat quality was not affected in this study but there was a tendency for the high dEB diet to have higher glycolytic potential and a lower incidence of PFN pork. Further research is necessary to understand the full impact alteration of dEB for three days prior to slaughter has on meat quality.

CHAPTER SEVEN

THE RELATIONSHIP BETWEEN EXSANGUINATION BLOOD LACTATE CONCENTRATION AND CARCASS QUALITY IN COMMERICIAL PIGS

ABSTRACT

One hundred twenty eight cross-bred barrows were used to determine the relationship between exsanguination blood lactate concentration ([LAC]) and carcass quality following standard commercial handling transportation and lairage conditions. Researcher-imposed stress treatments were not utilized in this study. The day prior to slaughter (approximately 20 hrs prior to exsanguination), feed was withdrawn from the experimental animals. After ten hours of feed withdrawal, pigs were loaded on a transport truck at the farm using low stress loading procedures and transported approximately 1 hr to a slaughter plant. At the plant, pigs were rested for eight hours after which time they were stunned with carbon dioxide and exsanguinated. A sample of exsanguination blood was taken and [LAC] was measured. The carcasses entered normal post-mortem processing and standard meat quality measurements were obtained. Pearson correlations were used to determine the relationships between [LAC] and the meat quality parameters. Exsanguination blood lactate concentration had a range of 4 to 19.7 mM and was negatively correlated to 60 min pH ($r = -0.32$, $P = 0.0004$) and positively correlated ($r = 0.21$, $P = 0.02$) to drip loss. These results suggest that exsanguination [LAC] is

predictive of the rate of early post-mortem metabolism, i.e. high [LAC] predicts a more rapid drop in early post-mortem pH resulting in greater drip loss. This study confirms results from previously published literature indicating that high [LAC] at slaughter is detrimental to ultimate meat quality. This research supports the development of an on-line system measuring exsanguination blood [LAC] to monitor and improve meat quality.

KEY WORDS: blood lactate, pork quality, slaughter, swine

INTRODUCTION

Increases in blood lactate concentration ([LAC]) associated with pre-slaughter stresses such as aggressive handling immediately prior to stunning have been shown to have detrimental effects on pork quality (Hambrecht et al., 2005a; Hambrecht et al., 2005b; Hambrecht et al., 2004b; Warriss et al., 1994). Hambrecht et al. (2004) determined that swine exposed to aggressive handling just prior to stunning had a higher [LAC] at slaughter and exhibited pork with higher drip loss. Although [LAC] was partially predictive of this decrease in meat quality, the effect of high [LAC] was compounded by high muscle glycolytic potential indicating the complexity of determining ultimate pork quality. Warriss (1994) has demonstrated that pork from pigs stressed immediately before slaughter had less acceptable eating quality than that from pigs handled carefully, despite there being no difference in the predictors of quality that were measured. Warriss et al. (1994) were able to demonstrate a correlation between the subjective assessment of stress level and the objective measures of stress and meat quality in a survey study of swine slaughter plants. High stress was associated with high exsanguination [LAC] and lower meat quality, i.e. decreased water holding capacity and lighter color. Another study did not find as strong a relationship between exsanguination

[LAC] and meat quality but did identify that there was a significant relationship between the stress of loading prior to transport and meat quality perhaps counter-acting any effects of pre-slaughter stress (Edwards et al., 2009c).

The objective of this study was to confirm at the relationship between exsanguination [LAC] and meat quality in commercial pigs with standard loading and transport procedures and without an imposed stress treatment.

MATERIALS AND METHODS

Prior to the initiation of these experiments, all animal use, handling, and sampling techniques described herein were approved by the Colorado State University Animal Care and Use Committee.

Animals, Housing and Feeding. Sixteen pens of eight (128) cross-bred barrows were used in this study. The pigs used in the trial were a cross between a Topigs Tempo boar and a Topigs C40 sow (Topigs International, Netherlands). All animals used in the study originated from the same farrowing unit. Barrows were housed in a finishing facility typical of the ones used in commercial practice in the local area (Quebec, Canada). Pens of test pigs were selected based on the following criteria: structurally sound, healthy and subjective average live weight. Animals in each test pen were identified by a unique numbered plastic ear tag. On the day before slaughter, a unique carcass tattoo number was applied to each pig as well to facilitate identification at the packing plant. Each pen was 8 m^2 with 8 test pigs in each pen (stocking density = 1 m^2 per pig). There was one feeder and one waterer per pen. Pigs were provided with *ad libitum* feed and water. The pigs used in this study were part of a feeding trial exploring the persistence of blood changes associated with electrolyte-supplemented diets. The two diets fed in the feeding

trial were either a Low (121 meq/kg) or High (375 meq/kg) dEB (dietary electrolyte balance) diet (n = 8). Both diets were formulated with 10 ppm Paylean® (Assayed concentration 6.8 to 9.8 ppm). These diets were fed for three days prior to slaughter. For details on the feeding trial, refer to Edwards et al. (2009).

Feed withdrawal, Transport and Lairage. Approximately twenty hours prior to the estimated slaughter time, feed was withdrawn from all test pens. Pigs remained in the barn without feed for 10 hours at which time they were loaded onto the trailer. Loading was low stress; the pigs were moved from the barn to the truck on a flat angle loading chute (width = 0.89 m; length = 3.35 m). The trailer was a straight deck with two decks. The stocking density was approximately 0.45 m^2 per pig. There were no internal ramps within the trailer between decks; a hydraulic lift raised the floor of the trailer to create the second deck. After loading, pigs were transported approximately 1 hour to the packing facility. They were rested at the slaughter plant for eight hours. Following lairage, they were stunned with carbon dioxide, exsanguinated and entered normal post-mortem processing.

Blood Sampling. An exsanguination blood sample was collected from each pig in a collection vessel (100 ml plastic cup) following carbon dioxide stunning and exsanguination. The blood was transferred to potassium oxalate sodium fluoride tubes to inhibit further glycolytic metabolism (Catalog #: 02-688-48, Fisher Scientific, Pittsburgh, PA). After collection, lactate was measured in blood samples using a hand held lactate analyzer (Lactate Scout, EKF Diagnostic, Magdeburg, Germany).

Carcass measurements. The following measures were recorded on all test pigs by the slaughter facility: hot carcass weight, dressing percent (HWT), cold carcass weight,

dressing percent (CWT) and percent lean. Percent lean was estimated using backfat and lean depth at the third/fourth last rib in the loin estimated with a Destron probe. Cold carcass weight of each pig was recorded approximately 22 hrs after slaughter, then the head was removed and the carcass was split into two sides. The right side of the carcass was used for meat quality evaluation.

Meat quality measurements. Muscle temperature and pH were taken at the third/fourth last rib at 60 min post-mortem approximately 20 min after carcasses were moved into the chill cooler. Muscle pH and temperature were measured with a pH meter (Oakton Instruments Model pH 100 Series, Nilis, IL) with a spear type electrode (Cole Parmer Instrument Company, Vernon Hills, IL) and an automatic temperature compensation probe. At approximately 22 hours post-mortem, chilled carcasses were transported to a carcass processing facility and fabricated into wholesale cuts. The loin from each test carcasss was removed from the fabrication line and a 2 cm loin chop was taken at the third/fourth last rib from each carcass for the following measurements: 24 hr pH and temperature , subjective muscle color (Japanese Color Standards; 1 = pale to 6 = dark color) (Nakai et al., 1975), objective color (Minolta L*, a*, b* values), 48 hr drip loss, muscle firmness (1 = firm, 3 = soft) and marbling (1 = devoid, 10 = abundant) (NPPC, 2000a). Minolta color was measured with a Minolta Chromameter CR 300 (Minolta Canada, Missisanga, Ontario, Canada) with a D65 light source with 0^{o} viewing angle geometry. Drip loss was determined using a modified "juice container" protocol (Christensen, 2003). Drip loss was conducted in triplicate using three muscle cores taken with a 25 mm diameter cork borer. Each core was weighed and placed into a plastic container (Christensen Aps Industrivaengetand, Hilleroed, Denmark). The containers

were stored at 4ºC and after forty-eight hours the samples were removed, carefully dabbed and weighed. Drip loss was calculated by determing the difference between the initial and final weight of each muscle core. A final drip loss value was determined by calculating the average drip loss of all three cores.

The remainder of the chops were packaged and frozen at -80ºC for glycolytic potential determination. Glycolytic potential was calculated using the following equation (Monin and Sellier, 1985): lactate concentration + 2(glucose + glucose-6-phosphate + glycogen). The extraction protocol of (Bergmeyer, 1974) was followed. Briefly, samples were homogenized in buffer with amyloglucosidase (Catalog #: A1602, Sigma Aldrich, St. Louis, MO) to degrade glycogen to glucose. A lactate kit (Catalog #: K607-100, Biovision, Inc., Mountain View, CA) was used to determine lactate concentration in the homogenized samples and a glucose kit (Catalog #: 10009582, Cayman Chemical Company, Detroit, MI) was used to determine glucose concentration (glycogen plus free glucose). The amount of glucose-6-phosphate in the muscle homogenates was negligible and therefore was not included in the analysis. The samples were analyzed on a BioTEK Synergy II plate reader (BioTek Instruments Incorporated, Winooksi, VT).

Statistical Analysis. Pearson correlations were performed to determine relationships between exsanguination blood lactate concentration and all carcass and meat quality measurements. Individual animal was the experimental unit.

RESULTS AND DISCUSSION

Tables 7.1 and 7.2 show the characterization of the population of pigs that were used in this experiment. This population is typical of commercial swine raised in North America. Pearson correlations between meat quality and carcass characteristics and

exsanguination blood lactate concentration are shown in Table 7.3. There were several inter-relationships between meat quality measurements that were expected providing confidence in the validity of the carcass data. For example, drip loss was positively correlated with L* (r = 0.46, *P* = 0.0001) and glycolytic potential (r = 0.34, *P* = 0.001) and negatively correlated with Japanese color score (r = -0.37, *P* = 0.001), 60 min pH (r = -0.43, *P* = 0.0001) and 24 hr pH (r = -0.37, *P* =0.0001). In addition, 24 hr pH was highly correlated with L*, a*, b*, muscle color and drip loss (P<0.001) and glycolytic potential was highly correlated with L*, b*, muscle color, drip loss and 24 hr pH (P<0.001). An inter-correlation that was not expected was the positive correlation between 60 min pH and 60 min temperature (*P* = 0.03). A more rapid drop in early post-mortem pH would be expected to be accompanied by increased temperature. This temperature measurement was taken approximately 20 minutes after carcasses had entered the cooler. Carcasses could be cooling at different rates dependent on location within the cooler, perhaps partially responsible for the unexpected correlation.

Pre-slaughter stress has been previously reported to be related to decreases in pork quality (Grandin, 1980, 1986; Hambrecht et al., 2004a; Hambrecht et al., 2005a; Hambrecht et al., 2004b; Warriss, 1994; Warriss et al., 1994) making an animal's experience immediately prior to slaughter important not only for maintaining high levels of animal welfare but also for maintaining meat quality. In this current study, a relationship between blood lactate concentration at slaughter, an indicator of stress in pigs, and certain meat quality characteristics was identified. Exsanguination blood lactate concentration was positively correlated (r = 0.21, *P* = 0.02) to drip loss and negatively correlated to 60 min pH (r = -0.32, *P* = 0.0004). These results suggest that

exsanguination blood lactate is predictive of the rate of post-mortem metabolism, i.e. high blood lactate predicts a rapid drop in early post-mortem pH resulting in greater drip loss. Figures 7.1 shows the direct relationship between blood lactate and 60 min pH. Figure 7.2 demonstrates that drip loss at a give blood lactate concentration is greater as glycolytic potential increases (> 100 umol/g of muscle tissue), consistent with the work of Hambrecht and co-workers (2004b) When the animals enter the post-mortem processing at a higher metabolic rate which can be caused by pre-slaughter stress, their meat quality may be compromised. The relationship between drip loss and [LAC] has been reported in previously published literature (Hambrecht et al., 2005a). Additionally, Hambrecht et al. (2004b) determined that high stress can cause decreased 30 min pH. If blood lactate concentrations could be kept at a minimum through very gentle handling just prior to stunning and exsanguination, this would predict a slow metabolic rate early post-mortem, slowing the drop in early post-mortem pH, resulting in improved meat quality.

Previous research has demonstrated that animals exposed to high pre-slaughter stress have lower glycolytic potential (Hambrecht et al., 2005b). The animals use their energy stores thus reducing the glycolytic potential of the muscles. Another study has demonstrated that the use of electric prod, a form of handling stress, prior to slaughter also decreases glycogen (D'Souza et al., 1998). Although not significant, the results of this study indicate a slight trend towards a negative correlation between [LAC] and glycolytic potential ($r = -0.14$, $P = 0.15$). However, exsanguination [LAC] was significantly correlated to the lactate content in the muscle ($r = -0.19$, $P = 0.05$). This result is contradictory to that found in Hambrecht et al. (2005b) in which it was shown

that highly stressed pigs had higher muscle lactate content and lower muscle glycogen content. In the Hambrecht study however, the results were also dependent on muscle type, a factor not explored in the current study. When pigs are stressed they produce more lactate in the muscle and the body compensates by increasing lactate clearance causing an increase in blood lactate concentration. Dependent on how these samples were handled, the reaction to stress could be expressed both by an increase in muscle lactate content or a decrease. Several studies by Hambrecht et al. (2004b, 2005a) have demonstrated that both [LAC] and glycolytic potential help explain a large portion of the variability in drip loss and L* emphasizing the importance of the relationship between the [LAC], glycolytic potential and ultimate meat quality.

Results indicate a positive correlation between exsanguination [LAC] and hot carcass dressing percentage ($r = 0.25$, $P = 0.01$) and a trend towards a positive correlation between percentage lean on the carcass and exsanguination [LAC] ($r = 0.18$, $P = 0.06$). This relationship was not expected but could be related to a greater susceptibility of leaner pigs to handling stress (Grandin, 1993). More heavily muscled pigs, indicated by the higher lean percentage could respond more significantly to pre-slaughter stress resulting in increased blood lactate concentrations at slaughter. Handling stresses were not documented at an individual animal level in this study. Further research is needed to individually quantify the response to pre-slaughter stress relate that back to the relative level of muscling.

IMPLICATIONS

This study was able to confirm previous research relating pre-slaughter stress and thus high blood lactate concentrations with an increased rate of early post-mortem

metabolism and decreased pork quality. Unlike previous reports, this study confirms this relationship using standard transportation, lairage and handling procedures. Blood lactate concentration has been shown to be an indicator of handling stress in swine (Edwards, et al. 2009b). Not only can [LAC] be used to judge the adequacy of animal handling systems in minimizing stress, it can also be used to monitor pork quality. By minimizing the stress that pigs experience immediately prior to slaughter the industry may be able to improve pork quality in addition to improving animal welfare. The results of this study support the development of an on-line system measuring exsanguination blood lactate concentration to monitor and improve meat quality.

CHAPTER EIGHT

SUMMARY

These studies reported the impact that pre-slaughter management has on physiological parameters of swine throughout the marketing process. Blood lactate concentration and rectal temperature change throughout the marketing process, increasing substantially during times of intense handling, i.e. loading and pre-slaughter handling, and returned to baseline levels during periods of rest, i.e. transport and lairage. Handling during loading and just prior to exsanguination resulted in the largest increases in blood lactate concentration and thus should be the focus of efforts to improve animal welfare and handling during the marketing process. These studies provided evidence that management factors such as lairage and distance moved to the stunner are effecting blood lactate concentrations in an unexpected manner warranting more focus on this area of research.

One of the areas of lactate increase, loading, had an impact on meat quality. There are two studies in this dissertation that demonstrate conflicting results on the effect of exsanguination lactate on meat quality. One of the papers indicated that exsanguination lactate did not have the same relationship to meat quality that has been previously documented in published studies. A strong relationship between blood lactate concentration at loading and meat quality was found in this study. With a substantial increase in blood lactate concentration early during the marketing process and the

resultant increase in 24 h muscle pH, the effects of exsanguination blood lactate concentration on meat quality were not seen. As handling improves at the barn, the importance of maintaining low blood lactate concentration at exsanguination through careful animal handling will very likely have a greater impact on meat quality. The second study did indicate that high blood lactate at exsanguination had a negative impact on meat quality. In this study the animals were stunned using a carbon dioxide stunning system utilizing a group handling system to move pigs into the stunning compartment so the pre-stun handling was calm and careful as in the first study. One significant difference between the studies was the manner in which the pigs were handled during loading. In this second study, pigs were loaded onto a truck but did not have to climb and ramps; the upper deck of the trailer was reached with a hydraulic lift. In the first study the pigs were moved up a ramp onto the upper deck of the truck. The difference in loading, could have had an impact on the role that exsanguination blood lactate played in determining ultimate meat quality.

Additionally, this research was able to identify specific behaviors and handling events that added to the increase in blood lactate concentration during pre-stun handling. By being able to identify certain aspects of animal behavior and pre-slaughter handling that affect blood lactate concentration, we can improve our animal handling and training protocols to minimize the occurrence of these handling events. This research provides evidence that blood lactate concentration can be used as a quantitative tool to improve animal handling and enhance commercial packing plant auditing systems.

Electrolyte supplementation was also explored as a potential technique to reduce the incidence of fatigued animals during the marketing process. This pilot study

determined that the persistence of blood changes associated with feeding a high electrolyte diet for three days prior to slaughter was not maintained after a 10 h feed withdrawal. Due to the wide range of feed withdrawal protocols and transport distances used in the swine industry, it is necessary to further explore this technique looking at ways to extend the buffering effects of the electrolytes.

These experiments indicate that blood lactate concentration does respond to alterations in pre-slaughter swine management and may be used as a tool to help develop effect systems of pre-slaughter management practices. By understanding how an animal is physiologically responding to movement, the environment and handling in the pre-slaughter area, there is an opportunity to manipulate the current system to better complement the animals' physiological system thus improving animal welfare.

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Table 3.1. Approximate timeline of events followed for each slaughter day during both Exp. 1 & 2. This table illustrates the staggered arrival of trucks at the packing facility to carry out the plant protocols.

Time	1st Truck	2nd Truck
Day prior to slaughter (at farm)		
1500 h	Animal selection, identification and baseline measurement (Sampling Point 1)	
1700 h		
1900 h		Animal selection, identification and baseline measurement (Sampling Point 1)
Slaughter day		
0030 h	Begin loading	
0100 h	Sample on truck (Sampling Point 2)	
0230 h		Begin loading
0300 h	Truck departs farm	Sample on truck (Sampling Point 2)
0400 h		Truck departs farm
0530 h	Arrive at plant	
0600 h	Sample and unload pigs (Sampling Point 3)	
0630 h	Start lairage (Sampling Point 4)	Arrive at plant
0700 h	Sample and terminate short lairage (Sampling Point 5)	Sample and unload pigs (Sampling Point 3)
	Move animals to crowd pen (Sampling Point 6)	Start lairage (Sampling Point 4)
	Exsanguination (Sampling Point 7)	Sample and terminate short lairage (Sampling Point 5)
		Move animals to crowd pen (Sampling Point 6)
		Exsanguination (Sampling Point 7)
1200 h	Sample and terminate long lairage (Sampling Point 5)	
	Move animals to crowd pen (Sampling Point 6)	
	Exsanguination (Sampling Point 7)	
1300 h		Sample and terminate long lairage (Sampling Point 5)
		Move animals to crowd pen (Sampling Point 6)
		Exsanguination (Sampling Point 7)

Table 3.2. Experiment 1. Least Square Means (LSM) of blood lactate concentration ([LAC], mM) for the main effects of DIS, REST and STUN at all sampling points (1 = baseline, 2 = post-unload, 3 = pre-unload, 4 = post-unload, 5 = post lairage, 6 = post movement, 7 = exsanguination). Animals that were not able to complete the treatments are not included in this analysis. Within a column, the value at each sampling point represents the LSM of 27-32 animals.

	DIS[1]		**REST**[2]		**STUN**[3]		**TIME**	Day				P <	
Sampling Point	Short	Long	Short	Long	Short	Long		1	2	SEM	N	Day* Time	STUN* Time
[LAC]													
1	3.8	3.5	3.6	3.6	3.6	3.6	3.6	3.4	3.9	0.4	64	0.31	0.88
2	4.8	4.7	4.7	4.8	4.7	4.8	4.7	4.9	4.6	0.4	63	0.52	0.85
3	3.8	3.5	3.4	3.9	3.5	3.7	3.6	**5.5**	**2.4**	0.3	63	**0.001**	0.72
4	2.6	2.4	2.5	2.5	2.3	2.7	2.5	2.7	2.3	0.3	60	0.24	0.21
5	1.9	2.3	2.0	2.1	2.0	2.2	2.1	2.0	2.1	0.1	59	0.77	0.39
6	2.6	2.4	2.4	2.6	2.4	2.6	2.5	2.3	2.6	0.2	58	0.31	0.52
7	6.0	7.1	6.2	6.9	**7.6**	**5.8**	6.6	7.1	6.2	0.4	56	0.27	**0.04**

[1]Distance moved at the farm, Short = approx. 15 m, Long = approx. 46 m
[2]Length of lairage at the plant, Short = approx. 30 min, Long = approx. 4.5 h
[3]Distance moved to the stunner, Short = approx. 20 m, Long = approx. 300 m

Table 3.3. Experiment 2. Least Square Means (LSM) of blood lactate concentration ([LAC], mM) for the main effects of DIS, REST and STUN for each sampling point (1=baseline, 2 = post-load, 3=pre-unload, 4=post-unload, 5=post lairage, 6=post movement, 7=exsanguination). Within a column, the value at each sampling point represents the LSM of 69-72 animals.

	DIS[1]		**REST**[2]		**STUN**[3]		**TIME**			P <		
Sampling Point	Short	Long	Short	Long	Short	Long		SEM	N	DIS*Time	REST*Time	STUN*Time
[LAC]												
1	3.7	3.3	3.5	3.6	3.4	3.6	3.5	0.2	141	0.16	0.55	0.46
2	**6.0**	**8.3**	7.1	7.2	6.9	7.2	7.1	0.4	144	**0.0001**	0.77	0.43
3	2.8	3.1	**2.7**	**3.2**	2.9	3.0	3.0	0.2	142	0.28	**0.05**	0.72
4	**3.9**	**4.8**	4.5	4.3	4.5	4.3	4.4	0.2	144	**0.01**	0.62	0.53
5	3.4	3.2	**3.0**	**3.6**	3.2	3.4	3.3	0.2	144	0.71	**0.02**	0.74
6	4.4	4.2	**3.8**	**4.7**	4.4	4.1	4.3	0.2	142	0.67	**0.01**	0.40
7	6.3	7.1	**5.5**	**8.1**	**7.2**	**6.0**	6.6	0.3	142	0.19	**0.0001**	**0.02**

[1]Distance moved at the farm, Short = approx. 15 m, Long = approx. 46 m
[2]Length of lairage at the plant, Short = approx. 30 min, Long = approx. 5 h
[3]Distance moved to the stunner, Short = approx. 20 m, Long = approx. 300 m

Table 3.4. Experiment 1. Least Square Means (LSM) of changes in blood lactate concentration between sampling points (2-1=loading, 3-2 = transport, 4-3 = unloading, 5-4 = lairage, 6-5 = movement to stun, 7-6 = preslaughter handling) associated with main effects of DIS, REST and STUN. Analysis does not include animals that did not complete the treatments. Within a column, the value at each sampling point represents the LSM of 27-32 animals.

	Treatments								*P*<							
	DIS[1]		REST[2]		STUN[3]		Day									
	Short	Long	Short	Long	Short	Long	1	2	DIS	REST	STUN	STUN* DIS	STUN* REST	DIS* REST	REST* STUN* DIS	Day
Loading	1.8	1.8	-	-	-	-	-	-	0.98	-	-	-	-	-	-	-
Transport	-1.4	-1.9	-	-	-	-	**0.4**	**-3.6**	0.61	-	-	-	-	-	-	**0.0001**
Unloading	-1.0	-1.5	-	-	-	-	**-2.7**	**0.1**	0.36	-	-	-	-	-	-	**0.0001**
Lairage	-0.9	-0.3	-0.5	-0.6	-	-	-	-	0.23	0.84	-	-	-	-	-	-
Movement to Stun	0.6	0.4	0.5	0.5	0.4	0.6	-	-	0.72	0.95	0.72	-	-	-	-	-
Pre-slaughter Handling	4.3	4.8	**3.6**	**5.6**	**5.4**	**3.7**	-	-	0.46	**0.003**	**0.01**	0.59	0.14	0.53	0.06	-

[1]Distance moved at the farm, Short = approx. 15 m, Long = approx. 46 m
[2]Length of lairage at the plant, Short = approx. 30 min, Long = approx .4.5 h
[3]Distance moved to the stunner, Short = approx. 20 m, Long = approx. 300 m

Table 3.5. Experiment 2. Least Square Means (LSM) of changes in blood lactate concentration between time points (2-1=loading, 3-2 = transport, 4-3 = unloading, 5-4 = lairage, 6-5 = movement to stun, 7-6 = pre-slaughter handling) associated with main effects of DIS REST and STUN. Analysis does not include animals that did not complete the treatments. Within a column, the value at each sampling point represents the LSM of 68-72 animals for treatment effects and 46-48 animals for day effects.

	Treatments											*P*<						
	DIS[1]		REST[2]		STUN[3]		Day			Gender								
	Short	Long	Short	Long	Short	Long	1	2	3	Barrow	Gilt	DIS	REST	STUN	STUN* REST	DIS* REST	Day	Gender
Loading	**2.8**	**5.5**	-	-	-	-	3.2	5.4	3.8	-	-	**0.0002**	-	-	-	-	0.05	-
Transport	**-2.9**	**-5.1**	-	-	-	-	-	-	-	**-5.1**	**-2.8**	**0.002**	-	-	-	-	-	**0.01**
Unloading	1.5	1.7	-	-	-	-	-	-	-	-	-	0.78	-	-	-	-	-	-
Lairage	-1.0	-1.4	**-2.1**	**-0.4**	-	-	-0.6	-2.1	-1.0	-	-	0.41	**0.001**	-	-	-	0.04	-
Movement to Stun	0.6	0.5	0.4	0.7	0.8	0.4	-	-	-	**1.4**	**-0.3**	0.88	0.46	0.43	0.005	0.03	-	**0.02**
Pre-slaughter Handling	2.8	3.8	**2.6**	**4.3**	**3.9**	**2.7**	-	-	-	**4.2**	**2.4**	0.12	**0.001**	**0.05**	0.03	-	-	**0.01**

[1]Distance moved at the farm, Short = approx. 15 m, Long = approx. 46 m
[2]Length of lairage at the plant, Short = approx. 30 min, Long = approx. 4.5 h
[3]Distance moved to the stunner, Short = approx. 20 m, Long = approx. 300 m

Table 3.6. Experiment 1. Least Square Means (LSM) of rectal temperature (TEMP, C) for the main effects of DIS, REST and STUN at all sampling points (1 = baseline, 2 = post-unload, 3 = pre-unload, 4 = post-unload, 5 = post lairage, 6 = post movement, 7 = exsanguination). Animals that were not able to complete the treatments are not included in this analysis. Within a column, the value at each sampling point represents the LSM of 27-32 animals for treatment effects and 56-64 animals for time effects.

	DIS[1]		**REST**[2]		**STUN**[3]		**TIME**			P <
Sampling Point	Short	Long	Short	Long	Short	Long		SEM	N	STUN*Time
TEMP										
1	39.4	39.5	39.4	39.4	39.4	39.4	39.4	0.1	64	0.75
2	39.9	39.7	39.7	39.9	39.8	39.8	39.8	0.1	63	0.68
3	39.4	39.3	39.3	39.5	39.4	39.3	39.3	0.1	63	0.20
4	39.9	39.8	39.8	39.9	39.9	39.8	39.8	0.1	59	0.31
5	39.6	39.5	39.5	39.6	39.5	39.6	39.6	0.1	59	0.36
6	40.0	39.9	39.9	40.0	**39.8**	**40.1**	39.9	0.1	58	**0.002**
7	39.8	39.7	39.7	39.8	**39.6**	**39.8**	39.7	0.1	55	**0.02**

[1]Distance moved at the farm, Short = approx. 15 m, Long = approx. 46 m
[2]Length of lairage at the plant, Short = approx. 30 min, Long = approx. 4.5 h
[3]Distance moved to the stunner, Short = approx. 20 m, Long = approx. 300 m

Table 3.7. Experiment 2. Least Square Means (LSM) of rectal temperature (TEMP, C) for the main effects of DIS, REST and STUN for each sampling point (1=baseline, 2 = post-load, 3=pre-unload, 4=post-unload, 5=post lairage, 6=post movement, 7=exsanguination). Within a column, the value at each sampling point represents the LSM of 68-72 animals for treatment effects and 140-144 animals for time effects.

	DIS[1]		**REST**[2]		**STUN**[3]		**TIME**			P <	
Sampling Point	Short	Long	Short	Long	Short	Long		SEM	N	REST*Time	STUN*Time
TEMP											
1	40.1	39.9	39.9	40.1	40.1	39.9	40.0	0.10	141	0.28	0.47
2	40.3	40.3	40.3	40.4	40.3	40.4	40.3	0.10	144	0.29	0.65
3	38.4	38.3	38.3	38.4	38.3	38.4	38.4	0.10	142	0.29	0.29
4	39.6	39.6	39.6	39.7	39.7	39.6	39.6	0.10	144	0.67	0.10
5	39.4	39.3	39.4	39.3	39.3	39.4	39.3	0.10	143	0.38	0.35
6	40.0	39.9	40.1	39.9	**39.8**	**40.2**	39.9	0.10	141	0.25	**0.001**
7	40.1	40.1	**40.0**	**40.2**	**39.9**	**40.3**	40.1	0.10	140	**0.02**	**0.001**

[1]Distance moved at the farm, Short = approx. 15 m, Long = approx. 46 m
[2]Length of lairage at the plant, Short = approx. 30 min, Long = approx. 4.5 h
[3]Distance moved to the stunner, Short = approx. 20 m, Long = approx. 300 m

Table 3.8. Experiment 1. Least Square Means (LSM) of changes in rectal temperature (ºC) between time points (2-1=loading, 3-2 = transport, 4-3 = unloading, 5-4 = lairage, 6-5 = movement to stun, 7-6 = preslaughter handling) associated with main effects in Experiment 1. Analysis does not include animals that did not complete the treatments. Within a column, the value at each sampling point represents the LSM of 24-32 animals.

	Treatments										*P*<								
	DIS[1]		REST[2]		STUN[3]		Day		Gender										
	Short	Long	Short	Long	Short	Long	1	2	Barrow	Gilt	DIS	REST	STUN	STUN* DIS	STUN* REST	DIS* REST	REST* STUN* DIS	Day	Gender
Loading	0.5	0.2	-	-	-	-	0.2	0.5	-	-	0.12	-	-	-	-	-	-	0.08	-
Transport	-0.5	-0.4	-	-	-	-	-	-	-	-	0.95	-	-	-	-	-	-	-	-
Unloading	0.5	0.6	-	-	-	-	-	-	**0.7**	**0.4**	0.54	-	-	-	-	-	-	-	**0.04**
Lairage	-0.3	-0.3	-0.3	-0.3	-	-	**-0.2**	**-0.4**	-	-	0.97	0.97	-	-	-	-	-	**0.03**	-
Movement to Stun	0.4	0.4	0.4	0.4	**0.3**	**0.5**	-	-	-	-	0.65	0.75	**0.02**	-	-	-	-	-	-
Pre-slaughter Handling	-0.2	-0.2	-0.2	-0.1	-0.2	-0.2	-0.1	-0.2	-	-	0.63	0.09	0.41	0.46	0.72	0.65	0.05	0.09	-

[1]Distance moved at the farm, Short = approx. 15 m, Long = approx. 46 m
[2]Length of lairage at the plant, Short = approx. 30 min, Long = approx .4.5 h
[3]Distance moved to the stunner, Short = approx. 20 m, Long = approx. 300 m

Table 3.9. Experiment 2. Least Square Means of changes in rectal temperature (°C) between time points (2-1=loading, 3-2 = transport, 4-3 = unloading, 4-5 = lairage, 6-5 = movement to stun, 7-6 = preslaughter handling) associated with main effects in Experiment 2. Analysis does not include animals that did not complete the treatments. Within a column, the value at each sampling point represents the LSM of 65-72 animals for treatment effects and 44-48 animals for day effects.

	Treatments											*P*<						
	DIS[1]		REST[2]		STUN[3]		Day			Gender								
	Short	Long	Short	Long	Short	Long	1	2	3	Barrow(n)	Gilt(n)	DIS	REST	STUN	STUN*REST	DIS* REST	Day	Gender
Loading	0.3	0.4	-	-	-	-	-	-	-	-	-	0.48	-	-	-	-	-	-
Transport	-1.6	-1.8	-	-	-	-	-	-	-	**-2.1(118)**	**-1.3(24)**	0.39	-	-	-	-	-	**0.0001**
Unloading	0.9	0.9	-	-	-	-	0.7	0.9	1.2	**0.5(118)**	**1.4(24)**	0.70	-	-	-	-	0.09	**0.0001**
Lairage	-0.1	-0.2	-0.1	-0.2	-	-	-	-	-	**-0.3(120)**	**-0.1(23)**	0.27	0.11	-	-	0.08	-	**0.0003**
Movement to Stun	0.3	0.4	0.4	0.3	**0.2**	**0.4**	0.5	0.3	0.2	**0.7(120)**	**-0.1(21)**	0.52	0.67	**0.02**	-	-	0.10	**0.0001**
Pre-slaughter Handling	0.3	0.4	**0.2**	**0.6**	0.4	0.4	**0.2**	**0.6**	**0.4**	**-0.01(116)**	**0.8(21)**	0.32	**0.0001**	0.83	0.05	-	**0.0002**	**0.0001**

[1]Distance moved at the farm, Short = approx. 15 m, Long = approx. 46 m
[2]Length of lairage at the plant, Short = approx. 30 min, Long = approx. 4.5 h
[3]Distance moved to the stunner, Short = approx. 20 m, Long = approx. 300 m

Table 4.1. Definitions of behavior and handling parameters

Behavioral/Handling Parameter	Definition
Electric prod use	An electric prod touches any portion of the animal's body
Vocalization with prod use	Animal vocalizes as the electric prod makes contact with its body
Falling	Animal loses an upright position in which part of the body (other than the limbs) touches the ground.
Jamming	Any action that involves two or more animals attempting to enter the same space
Rearing	Any action in which both of the animal's front limbs lose contact with the ground
Turning back	Animal turns back towards the direction it was coming from
Backing up	Animal takes at least two steps in the backwards direction
Rooting	Animal uses its snout to push underneath the hind quarters of the animal in front of it
Being rooted	Animal's hindquarters are lifted and pushed by the snout of the animal behind it
Duration of time	The number of seconds that the animal remains in the area designated by the entrance to the crowd pen and the entrance to the V-restrainer
Vocalization	A score of 1 indicates that the animal did not vocalize during movement through the handling area. A score of 2 indicates that the animal did vocalize during handling, not necessarily in response to an electric prod.

Table 4.2. Experiment 1. Minimum and maximum values for instances of listed handling and behavior parameters and Pearson correlations between exsanguination blood lactate concentration and rectal temperatures.

Behavior/Handling Parameter	Minimum Value (# of instances /pig)	Maximum Value (# of instances /pig)	Blood Lactate Concentration		Rectal Temperature	
			r-value	p-value	r-value	p-value
Electric Prod Use	0	12	0.12	0.30	0.10	0.41
Vocalization with Prod	0	3	0.07	0.56	-0.11	0.33
Jamming	0	1	0.23	0.05	0.18	0.12
Backing up	0	4	0.27	0.02	-0.18	0.12
Turning back	0	3	-0.05	0.66	-0.14	0.22
Rearing	0	3	0.25	0.03	0.08	0.50
Rooting	0	1	0.01	0.94	-0.12	0.28
Being Rooted	0	2	0.08	0.51	0.02	0.87
Duration (s)	11	377	-0.03	0.80	-0.26	0.03

Table 4.3.Experiment 1. Single degree of freedom contrast significance and means of animals experiencing (+) listed handling parameters and those not experiencing (-) the listed handling parameters.

Contrast Groups	**Blood Lactate Concentration (mM)** (Mean ± SE)	n	**Rectal Temperature (F)** (Mean ± SE)	n	**Contrast** **Lactate p-value**	**Temperature p-value**
Electric Prod Use						
+	7.3 ± 0.55	35	103.5 ± 0.15	34	0.96	0.64
-	7.4 ± 0.51	41	103.6 ± 0.13	41		
Jamming						
+	8.5 ± 0.68	22	103.8 ± 0.18	22	0.05	0.12
-	6.9 ± 0.43	54	103.5 ± 0.12	53		
Rearing						
+	8.7 ± 0.89	13	103.6 ± 0.24	13	0.11	0.93
-	7.1 ± 0.40	63	103.6 ±0.12	62		
Back up						
+	9.0 ± 0.76	17	103.3 ± 0.21	17	0.01	0.17
-	6.9 ± 0.41	59	103.6 ± 0.11	58		
Turn back						
+	7.6 ± 0.77	18	103.4 ± 0.20	18	0.73	0.30
-	7.3 ± 0.43	58	103.6 ± 0.11	57		
Rear, Jam, Backup or Electric Prod						
+	7.8 ±0.43	55	103.6 ±0.12	54	0.05	0.74
-	6.2 ± 0.69	21	103.5 ±0.19	21		
Rear, Jam or Backup						
+	8.3 ± 0.48	41	103.6 ± 0.13	41	0.005	0.84
-	6.2 ± 0.52	35	103.5 ± 0.15	34		
Rear, Jam						
+	8.2 ± 0.58	30	103.7 ± 0.16	30	0.06	0.30
-	6.8 ± 0.47	46	103.5 ± 0.13	45		
Rooting Behavior						
+	7.3 ± 0.54	36	103.5 ± 0.15	35	0.89	0.50
-	7.4 ± 0.52	40	103.6 ± 0.14	40		
Duration						
>2 min	7.2 ± 0.54	34	103.4 ± 0.14	33	0.69	0.24
<2 min	7.5 ± 0.52	40	103.7 ± 0.14	40		

Table 4.4. Experiment 2. Minimum and maximum values for instances of listed handling and behavior parameters and Pearson correlations between exsanguination blood lactate concentration and rectal temperatures in the entire handling area, the crowd pen and the single-file chute.

Behavior/Handling Parameter	Minimum Value (# of instances /pig)	Maximum Value (# of instances /pig)	Blood Lactate Concentration		Rectal Temperature	
			r-value	p-value	r-value	p-value
ENTIRE HANDLING AREA						
Electric Prod Use	0	8	0.16	0.06	0.12	0.17
Vocalization with Prod	0	6	0.23	0.006	0.07	0.40
Jamming	0	8	0.12	0.16	0.12	0.15
Backing up	0	5	-0.05	0.57	0.08	0.32
Turning back	0	3	0.07	0.42	-0.04	0.61
Rearing	0	3	0.09	0.31	0.02	0.84
Rooting	0	1	0.12	0.14	-0.05	0.55
Being Rooted	0	1	-0.03	0.73	-0.02	0.79
Duration (s)	3	455	-0.06	0.45	0.07	0.45
CROWD PEN						
Electric Prod Use	0	7	0.18	0.04	0.17	0.05
Vocalization with Prod	0	5	0.28	0.0009	0.11	0.20
Jamming	0	7	0.03	0.68	0.13	0.14
Backing up	0	5	-0.04	0.62	0.11	0.19
Turning back	0	3	0.07	0.44	-0.05	0.56
Rearing	0	3	0.01	0.89	0.009	0.91
SINGLE-FILE CHUTE						
Electric Prod Use	0	6	0.07	0.41	0.02	0.84
Vocalization with Prod	0	2	0.07	0.38	0.00	1.00
Jamming	0	2	0.24	0.005	-0.001	0.99
Backing up	0	2	-0.02	0.77	-0.03	0.77
Turning back	0	1	0.04	0.67	0.04	0.67
Rearing	0	2	0.15	0.08	0.02	0.85
Rooting	0	1	0.12	0.14	-0.05	0.55
Being Rooted	0	1	-0.03	0.73	-0.02	0.79

[1]Vocalization was scored as present or absent, not as the number of times per animal.

Table 4.5. Experiment 2. Single degree of freedom contrast significance and means of animals experiencing (+) listed handling parameters and those not experiencing (-) the listed handling parameters in the entire handling area.

Contrast Statement	Blood Lactate Concentration (mM) (Mean ± SEM)	n	Rectal Temperature (F) (Mean ± SEM)	n	Contrast Lactate p-value	Contrast Temperature p-value
ENTIRE HANDLING AREA						
Electric Prod Use						
+	7.9 ± 0.44	71	104.1 ± 0.15	71	0.13	0.97
-	6.9 ± 0.45	69	104.1 ± 0.15	69		
Vocalization w/ Prod						
+	7.9 ± 0.52	52	104.1 ± 0.17	52	0.23	0.65
-	7.1 ± 0.40	88	104.0 ± 0.13	88		
Jamming						
+	7.5 ± 0.32	133	104.1 ± 0.11	133	0.22	0.81
-	5.7 ± 1.41	7	104.0 ± 0.48	7		
Rearing						
+	7.7 ± 0.78	117	104.0 ± 0.26	117	0.72	0.89
-	7.3 ± 0.35	23	104.1 ±0.11	23		
Back up						
+	7.4 ± 0.41	82	104.1 ± 0.14	82	0.87	0.57
-	7.3 ± 0.49	58	104.0 ± 0.16	58		
Turn back						
+	7.9 ± 0.50	57	104.0 ± 0.17	57	0.24	0.73
-	7.1 ± 0.41	83	104.1 ± 0.14	83		
Rear, Jam						
+	7.5 ± 0.32	133	104.1 ± 0.11	133	0.22	0.81
-	5.7 ± 1.4	7	104.0 ± 0.48	7		
Rooting Behavior						
+	8.4 ± 0.73	26	103.9 ± 0.25	26	0.14	0.55
-	7.2 ± 0.35	114	104.1 ± 0.12	114		
Being Rooted						
+	7.3 ± 0.54	49	104.0 ± 0.18	49	0.73	0.79
-	7.5 ± 0.39	91	104.1 ± 0.13	91		
Duration						
>2 min	7.2 ± 0.54	47	104.2 ± 0.19	47	0.70	0.36
<2 min	7.4 ± 0.40	85	104.0 ± 0.14	85		
Vocalization						
+	7.9 ± 0.45	69	104.3 ± 0.15	69	0.09	0.01
-	6.9 ± 0.44	71	103.8 ± 0.15	71		
CROWD PEN						
Electric Prod Use						
+	9.7 ± 1.11	11	104.7 ± 0.37	11	0.03	0.08
-	7.2 ± 0.33	129	104.0 ± 0.11	129		
Vocalization with Prod Use						
+	9.9 ± 1.40	7	104.8 ± 0.47	7	0.07	0.11
-	7.3 ± 0.32	133	104.0 ± 0.11	133		
Jamming						
+	7.5 ± 0.33	130	104.1 ± 0.11	130	0.32	0.86
-	6.3 ± 1.18	10	104.0 ± 0.40	10		
Rearing						
+	7.4 ± 0.34	17	104.1 ± 0.11	17	0.85	0.75
-	7.2 ± 0.91	123	104.0 ±0.30	123		
Back up						
+	7.5 ± 0.47	65	104.2 ± 0.15	65	0.82	0.16
-	7.3 ± 0.43	75	103.9 ± 0.14	75		
Turn back						
+	7.9 ± 0.50	57	104.0 ± 0.17	57	0.24	0.73
-	7.1 ± 0.41	83	104.1 ± 0.14	83		
Rear, Jam, Backup or Electric Prod						
+	7.4 ±0.32	135	104.1 ±0.11	135	0.43	0.81
-	6.1 ± 1.68	5	104.2 ±0.56	5		
Rear, Jam or Backup						
+	7.4 ± 0.32	134	104.1 ±0.11	134	0.67	0.66
-	6.8 ± 1.53	6	103.9 ±0.51	6		
Rear, Jam						
+	7.5 ± 0.33	130	104.1 ± 0.11	130	0.32	0.86
-	6.3 ± 1.18	10	104.0 ± 0.40	10		
Vocalization						
+	8.4 ± 0.91	17	104.8 ± 0.30	17	0.24	0.008
-	7.3 ± 0.34	123	104.0 ± 0.11	123		
SINGLE-FILE CHUTE						
Electric Prod Use						
+	7.7 ± 0.45	68	104.1 ± 0.15	68	0.31	0.86
-	7.1 ± 0.44	72	104.1 ± 0.15	72		
Jamming						
+	8.7 ± 0.56	42	104.1 ± 0.19	42	0.005	0.88
-	6.8 ± 0.37	98	104.1 ± 0.13	98		
Rearing						
+	8.9 ± 1.53	6	104.2 ± 0.51	6	0.33	0.80
-	7.3 ± 0.32	134	104.1 ±0.11	134		
Back up						
+	7.1 ± 0.63	36	104.0 ± 0.21	36	0.60	0.83
-	7.5 ± 0.37	104	104.1 ± 0.12	104		
Rear, Jam, Backup or Electric Prod						
+	8.0 ±0.35	105	104.1 ±0.12	105	0.002	0.51
-	5.7 ± 0.61	35	104.0 ±0.21	35		
Rear, Jam or Backup						
+	8.1 ± 0.44	69	104.1 ± 0.15	69	0.03	0.92
-	6.7 ± 0.44	71	104.1 ± 0.15	71		
Rear, Jam						
+	8.7 ± 0.54	46	104.1 ± 0.19	46	0.003	0.97
-	6.8 ± 0.38	94	104.1 ± 0.13	94		
Vocalization						
+	8.1 ± 0.47	61	104.3 ± 0.16	61	0.05	0.06
-	6.9 ± 0.42	79	103.9 ± 0.14	79		

Table 5.1. Experiment 1: Pearson correlations for all meat quality measurements, exsanguination blood lactate concentration (Final LAC, mmol) and rectal temperature (Final TEMP, F). The last three columns represent the raw mean, minimum and maximum values for each listed parameter.

Item r-value P < (n)	Final LAC	Final TEMP	45 min pH	45 min TEMP	24 hr pH	Blood Splash	Visual Color	L*	b*	a*	Drip Loss	GP[1]	Lactate[2]	Glycogen[3]	Mean	Min	Max
Final LAC	1.00	0.30	0.03	-0.05	0.23	-0.01	0.04	-0.10	-0.14	-0.10	-0.13	-0.11	-0.11	-0.08	7.4	1.1	18.4
		0.01	0.79	0.69	0.05	0.93	0.73	0.38	0.23	0.38	0.27	0.37	0.38	0.52			
	(79)	(77)	(74)	(75)	(73)	(73)	(73)	(73)	(72)	(72)	(72)	(67)	(69)	(67)			
Final TEMP		1.00	-0.01	0.25	0.23	-0.03	0.02	-0.06	-0.24	-0.13	-0.07	0.04	0.05	0.02	103.6	101.6	105.6
			0.93	0.03	0.05	0.79	0.83	0.60	0.04	0.29	0.56	0.74	0.69	0.87			
		(78)	(72)	(73)	(71)	(71)	(71)	(71)	(70)	(71)	(71)	(66)	(68)	(66)			
45 min pH	-	-	1.00	0.01	0.29	-0.07	0.05	-0.25	-0.35	-0.35	-0.59	-0.02	0.02	-0.11	6.39	5.9	6.7
				0.92	0.01	0.53	0.62	0.03	0.003	0.003	0.0001	0.90	0.87	0.37			
			(75)	(74)	(72)	(72)	(72)	(72)	(71)	(71)	(71)	(66)	(68)	(65)			
45 min TEMP	-	-	-	1.00	0.06	0.15	-0.21	0.18	-0.11	0.04	0.09	-0.08	-0.06	0.003	103.7	97.5	107.8
					0.63	0.20	0.07	0.13	0.36	0.73	0.48	0.51	0.64	0.98			
				(76)	(72)	(72)	(72)	(72)	(71)	(71)	(71)	(67)	(69)	(66)			
24 hr pH	-	-	-	-	1.00	-0.15	0.23	-0.43	-0.69	-0.41	-0.56	-0.22	-0.13	-0.30	5.75	5.6	6.1
						0.22	0.05	0.0002	0.0001	0.0003	0.0001	0.07	0.30	0.01			
					(74)	(73)	(73)	(73)	(72)	(72)	(72)	(67)	(69)	(66)			
Blood splash	-	-	-	-	-	1.00	0.10	-0.10	0.08	0.25	0.14	0.12	0.10	0.14	.42	0	1
							0.40	0.42	0.52	0.03	0.25	0.34	0.41	0.26			
						(74)	(74)	(74)	(73)	(73)	(73)	(68)	(70)	(67)			
Visual Color	-	-	-	-	-	-	1.00	-0.84	-0.42	0.18	-0.43	-0.11	-0.11	-0.05	3	1.5	4
								0.0001	0.0002	0.13	0.0001	0.37	0.35	0.70			
							(74)	(74)	(73)	(73)	(73)	(68)	(70)	(67)			
L*	-	-	-	-	-	-	-	1.00	0.68	-0.06	0.62	0.24	0.21	0.13	45.17	40.02	51.45
									0.0001	0.60	0.0001	0.05	0.09	0.28			
								(74)	(73)	(73)	(73)	(68)	(70)	(67)			
b*	-	-	-	-	-	-	-	-	1.00	0.35	0.60	0.25	0.16	0.33	7.57	5.6	9.75
										0.003	0.0001	0.04	0.46	0.01			
									(73)	(72)	(72)	(67)	(69)	(66)			
a*	-	-	-	-	-	-	-	-	-	1.00	0.25	0.16	0.09	0.23	-1.43	-3.73	0.55
											0.04	0.20	0.46	0.07			
										(73)	(72)	(67)	(69)	(66)			
Drip Loss	-	-	-	-	-	-	-	-	-	-	1.00	0.24	0.19	0.30	2.6	0.10	6.5
												0.05	0.12	0.01			
											(73)	(67)	(69)	(66)			
GP[1]	-	-	-	-	-	-	-	-	-	-	-	1.00	0.96	0.20	99.4	57.0	129.1
													0.001	0.10			
												(68)	(68)	(67)			
Lactate[2]	-	-	-	-	-	-	-	-	-	-	-	-	1.00	-0.11	91.8	48	120
														0.40			
													(70)	(67)			
Glycogen[3]	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00	2.0	0.5	10.3
														(67)			

[1]GP = glycolytic potential = Lactate content + (2 x Glycogen + Glucose-6-Phosphate + Glucose content); [2]Lactate = Lactate content; [3]Glycogen = Glucose + Glycogen + Glucose-6-Phosphate content

Table 5.2. Experiment 2: Pearson correlations for all meat quality measurements, exsanguination blood lactate concentration (Final LAC, mmol/L) and rectal temperature (Final TEMP, F). The last three columns represent the raw mean, minimum and maximum values for each listed parameter.

Item r-value P < (n)	Final LAC	Final TEMP	45 min pH	24 hr pH	Visual Color	L*	a*	b*	Drip Loss	GP[1]	Lactate[2]	Glycogen[3]	Mean	Min	Max
Final LAC	1.00	0.21	-0.25	0.06	0.12	-0.22	-0.19	0.07	-0.20	-0.12	-0.04	-0.24	7.4	1.4	20.6
mM		0.02	0.004	0.47	0.28	0.01	0.03	0.43	0.03	0.23	0.70	0.01			
	(139)	(139)	(132)	(127)	(87)	(127)	(127)	(126)	(125)	(104)	(111)	(107)			
Final TEMP,		1.00	-0.04	0.06	0.01	-0.04	-0.001	0.09	0.04	-0.03	-0.001	-0.14	104.1	101.0	106.1
F			0.68	0.51	0.93	0.64	0.99	0.31	0.64	0.75	0.99	0.16			
		(139)	(132)	(127)	(87)	(127)	(127)	(126)	(125)	(104)	(111)	(107)			
45 min pH	-	-	1.00	0.22	-0.15	0.05	0.08	-0.12	-0.12	0.01	0.09	-0.14	6.48	5.97	7.12
				0.01	0.17	0.57	0.35	0.17	0.18	0.95	0.33	0.14			
			(136)	(129)	(87)	(129)	(129)	(128)	(126)	(104)	(111)	(108)			
24 hr pH	-	-	-	1.00	0.09	-0.29	-0.02	-0.21	-0.40	-0.33	-0.27	-0.27	5.72	5.15	6.32
					0.40	0.001	0.80	0.02	0.001	0.0007	0.004	0.004			
				(131)	(88)	(129)	(129)	(128)	(126)	(105)	(112)	(109)			
Visual Color	-	-	-	-	1.00	-0.65	-0.45	0.18	-0.45	-0.07	-0.10	-0.07	3	2	4
						0.0001	0.0001	0.08	0.0001	0.55	0.40	0.56			
					(89)	(89)	(89)	(89)	(87)	(72)	(78)	(74)			
L*	-	-	-	-	-	1.00	0.68	-0.27	0.51	0.11	0.10	0.20	45.31	39.39	50.97
							0.0001	0.002	0.0001	0.26	0.30	0.04			
						(131)	(131)	(130)	(128)	(106)	(112)	(110)			
a*	-	-	-	-	-	-	1.00	0.16	0.27	0.11	0.13	0.14	-0.81	-2.7	1.62
								0.07	0.002	0.26	0.19	0.15			
							(131)	(130)	(128)	(106)	(113)	(110)			
b*	-	-	-	-	-	-	-	1.00	0.08	0.03	0.13	-0.10	7.68	5.33	9.93
									0.39	0.73	0.18	0.30			
								(130)	(128)	(106)	(112)	(109)			
Drip Loss, %	-	-	-	-	-	-	-	-	1.00	0.26	0.21	0.37	2.24	0.10	7.11
										0.008	0.03	0.0001			
									(128)	(104)	(110)	(108)			
GP[1]	-	-	-	-	-	-	-	-	-	1.00	0.94	0.46	98.2	68.0	132.6
											0.0001	0.0001			
										(106)	(106)	(106)			
Lactate[2]	-	-	-	-	-	-	-	-	-	-	1.00	0.14	89.7	57.5	117.5
												0.16			
											(112)	(106)			
Glycogen[3]	-	-	-	-	-	-	-	-	-	-	-	1.00	2.0	0.4	7.5
												(110)			

[1]GP = glycolytic potential = Lactate content + (2 x Glycogen + Glucose-6-Phosphate + Glucose content); [2]Lactate = Lactate content; [3]Glycogen = Glucose + Glycogen + Glucose-6-Phosphate content

Table 5.3. Experiment 1: Pearson correlations for all meat quality measurements and blood lactate concentrations taken at sample points 1-7 (1 = baseline, 2 = post-load, 3 = pre-unload, 4 = post-unload, 5 = post lairage, 6 = post movement to stun, 7 = exsanguination).

Item: r *P* (n)							
Sample Point	1	2	3	4	5	6	7
[LAC][1] (mM)	3.6 ± 0.4	4.7 ± 0.4	3.6 ± 0.3	2.5 ± 0.3	2.1± 0.1	2.5 ± 0.2	6.6 ± 0.4
Range (min-max; mM)	1.8 – 13.4	1.2 – 16.9	1.2 – 11.1	1.2 – 11.3	0.8 – 5.7	1.1 – 10.1	1.1 – 18.4
45 min pH	0.08	0.17	0.04	0.14	0.12	0.15	0.03
	0.46	0.14	0.73	0.22	0.31	0.20	0.79
	(74)	(74)	(74)	(74)	(75)	(75)	(74)
45 min TEMP	-0.12	0.12	0.28	0.14	-0.01	-0.02	-0.05
	0.32	0.31	0.02	0.23	0.92	0.89	0.69
	(75)	(75)	(75)	(75)	(76)	(76)	(75)
24 h pH	-0.03	0.36	0.22	0.22	0.16	0.23	0.23
	0.81	0.002	0.07	0.06	0.19	0.05	0.05
	(73)	(73)	(73)	(73)	(74)	(73)	(73)
Splash	0.05	0.02	-0.23	-0.22	0.09	-0.06	-0.01
	0.67	0.90	0.05	0.06	0.41	0.63	0.93
	(72)	(72)	(73)	(73)	(74)	(74)	(73)
Visual Color Score	0.05	0.22	-0.09	0.08	0.04	0.02	0.04
	0.66	0.07	0.47	0.50	0.72	0.88	0.73
	(73)	(73)	(73)	(73)	(74)	(74)	(73)
L*	-0.14	-0.25	0.10	-0.12	-0.06	-0.11	-0.10
	0.25	0.03	0.38	0.31	0.62	0.37	0.38
	(73)	(73)	(73)	(73)	(74)	(74)	(73)
b*	-0.06	-0.20	-0.16	-0.25	-0.16	-0.07	-0.14
	0.64	0.10	0.17	0.04	0.17	0.54	0.23
	(72)	(72)	(72)	(72)	(73)	(73)	(72)
a*	0.16	-0.003	-0.28	-0.26	-0.16	-0.08	-0.10
	0.18	0.98	0.02	0.03	0.18	0.51	0.38
	(72)	(72)	(72)	(72)	(73)	(74)	(72)
Drip Loss	-0.07	-0.28	0.004	-0.12	-0.10	-0.11	-0.13
	0.54	0.02	0.98	0.33	0.42	0.37	0.27
	(72)	(72)	(72)	(72)	(73)	(73)	(72)
Glycolytic Potential[2]	0.05	-0.18	-0.16	-0.09	0.13	-0.03	-0.11
	0.66	0.14	0.21	0.48	0.29	0.80	0.37
	(67)	(66)	(66)	(66)	(67)	(67)	(67)
Lactate Content	0.07	-0.14	-0.14	-0.01	0.11	-0.002	-0.11
	0.58	0.25	0.25	0.91	0.35	0.99	0.38
	(69)	(69)	(69)	(69)	(70)	(70)	(69)
Glycogen Content[3]	-0.06	-0.20	-0.07	-0.25	0.06	-0.12	-0.08
	0.63	0.11	0.56	0.05	0.65	0.35	0.52
	(67)	(66)	(66)	(66)	(67)	(67)	(67)

[1][LAC] = Least Square Mean ± Standard Error of the Mean of blood lactate concentration at each of the sample points from Edwards et al., 2009b; [2]Glycolytic Potential = Lactate + 2(Glycogen + Glucose-6-Phosphate + Glucose); [3]Glycogen Content = Glycogen + Glucose-6-Phosphate + Glucose in a sample of the longissimus muscle

Table 5.4. Experiment 2: Pearson correlations for all meat quality measurements and blood lactate concentration taken at sample points 1-7 (1=baseline, 2=post-load, 3=pre-unload, 4=post-unload, 5= post lairage, 6= post movement, 7 = exsanguination).

Item r *P* (n)							
Sample Point	1	2	3	4	5	6	7
[LAC][1] (mM)	3.5 ± 0.2	7.1 ± 0.4	3.0 ± 0.2	4.4 ± 0.2	3.3 ± 0.2	4.3 ± 0.2	6.6 ± 0.3
Range (min-max; mM)	1.2 – 11	1.5 – 24.3	0.8 – 12.2	1.3 – 15.3	1.1 – 14.5	1.2 – 18.7	1.4 – 20.6
45 min pH	0.10	0.12	-0.01	-0.01	-0.001	-0.07	-0.25
	0.27	0.18	0.90	0.90	0.99	0.43	0.004
	(133)	(136)	(134)	(136)	(136)	(135)	(132)
24 h pH	0.28	0.40	0.09	0.24	0.08	0.12	0.06
	0.001	0.0001	0.32	0.005	0.39	0.17	0.47
	(128)	(131)	(129)	(131)	(131)	(130)	(127)
Visual Color Score	-0.21	0.12	-0.07	0.04	-0.03	0.07	0.12
	0.05	0.27	0.53	0.68	0.80	0.49	0.28
	(88)	(89)	(87)	(89)	(89)	(88)	(87)
L*	-0.10	-0.29	-0.07	-0.10	-0.001	-0.19	-0.22
	0.27	0.001	0.40	0.25	0.95	0.03	0.01
	(128)	(131)	(129)	(131)	(131)	(130)	(127)
b*	0.03	-0.08	-0.03	-0.11	0.02	-0.06	0.07
	0.75	0.37	0.76	0.23	0.84	0.52	0.43
	(127)	(130)	(128)	(130)	(130)	(129)	(126)
a*	0.02	-0.12	-0.08	-0.06	-0.03	-0.21	-0.19
	0.83	0.16	0.35	0.53	0.76	0.02	0.03
	(128)	(131)	(129)	(131)	(131)	(130)	(127)
Drip Loss	-0.08	-0.28	-0.02	-0.20	-0.07	-0.14	-0.20
	0.41	0.002	0.81	0.02	0.41	0.10	0.03
	(125)	(128)	(126)	(128)	(128)	(127)	(125)
Glycolytic Potential[2]	-0.16	-0.16	-0.05	0.01	-0.14	-0.09	-0.12
	0.10	0.11	0.65	0.95	0.16	0.36	0.23
	(104)	(106)	(105)	(106)	(106)	(105)	(104)
Lactate Content	-0.10	-0.12	-0.03	0.02	-0.09	-0.09	-0.04
	0.29	0.22	0.72	0.80	0.35	0.36	0.70
	(110)	(113)	(111)	(113)	(112)	(112)	(111)
Glycogen Content[3]	-0.10	-0.21	-0.04	-0.09	-0.14	-0.16	-0.24
	0.30	0.03	0.67	0.35	0.15	0.10	0.01
	(108)	(110)	(109)	(110)	(110)	(109)	(107)

[1][LAC] = Least Square Mean ± Standard Error of the Mean blood lactate concentration at each of the listed time points adapted from Edwards et al., 2009b.

[2]Glycolytic Potential = Lactate + 2(Glycogen + Glucose-6-Phosphate Glucose)

[3]Glycogen Content = Glycogen + Glucose-6-Phosphate + Glucose in a sample of the longissimus muscle

Table 5.5. Experiment 1: Pearson correlations for meat quality measurements and the changes in blood lactate concentration between sample points 1-7 (2-1 = loading; 3-2 = transport; 4-3 = unloading/movement; 5-4 = lairage; 6-5 = movement to circle corral; 7-6 = circle corral to stun) .

Item: r *P* (n)						
Sample Point	1	2	3	4	5	6
Δ [LAC][1] (mM)	1.7 ± 0.5	-1.6 ± 0.5	-1.2 ± 0.3	-0.7 ± 0.3	0.5 ± 0.2	4.6 ± 0.3
Min Reduction	-10.6	-15.2	-8.3	-8.5	-4.4	0
Max Increase	14.7	8.4	9.5	3.9	7	16.8
Final [LAC]	0.35	-0.23	0.002	-0.06	0.35	0.88
	0.002	0.05	0.99	0.60	0.003	0.0001
	(76)	(76)	(72)	(73)	(71)	(70)
Final TEMP	0.21	-0.12	0.03	-0.15	0.30	0.09
	0.07	0.29	0.79	0.22	0.01	0.46
	(75)	(75)	(71)	(72)	(70)	(69)
45 min pH	0.14	-0.11	0.14	-0.14	-0.05	-0.03
	0.25	0.35	0.24	0.26	0.68	0.81
	(73)	(73)	(70)	(70)	(68)	(66)
45 min TEMP	0.21	0.06	-0.19	-0.09	0.03	-0.02
	0.07	0.60	0.11	0.44	0.80	0.88
	(74)	(74)	(70)	(71)	(69)	(67)
24 hr pH	0.39	-0.16	-0.05	-0.10	0.02	0.19
	0.0006	0.19	0.69	0.39	0.89	0.13
	(70)	(72)	(70)	(71)	(69)	(67)
Visual Color	0.20	-0.21	0.09	0.05	-0.18	0.01
	0.09	0.08	0.45	0.69	0.14	0.92
	(72)	(72)	(69)	(70)	(68)	(66)
Blood Splash	-0.02	-0.17	-0.02	0.30	-0.04	0.06
	0.90	0.15	0.85	0.01	0.77	0.62
	(72)	(72)	(69)	(70)	(68)	(66)
Drip Loss	-0.25	0.21	-0.05	0.01	0.03	-0.10
	0.04	0.19	0.66	0.96	0.82	0.41
	(71)	(71)	(69)	(70)	(67)	(65)
L*	-0.18	0.21	-0.14	-0.03	0.08	-0.06
	0.12	0.07	0.25	0.82	0.50	0.64
	(72)	(72)	(69)	(70)	(68)	(66)
b*	-0.18	0.04	0.01	0.06	0.12	-0.07
	0.14	0.75	0.94	0.64	0.33	0.56
	(71)	(71)	(68)	(69)	(67)	(65)
a*	-0.11	-0.17	0.04	0.14	0.03	-0.06
	0.37	0.15	0.75	0.24	0.82	0.66
	(71)	(71)	(68)	(69)	(67)	(65)
Glycolytic Potential[1]	-0.24	-0.003	0.11	0.15	-0.09	-0.15
	0.05	0.98	0.38	0.24	0.50	0.27
	(67)	(66)	(63)	(64)	(62)	(60)
Lactate Content	-0.20	-0.03	0.14	0.07	-0.06	-0.16
	0.11	0.80	0.26	0.59	0.65	0.22
	(68)	(68)	(65)	(66)	(64)	(60)
Glycogen Content[2]	-0.18	0.12	-0.10	0.23	-0.10	-0.02
	0.15	0.32	0.45	0.07	0.44	0.89
	(67)	(66)	(63)	(64)	(62)	(60)
a*	-0.11	-0.17	0.04	0.14	0.03	-0.06
	0.37	0.15	0.75	0.24	0.82	0.66
	(71)	(71)	(68)	(69)	(67)	(65)

[1]Δ in [LAC] = Raw Means ± Standard Error of the Mean of change in blood lactate concentration between sample points from Edwards et al., 2009b.

[2]Glycolytic Potential = Lactate + 2(Glycogen + Glucose-6-Phosphate Glucose)

[3]Glycogen Content = Glycogen + Glucose-6-Phosphate + Glucose in a sample of the longissimus muscle

Table 5.6. Experiment 2: Pearson correlations for meat quality measurements and the changes in blood lactate concentration between sample points 1-7 (2-1 = loading; 3-2 = transport; 4-3 = unloading/movement; 5-4 = lairage; 6-5 = movement to circle corral; 7-6 = circle corral to stun).

Item: r *P* (n)						
Sample Point	1	2	3	4	5	6
Δ [LAC][1] (mM)	4.1 ± 0.4	-4.7 ± 0.4	1.6 ± 0.2	-1.2 ± 0.3	1.1 ± 0.3	2.7 ± 0.3
Min Reduction	-7.1	-19.2	-8.4	-11.9	-11.7	-12.5
Max Increase	21.8	4.6	13.8	7.3	15.5	16.9
Final LAC	0.12	-0.18	0.03	0.11	0.16	0.71
	0.18	0.04	0.74	0.19	0.06	0.0001
	(136)	(137)	(137)	(139)	(138)	(139)
Final TEMP	0.05	0.06	-0.25	0.26	-0.17	0.20
	0.55	0.48	0.003	0.001	0.04	0.02
	(136)	(137)	(137)	(139)	(138)	(139)
45 min pH	0.06	-0.12	0.003	0.008	-0.06	-0.20
	0.46	0.16	0.98	0.92	0.47	0.02
	(133)	(134)	(134)	(136)	(135)	(132)
24 hr pH	0.24	-0.36	0.06	-0.15	0.06	-0.03
	0.01	0.001	0.07	0.08	0.46	0.74
	(128)	(129)	(129)	(131)	(130)	(127)
Visual Color	0.20	-0.15	0.08	-0.05	0.09	0.02
	0.06	0.18	0.46	0.63	0.39	0.82
	(88)	(87)	(87)	(89)	(88)	(87)
Drip Loss	-0.23	0.26	-0.16	0.13	-0.10	-0.08
	0.01	0.003	0.07	0.14	0.28	0.39
	(125)	(126)	(126)	(128)	(125)	(127)
L*	-0.24	0.35	-0.04	0.08	-0.19	-0.06
	0.01	0.004	0.65	0.34	0.03	0.51
	(128)	(129)	(129)	(131)	(130)	(127)
b*	-0.09	0.06	-0.07	0.10	-0.07	0.10
	0.33	0.47	0.44	0.25	0.46	0.27
	(127)	(128)	(128)	(130)	(129)	(126)
a*	-0.14	0.09	0.006	0.03	-0.19	-0.04
	0.12	0.33	0.05	0.72	0.03	0.65
	(128)	(129)	(129)	(131)	(130)	(127)
Glycolytic Potential[1]	-0.08	0.14	0.03	-0.08	-0.004	-0.05
	0.44	0.16	0.75	0.40	0.97	0.61
	(104)	(105)	(105)	(106)	(105)	(104)
Lactate Content	-0.06	0.10	0.06	-0.07	0.02	-0.009
	0.51`	0.29	0.56	0.46	0.80	0.93
	(110)	(111)	(111)	(113)	(112)	(111)
Glycogen Content[2]	-0.15	0.19	-0.06	-0.001	-0.07	-0.12
	0.13	0.05	0.54	0.99	0.48	0.21
	(108)	(109)	(109)	(110)	(109)	(107)

[1]Δ in [LAC]= Raw Means ± Standard Error of the Mean of change in rectal temperature between sample points

[2]Glycolytic Potential = Lactate + 2(Glycogen + Glucose-6-Phosphate Glucose)

[3]Glycogen Content = Glycogen + Glucose-6-Phosphate + Glucose in a sample of the longissimus muscle

Table 5.7. Experiment 1: Pearson correlations for all meat quality measurements and rectal temperatures taken at time points 1-7 (1 = baseline, 2 = post-load, 3 = pre-unload, 4 = post-unload, 5 = post lairage, 6 = post movement, 7 = exsanguination).

Item: r *P* (n)							
Sample Point	1	2	3	4	5	6	7
TEMP[1] (C)	39.4 ± 0.05	39.8 ± 0.05	39.3 ± 0.05	39.8 ± 0.05	39.6 ± 0.05	39.9 ± 0.05	39.7 ± 0.05
Range (min-max; C)	38.4 – 40.6	39.0 – 41.1	38.2 – 40.5	38.7 – 41.8	38.6 – 40.8	38.9 – 41.3	38.7 – 40.9
45 min pH	0.09	0.01	0.07	-0.03	-0.16	-0.07	-0.01
	0.46	0.93	0.57	0.81	0.18	0.58	0.93
	(74)	(73)	(74)	(75)	(75)	(75)	(72)
45 min TEMP	0.08	0.08	0.04	0.16	0.10	0.10	0.25
	0.50	0.51	0.74	0.18	0.40	0.39	0.03
	(75)	(74)	(75)	(76)	(76)	(76)	(73)
24 h pH	0.01	0.21	0.26	0.29	0.13	0.18	0.23
	0.93	0.07	0.03	0.01	0.26	0.13	0.05
	(73)	(72)	(73)	(74)	(74)	(74)	(71)
Splash	0.09	-0.07	-0.02	0.11	0.11	-0.01	-0.03
	0.43	0.55	0.84	0.35	0.37	0.95	0.79
	(73)	(72)	(73)	(74)	(74)	(74)	(71)
Visual Color	-0.16	0.11	0.30	0.29	0.13	0.13	0.02
Score	0.19	0.37	0.01	0.01	0.27	0.26	0.83
	(73)	(72)	(73)	(74)	(74)	(68)	(71)
L*	0.13	-0.14	-0.30	-0.34	-0.08	-0.16	-0.06
	0.29	0.24	0.01	0.003	0.51	0.18	0.60
	(73)	(72)	(73)	(74)	(74)	(74)	(71)
b*	-0.13	-0.18	-0.35	-0.37	-0.16	-0.27	-0.24
	0.28	0.13	0.002	0.001	0.16	0.02	0.04
	(72)	(71)	(72)	(73)	(73)	(73)	(70)
a*	-0.06	-0.12	-0.08	0.02	0.10	-0.04	-0.13
	0.57	0.33	0.50	0.83	0.40	0.76	0.29
	(72)	(71)	(72)	(73)	(73)	(73)	(71)
Drip Loss	0.13	-0.14	-0.26	-0.19	0.04	-0.09	-0.07
	0.29	0.24	0.03	0.11	0.72	0.45	0.56
	(72)	(71)	(72)	(73)	(73)	(73)	(71)
Glycolytic	0.05	-0.16	-0.09	-0.04	0.03	-0.12	0.04
Potential[1]	0.71	0.20	0.48	0.73	0.79	0.32	0.74
	(66)	(65)	(66)	(67)	(67)	(67)	(66)
Lactate	0.10	-0.15	-0.06	0.01	0.05	-0.12	0.05
Content	0.43	0.22	0.62	0.96	0.65	0.32	0.69
	(69)	(68)	(69)	(70)	(70)	(70)	(68)
Glycogen	-0.04	0.10	-0.09	-0.11	0.04	-0.03	0.02
Content[2]	0.75	0.43	0.49	0.37	0.77	0.83	0.87
	(66)	(65)	(66)	(67)	(67)	(67)	(66)

[1]TEMP= Least Square Mean ± Standard Error of the Mean of rectal temperature at each of the sample points from Edwards, 2009

[2]Glycolytic Potential = Lactate + 2(Glycogen + Glucose-6-Phosphate + Glucose)

[3]Glycogen Content = Glycogen + Glucose-6-Phosphate + Glucose in a sample of the longissimus muscle

Table 5.8. Experiment 2: Pearson correlations for all meat quality measurements and rectal temperatures taken at time points 1-7 (1=baseline, 2=post-load, 3=pre-unload, 4=post-unload, 5= post lairage, 6= post movement, 7 = exsanguination).

Item: r *P* (n)							
Sample Point	1	2	3	4	5	6	7
TEMP[1] (C)	40.0 ± 0.05	40.3 ± 0.05	38.4 ± 0.05	39.6 ± 0.05	39.6 ± 0.05	39.9 ± 0.05	39.7 ± 0.05
Range (min-max; C)	38.9 – 41.0	39.4 – 41.6	35.7 – 40.0	37.9 – 41.2	37.9 – 40.9	38.4 – 41.4	38.3 – 41.2
45 min pH	0.06	0.03	0.008	-0.05	-0.003	0.04	-0.04
	0.46	0.76	0.93	0.56	0.97	0.61	0.68
	(133)	(136)	(134)	(136)	(135)	(134)	(132)
24 h pH	0.18	0.36	-0.10	0.08	-0.07	0.19	0.06
	0.05	0.0001	0.24	0.34	0.42	0.03	0.51
	(128)	(131)	(129)	(131)	(130)	(129)	(127)
Visual Color	-0.11	0.02	-0.02	0.12	0.07	0.10	0.01
Score	0.29	0.85	0.88	0.26	0.49	0.37	0.93
	(88)	(89)	(87)	(89)	(88)	(87)	(87)
L*	-0.07	-0.11	0.03	-0.11	0.02	-0.15	-0.04
	0.43	0.22	0.75	0.21	0.78	0.08	0.64
	(128)	(131)	(129)	(131)	(130)	(129)	(127)
b*	-0.02	-0.04	-0.10	-0.06	-0.03	0.14	-0.001
	0.82	0.65	0.29	0.49	0.75	0.11	0.99
	(127)	(130)	(128)	(130)	(129)	(128)	(127)
a*	0.11	0.10	-0.01	-0.11	-0.02	0.02	0.09
	0.21	0.24	0.91	0.21	0.86	0.83	0.31
	(128)	(131)	(129)	(131)	(130)	(129)	(126)
Drip Loss	-0.13	-0.27	0.04	-0.22	-0.09	-0.05	0.04
	0.16	0.003	0.65	0.01	0.33	0.61	0.64
	(125)	(128)	(126)	(128)	(127)	(126)	(125)
Glycolytic	0.05	-0.09	0.06	-0.13	0.07	-0.06	-0.03
Potential [1]	0.62	0.35	0.55	0.19	0.51	0.56	0.75
	(104)	(106)	(105)	(106)	(105)	(104)	(104)
Lactate	0.02	-0.06	0.06	-0.05	0.11	-0.03	-0.001
Content	0.87	0.53	0.52	0.63	0.26	0.78	0.99
	(110)	(112)	(111)	(112)	(112)	(110)	(111)
Glycogen	-0.001	-0.10	0.01	-0.29	-0.18	-0.18	-0.14
Content [2]	0.99	0.32	0.95	0.002	0.07	0.07	0.16
	(108)	(110)	(109)	(110)	(109)	(108)	(107)

[1]TEMP= Least Square Mean ± Standard Error of the Mean of rectal temperature at each of the sample points from Edwards et al., 2009b

[2]Glycolytic Potential = Lactate + 2(Glycogen + Glucose-6-Phosphate + Glucose)

[3]Glycogen Content = Glycogen + Glucose-6-Phosphate + Glucose in a sample of the longissimus muscle

Table 5.9. Experiment 1: Pearson correlations for meat quality measurements and the rectal temperature between sample points 1-7 (2-1 = loading; 3-2 = transport; 4-3 = unloading/movement; 5-4 = lairage; 6-5 = movement to circle corral; 7-6 = circle corral to stun).

Item: r *P* (n)						
Sample Point	1	2	3	4	5	6
Δ TEMP[1] (C)	0.3 ± 0.05	-0.8 ± 0.05	1.0 ± 0.05	-0.6 ± 0.05	0.7 ± 0.05	-0.3 ± 0.05
Min Reduction	-0.9	-2.2	-0.2	-1.6	-0.4	-0.7
Max Increase	1.9	1.0	1.7	0.3	1.3	0.4
Final LAC	0.01	-0.09	0.19	-0.08	0.05	0.09
	0.91	0.44	0.12	0.51	0.70	0.43
	(68)	(69)	(70)	(71)	(71)	(70)
Final TEMP	0.03	-0.02	0.17	0.02	0.37	0.23
	0.80	0.88	0.15	0.89	0.001	0.05
	(67)	(68)	(69)	(70)	(70)	(70)
45 min pH	-0.05	0.06	-0.12	-0.09	0.07	-0.11
	0.68	0.65	0.35	0.44	0.59	0.38
	(71)	(71)	(69)	(70)	(68)	(65)
	-0.06	-0.03	0.15	0.03	0.03	0.16
45 min TEMP	0.61	0.79	0.24	0.83	0.83	0.19
	(65)	(66)	(67)	(68)	(68)	(66)
24 hr pH	0.14	0.07	0.05	-0.19	0.11	-0.05
	0.26	0.55	0.71	0.11	0.38	0.69
	(70)	(71)	(70)	(71)	(69)	(66)
Visual Color	0.20	0.19	-0.02	-0.18	-0.02	-0.13
	0.10	0.11	0.89	0.14	0.89	0.32
	(70)	(71)	(69)	(70)	(68)	(65)
	-0.12	0.02	0.17	-0.02	-0.09	0.09
Blood Splash	0.34	0.86	0.17	0.88	0.46	0.48
	(70)	(71)	(69)	(70)	(68)	(65)
Drip Loss	-0.19	-0.13	0.11	0.19	-0.13	0.21
	0.13	0.27	0.37	0.12	0.28	0.10
	(69)	(70)	(68)	(69)	(67)	(64)
L*	-0.20	-0.17	-0.06	0.28	-0.04	0.15
	0.10	0.15	0.65	0.02	0.75	0.22
	(70)	(71)	(69)	(70)	(67)	(65)
b*	-0.05	-0.16	-0.02	0.19	-0.10	0.10
	0.70	0.18	0.90	0.12	0.40	0.42
	(69)	(70)	(68)	(69)	(67)	(64)
a*	-0.04	0.02	0.14	-0.03	-0.08	-0.08
	0.75	0.89	0.26	0.83	0.52	0.54
	(69)	(64)	(68)	(69)	(67)	(64)
Glycolytic	-0.13	0.01	0.09	-0.03	-0.11	0.21
Potential[1]	0.30	0.95	0.47	0.81	0.41	0.11
	(65)	(65)	(63)	(64)	(62)	(59)
Lactate Content	-0.16	0.03	0.11	-0.05	-0.14	0.18
	0.19	0.81	0.38	0.69	0.28	0.15
	(66)	(67)	(63)	(64)	(64)	(61)
Glycogen Content[2]	0.11	-0.16	-0.01	0.11	-0.03	0.16
	0.40	0.21	0.96	0.39	0.82	0.24
	(65)	(65)	(63)	(64)	(62)	(59)

[1]Δ in TEMP= Raw Means ± Standard Error of the Mean of change in rectal temperature between sample points

[2]Glycolytic Potential = Lactate + 2(Glycogen + Glucose-6-Phosphate Glucose)

[3]Glycogen Content = Glycogen + Glucose-6-Phosphate + Glucose in a sample of the longissimus muscle

Table 5.10. Experiment 2: Pearson correlations for meat quality measurements and the changes in rectal temperature between sample points 1-7 (2-1 = loading; 3-2 = transport; 4-3 = unloading/movement; 5-4 = lairage; 6-5 = movement to circle corral; 7-6 = circle corral to stun).

Item: r *P* (n)						
Sample Point	1	2	3	4	5	6
Δ TEMP[1] (C)	0.3 ± 0.05	-2.0 ± 0.05	1.2 ± 0.05	-0.3 ± 0.05	0.6 ± 0.05	0.1 ± 0.05
Min Reduction	-0.6	-4.7	-0.9	-1.9	-1.9	-1.9
Max Increase	1.4	0.2	3.5	1.8	2.3	2.3
Final LAC	0.21	0.04	0.03	-0.24	-0.02	0.20
	0.01	0.67	0.73	0.004	0.84	0.02
	(137)	(138)	(138)	(139)	(137)	(137)
Final TEMP	0.12	0.16	-0.14	0.04	0.10	0.54
	0.15	0.06	0.10	0.64	0.25	0.0001
	(137)	(138)	(138)	(139)	(137)	(137)
45 min pH	-0.05	-0.003	-0.04	0.05	0.04	-0.08
	0.59	0.97	0.62	0.60	0.63	0.39
	(133)	(134)	(134)	(135)	(134)	(130)
24 hr pH	0.21	-0.27	0.18	-0.15	0.22	-0.10
	0.02	0.002	0.04	0.09	0.01	0.27
	(128)	(129)	(129)	(130)	(129)	(125)
Visual	0.12	-0.01	0.11	-0.05	0.02	-0.07
	0.26	0.90	0.29	0.66	0.82	0.53
	(88)	(87)	(87)	(88)	(87)	(85)
Drip Loss	-0.14	0.16	-0.20	0.13	0.03	0.08
	0.11	0.07	0.03	0.14	0.78	0.39
	(125)	(126)	(126)	(127)	(126)	(123)
L*	-0.05	0.08	-0.11	0.13	-0.16	0.05
	0.54	0.38	0.22	0.15	0.08	0.68
	(128)	(129)	(129)	(130)	(129)	(125)
b*	-0.04	-0.07	0.07	0.03	0.15	-0.05
	0.69	0.46	0.45	0.70	0.10	0.58
	(127)	(128)	(128)	(129)	(128)	(124)
a*	-0.02	-0.06	-0.07	0.09	0.03	-0.01
	0.87	0.52	0.46	0.29	0.74	0.87
	(128)	(129)	(129)	(130)	(129)	(125)
Glycolytic Potential[1]	-0.14	0.10	-0.15	0.17	-0.10	0.09
	0.15	0.31	0.12	0.08	0.31	0.39
	(104)	(105)	(105)	(105)	(104)	(102)
Lactate Content	-0.09	0.09	-0.10	0.14	-0.11	-0.02
	0.36	0.32	0.28	0.15	0.25	0.87
	(110)	(111)	(111)	(111)	(111)	(108)
Glycogen Content[2]	-0.09	0.05	-0.19	0.11	-0.02	0.06
	0.37	0.62	0.04	0.24	0.87	0.53
	(108)	(109)	(109)	(109)	(108)	(104)

[1]Δ in TEMP= Raw Means ± Standard Error of the Mean of change in rectal temperature between sample points

[2]Glycolytic Potential = Lactate + 2(Glycogen + Glucose-6-Phosphate Glucose)

[3]Glycogen Content = Glycogen + Glucose-6-Phosphate + Glucose in a sample of the longissimus muscle

Table 6.1. Composition of treatment diets.

Item	Low dEB (g/kg)	High dEB (g/kg)
Corn	738.45	702.45
Soybean meal	220	224
Fat	14	27
Limestone	7	12
Salt	5	0
Lysine HCl	2	2
Dicalcium phosphate	4.5	4.5
Swine Micro	1.5	1.5
Sodium bicarbonate	0	25
Calcium chloride	7	0
Phytase 5000L	0.05	0.05
Paylean®	0.50	0.50
Iron oxide	0	1.0
Na + K - Cl (meq/kg)	121	375

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Table 6.2. Parameter ranges for classification of carcasses

Category	24 hr pH	Drip loss	Muscle color	L*
Dark Firm Dry (DFD)	> 6.10	< 2%	≥ 4	≤ 42
Trend DFD	5.8 – 6.1	< 5%	3 - 4	42 - 45
Normal	5.6 – 5.8	2 – 5%	3	42 - 50
Red Soft Exudative (RSE)	5.6 – 5.8	> 5%	3	42 - 50
Pale Firm Normal (PFN)	5.5 – 5.8	< 5%	< 3	> 50
Trend Pale Soft Exudative (PSE)	5.5 – 5.6	> 5%	2 – 3	≥ 50
PSE	< 5.5	> 5%	1 – 1.5	> 50

Table 6.3. Least Square Means for blood parameters of pigs fed the Low dEB treatment (121 meq/kg) and the High dEB treatment (375 meq/kg) at three sampling points: baseline (5 days prior to feed withdrawal), 0 h (prior to feed withdrawal after three days of being fed the treatment diet) and 10 h (10 h after feed withdrawal). Each of table values is derived from the mean of eight pens per treatment (n=8). Four barrows per pen were sampled for the baseline measurement and six barrows per pen were sampled for the other two measurements.

				P <		
Parameter	Low dEB[2] Trt	High dEB Trt	SEM	Trt	Time	Trt*Time
Glucose, mg/dL	83.5	83.6	0.89	0.96	0.001	0.02
Baseline	78.8	84.6	1.34	0.03		
0 h	83.3	78.6	1.32	0.08		
10 h	88.5	87.5	0.95	0.70		
BUN[1], mg/dL	17.1	17.5	0.25	0.49	0.07	0.27
Baseline	17.8	17.0	0.48	0.37		
0 h	17.6	18.3	0.34	0.46		
10 h	16.0	17.1	0.43	0.18		
Sodium, mmol/L	138.87	139.33	0.20	0.16	0.0001	0.49
Baseline	138.63	139.63	0.26	0.08		
0 h	140.0	140.13	0.22	0.82		
10 h	138.0	138.25	0.30	0.66		
Potassium, mmol/L	5.30	5.18	0.06	0.18	0.0001	0.41
Baseline	4.90	4.95	0.07	0.76		
0 h	5.33	5.10	0.07	0.18		
10 h	5.69	5.48	0.07	0.20		
Chloride, mmol/L	100.71	100.29	0.19	0.21	0.05	0.002
Baseline	99.50	100.63	0.35	0.05		
0 h	101.38	99.38	0.33	0.001		
10 h	101.25	100.88	0.26	0.51		
Anion Gap, mmol/L	9.46	9.17	0.20	0.45	0.48	0.02
Baseline	8.63	9.50	0.34	0.20		
0 h	10.13	8.38	0.36	0.01		
10 h	9.63	9.63	0.29	1.00		
Total CO_2, mmol/L	36.50	36.96	0.34	0.42	0.01	0.003
Baseline	37.75	36.50	0.50	0.21		
0 h	36.00	39.38	0.72	0.001		
10 h	35.75	35.00	0.42	0.45		
Hematocrit, % PCV	40.17	39.83	0.30	0.58	0.08	0.73
Baseline	39.63	38.63	0.55	0.34		
0 h	40.88	40.75	0.34	0.90		
10 h	40.00	40.13	0.49	0.90		
Hemoglobin, g/dL	13.60	13.56	0.09	0.82	0.08	0.87
Baseline	13.40	13.24	0.16	0.62		
0 h	13.86	13.83	0.12	0.91		
10 h	13.54	13.61	0.17	0.82		
pH	7.340	7.350	0.006	0.22	0.03	0.14
Baseline	7.368	7.355	0.007	0.39		
0 h	7.328	7.344	0.005	0.30		
10 h	7.323	7.352	0.010	0.06		
Partial CO_2, mmHg	64.75	64.02	0.81	0.63	0.06	0.07
Baseline	63.44	62.03	1.11	0.59		
0 h	65.01	68.98	1.23	0.14		
10 h	65.80	61.06	1.87	0.08		
Bicarbonate, mmol/L	34.40	35.03	0.33	0.25	0.006	0.002
Baseline	35.75	34.48	0.48	0.18		
0 h	33.81	37.39	0.64	0.001		
10 h	33.64	33.24	0.37	0.67		
Base Excess, mmol/L	8.63	9.50	0.35	0.13	0.004	0.002
Baseline	10.38	9.13	0.56	0.21		
0 h	7.88	11.75	0.67	0.0003		
10 h	7.63	7.63	0.35	1.00		

[1]BUN = Blood Urea Nitrogen.[2]dEB = dietary electrolyte balance

Table 6.4. Effect of Low (121 meq/kg) and High (375 meq/kg) dEB diets on blood parameters at exsanguination (Least Square Means). The means for each treatment represent the average of eight pens of six barrows each. This sample was taken approximately 20 hrs post feed withdrawal. Carbon dioxide stunning was used.

Parameter*	Low dEB Treatment (n = 8)	High dEB Treatment (n = 8)	SEM	*P* <
Glucose, mg/dL	160.4	170.5	6.92	0.48
BUN[1], mg/dL	20.38	20.25	0.32	0.84
Sodium, mmol/L	134.47	135.11	0.18	0.07
Chloride, mmol/L	103.72	104.79	0.25	0.02
Hematocrit, % PCV	45.55	45.64	0.38	0.91
Hemoglobin, g/dL	15.49	15.52	0.14	0.92
pH (**)	6.904 (6.889)	6.881 (6.881)	0.006 (0.003)	0.04 (0.25)
Lactate, mmol/L	9.2	8.6	0.15	0.06

[1]BUN = Blood Urea Nitrogen

*The following parameters were outside of the range of the measuring device due to carbon dioxide stunning: Potassium, Anion Gap, Base Excess, Bicarbonate, Total CO_2 and Partial CO_2.

**Three barrows were removed from this analysis, results shown in parentheses. These animals were not completely stunned and therefore had outlying blood pH values.

Table 6.5. Effect of Low (121 meq/kg) and High (375 meq/kg) dEB diets on growth performance characteristics (Least Square Means). The means for each treatment represent the average of eight pens of six barrows each.

Parameter	Low dEB Trt (n = 8)	High dEB Trt (n = 8)	SEM	*P* <
Average daily gain, kg/d	0.79	0.64	0.06	0.28
Average daily feed intake, kg	2.86	2.92	0.07	0.73
Gain/Feed	0.27	0.22	0.02	0.18
Initial Live Weight, kg	124.3	124.8	0.78	0.71
Final Live Weight, kg	126.7	126.8	0.80	0.93

Table 6.6. Effect of Low (121 meq/kg) and High (375 meq/kg) dEB diets on incidence of pars esophageal ulcers and meat quality in experimental animals (n = 8). There were six barrows per pen. Carbon dioxide stunning was used.

Parameter	Low dEB Trt	High dEB Trt	SEM	P <
Ulcer Score[1]	1.2	1.4	0.14	0.52
L *	51.38	51.10	0.27	0.62
b *	6.26	6.30	0.07	0.80
a *	8.63	8.80	0.12	0.52
pH 60 min	6.62	6.57	0.03	0.44
Temperature 60 min, ºC	32.1	32.4	0.45	0.74
Japanese Color Score	2.7	2.9	0.05	0.21
Drip Loss 48 h	3.5	3.6	0.15	0.73
Firmness[2]	1.4	1.4	0.05	0.93
Marbling	1.8	1.8	0.06	0.67
pH 24 h	5.68	5.68	0.01	0.78
Temperature 24h, ºC	1.86	1.87	0.08	0.99
Glycolytic Potential, µmol/g of tissue	101.2	105.0	1.00	0.05
Lactate Content, µmol/g of tissue	91.8	94.6	0.87	0.11
Glycogen Content, µmol/g of tissue	4.8	5.3	0.40	0.32
Hot Carcass Weight, kg	104.2	104.5	0.60	0.83
Cold Carcass Wt, kg	93.7	93.6	0.60	0.91
Dressing %, HWT/live wt	82.1	82.3	0.10	0.57
Dressing %, CWT/live wt	73.4	73.7	0.13	0.29
Carcass Shrink, %	10.3	10.5	0.12	0.62
Estimated Muscle, kg	67.4	65.4	0.66	0.14
Estimated % Muscle (muscle kg/HWT * 100)	64.8	63.0	0.80	0.27

[1]Ulcer Score: 0 = Normal, 1 = Moderate hyperkeratosis, 2 = Severe hyperkeratosis, 3 = Light ulcer, 4 = Moderate ulcer, 5 = Severe ulcer. Stomachs were collected, rinsed, opened along the greater curvature and assigned a score after slaughter.

[2]Firmness score: 1 = firm, 3 = soft.

[3]Glycolytic Potential = Lactate content + 2(Glucose Content)

[4]Glycogen Content = Glucose from glycogen breakdown plus free glucose

Table 6.7. Effect of Low (121 meq/kg) and High (375 meq/kg) dEB diets on meat quality classification in swine (Least Square Means). The values listed are the proportion of carcasses in each meat classification category. The experimental unit was pen and there were 6 barrows per pen.

Meat Classifications[1]	Low dEB Trt (n = 8)	High dEB Trt (n = 8)	SEM	*P*
Dark Firm Dry (DFD)	0	0	0	-
Trend DFD	0.02	0	0.01	0.33
Normal	0.39	0.50	0.05	0.32*
Red Soft Exudative (RSE)	0.00	0.03	0.01	0.15
Pale Firm Normal (PFN)	0.43	0.28	0.06	0.26*
Trend Pale Soft Exudative (PSE)	0.09	0.13	0.03	0.46
PSE	0.08	0.07	0.02	0.76

[1]Meat category classification system (Category = 24 hr ph, drip loss, color, L*): DFD = >6.10, < 2%, ≥ 4, ≤ 42; Trend DFD = 5.8-6.1, < 5%, 3-4, 42-45; Normal = 5.6-5.8, 2-5%, 3, 42-50; RSE = 5.6-5.8, > 5%, 3, 42-50; PFN = 5.5-5.8, <5%, <3, >50; Trend PSE = 5.5-5.6, >5%, 2-3, ≥ 50; PSE = <5.5, >5%, 1-1.5, >50.
* Using animal as the experimental unit, the p-values for the difference in proportion between diet treatmens for normal and PFN carcasses were 0.19 and 0.05, respectively.

Table 7.1. Characterization of the experimental population: Carcass characterisitics.

Item	Mean	Standard Deviation	Minimum	Maximum
Live wt.	126.6	8.4	106.0	143.0
Hot Carcass wt., kg	104.3	6.7	86.5	115.6
Chilled Carcass wt., kg[1]	93.5	6.2	77.8	104.0
Dressing percent (HWT), %	82.2	1.4	77.9	85.6
Dressing percent (CWT), %	73.6	1.3	69.7	75.8
Shrink, %	10.4	1.1	8.2	14.3
Estimated % Lean[2]	64.0	7.2	38.4	86.4
Ulcer score[3]	1	1	0	4

[1]Chilled Carcass wt was taken approximately 22 hours after slaughter

[2]Lean mass (calculated by the packing facility) divided by the hot carcass weight

[3]Stomachs were collected during slaughter and opened along the greater curvature. Stomachs were rinsed and assigned a score based on the following scoring system: 0 = normal, 1 = moderate hyperkeratosis, 2 = severe hyperkeratosis, 3 = light ulcer, 4 = moderate ulcer and 5 = severe ulcer

Table 7.2. Characterization of the experimental population: Pork quality.

Item	Mean	Standard Deviation	Minimum	Maximum
L*	51.21	2.52	45.13	57.74
a*	8.73	1.17	5.94	11.74
b*	6.28	1.00	4.05	9.47
60 min pH	6.59	0.22	6.06	7.04
60 min temperature, ºC	32.2	2.7	26.5	38.9
Muscle color[1]	2.8	0.6	1.0	4.0
Firmness[2]	1.4	0.5	1.0	3.0
Marbling[3]	1.8	0.6	1.0	3.0
24 hr pH	5.68	0.09	5.47	6.17
24 hr temperature, ºC	1.9	0.5	1.0	3.4
48 hr drip loss	3.55	1.52	0.69	7.77
Glycolytic potential[4]	103.1	9.8	78.3	130.5
Muscle lactate content	93.3	7.9	74.3	122.2
Muscle glucose content[5]	5.0	1.8	2.0	13.1

[1]Japanese color score taken at the 10th rib chop on the right side of the carcass
[2]1 = firm, 3 = soft.
[3]1 = devoid, 10 = abundant
[4]Glycolytic potential of the muscle = Lactate content + 2 (Glucose and Glycogen content)
[5]Free glucose and glucose broken down from glycogen

Table 7.3. Pearson correlations exhibiting the relationships between exsanguination blood lactate and meat quality parameters.

Parameter p (n)	Lactate	L*	a*	b*	pH 60 min	TEMP 60min	JCS	Drip Loss	Ulcer	Firm	Marble	HWT	CWT	Dressing HWT/live	Dressing CWT/live	Shrink	pH 24h	TEMP 24h	% Lean	GP[1]	Lactate Content	Glucose Content[2]
Lactate	1.0	0.09 0.31 (119)	-0.05 0.58 (119)	0.03 0.74 (119)	-0.32 0.0004 (118)	-0.18 0.05 (117)	-0.11 0.23 (119)	0.22 0.02 (119)	-0.17 0.07 (117)	0.13 0.17 (118)	0.02 0.86 (118)	-0.14 0.13 (112)	-0.11 0.30 (91)	0.25 0.01 (112)	0.20 0.06 (91)	0.01 0.96 (86)	0.01 0.90 (119)	-0.07 0.43 (119)	0.18 0.06 (107)	-0.14 0.15 (101)	-0.19 0.05 (107)	-0.10 0.31 (109)
L*		1.0 (118)	0.20 0.03 (119)	0.73 0.0001 (119)	-0.15 0.10 (118)	-0.002 0.99 (117)	-0.71 0.0001 (119)	0.46 0.0001 (120)	-0.07 0.46 (117)	-0.02 0.80 (118)	0.09 0.36 (118)	-0.03 0.72 (112)	-0.08 0.44 (91)	-0.06 0.51 (112)	-0.11 0.32 (91)	0.08 0.50 (86)	-0.61 0.0001 (119)	0.19 0.04 (119)	-0.09 0.34 (107)	0.42 0.0001 (101)	0.32 0.001 (107)	0.42 0.0001 (109)
a*			1.0 (118)	0.74 0.0001 (119)	-0.18 0.05 (118)	-0.14 0.14 (117)	-0.02 0.83 (119)	0.38 0.0001 (119)	0.04 0.67 (117)	0.09 0.34 (118)	0.14 0.14 (118)	-0.18 0.06 (112)	-0.18 0.08 (91)	-0.11 0.23 (112)	-0.18 0.09 (91)	0.06 0.58 (86)	-0.30 0.001 (119)	0.14 0.13 (119)	-0.03 0.78 (107)	0.21 0.03 (101)	0.12 0.23 (107)	0.36 0.0002 (109)
b*				1.0 (119)	-0.17 0.06 (118)	-0.08 0.40 (117)	-0.45 0.0001 (119)	0.51 0.0001 (119)	0.004 0.97 (117)	0.11 0.24 (118)	0.14 0.12 (118)	-0.16 0.09 (112)	-0.18 0.09 (91)	-0.08 0.41 (112)	-0.16 0.12 (92)	0.10 0.34 (87)	-0.56 0.0001 (119)	0.20 0.03 (119)	-0.06 0.57 (107)	0.37 0.0001 (101)	0.24 0.01 (107)	0.49 0.0001 (109)
pH 60 min					1.0 (118)	0.20 0.03 (117)	0.16 0.08 (118)	-0.43 0.0001 (118)	0.19 0.04 (116)	-0.24 0.01 (118)	-0.11 0.25 (118)	0.12 0.21 (111)	0.14 0.18 (90)	0.01 0.89 (111)	0.02 0.85 (90)	-0.03 0.78 (85)	0.14 0.12 (118)	0.02 0.86 (118)	-0.09 0.36 (106)	0.03 0.78 (100)	0.07 0.50 (106)	-0.001 0.99 (108)
TEMP 60 min						1.0 (117)	0.09 0.35 (117)	-0.22 0.02 (117)	0.13 0.17 (115)	-0.20 0.03 (117)	0.10 0.28 (117)	0.16 0.09 (110)	0.22 0.03 (89)	0.04 0.71 (110)	0.16 0.14 (89)	-0.08 0.47 (84)	0.05 0.56 (117)	-0.21 0.02 (117)	-0.12 0.24 (105)	0.02 0.88 (99)	0.08 0.44 (105)	-0.13 0.19 (107)
Japanese Color Score							1.0 (118)	-0.37 0.0001 (119)	0.05 0.62 (117)	-0.13 0.17 (118)	0.08 0.41 (118)	0.03 0.74 (112)	0.05 0.61 (91)	0.01 0.92 (112)	-0.02 0.86 (91)	-0.001 0.99 (86)	0.56 0.0001 (119)	-0.18 0.05 (119)	0.01 0.90 (107)	-0.34 0.001 (101)	-0.25 0.01 (107)	-0.33 0.0004 (109)
Drip Loss 48 h, %								1.0 (120)	-0.19 0.04 (117)	0.41 0.0001 (118)	-0.0004 1.00 (118)	-0.17 0.08 (112)	-0.13 0.22 (91)	-0.06 0.53 (112)	0.03 0.75 (91)	-0.09 0.41 (86)	-0.50 0.0001 (119)	0.10 0.27 (119)	0.06 0.55 (107)	0.34 0.001 (101)	0.20 0.04 (107)	0.43 0.0001 (109)
Ulcer Score									1.0 (117)	0.03 0.73 (116)	0.04 0.64 (116)	0.001 1.00 (110)	-0.01 0.93 (90)	-0.13 0.16 (110)	0.03 0.80 (90)	0.003 0.98 (85)	0.10 0.28 (117)	0.07 0.47 (117)	-0.09 0.36 (105)	0.002 0.99 (100)	0.05 0.62 (106)	-0.03 0.77 (108)
Firm										1.0 (118)	-0.09 0.32 (118)	-0.13 0.19 (111)	-0.13 0.22 (90)	-0.11 0.27 (111)	-0.02 0.83 (90)	-0.06 0.56 (85)	-0.12 0.21 (118)	0.09 0.35 (118)	0.07 0.48 (106)	0.04 0.71 (100)	-0.08 0.42 (106)	0.21 0.03 (108)
Marble											1.0 (118)	-0.05 0.63 (111)	-0.01 0.92 (90)	0.03 0.79 (111)	0.002 0.98 (90)	0.05 0.66 (85)	0.04 0.67 (118)	-0.19 0.04 (118)	-0.05 0.63 (106)	-0.04 0.71 (100)	-0.12 0.24 (106)	0.09 0.37 (108)
HWT												1.0 (112)	0.98 0.0001 (86)	0.11 0.24 (112)	0.14 0.20 (86)	-0.03 0.81 (86)	-0.12 0.21 (112)	0.03 0.77 (112)	-0.58 0.0001 (107)	0.18 0.07 (96)	0.21 0.04 (102)	0.04 0.73 (102)
CWT													1.0 (91)	0.09 0.41 (86)	0.22 0.04 (91)	-0.20 0.06 (86)	-0.08 0.46 (91)	0.02 0.83 (91)	-0.52 0.0001 (84)	0.11 0.36 (76)	0.14 0.21 (82)	-0.02 0.85 (81)
Dressing%, HWT/live														1.0 (112)	0.76 0.0001 (86)	0.23 0.03 (86)	0.04 0.67 (112)	-0.12 0.22 (112)	-0.03 0.78 (107)	-0.18 0.07 (96)	-0.16 0.11 (102)	-0.20 0.05 (102)
Dressing%, CWT/live															1.0 (90)	-0.46 0.0001 (86)	0.08 0.47 (91)	-0.17 0.11 (91)	0.05 0.66 (84)	0.14 0.23 (76)	-0.04 0.70 (82)	-0.29 0.01 (81)
Shrink																1.0 (87)	-0.01 0.95 (86)	0.01 0.94 (86)	-0.01 0.95 (84)	0.04 0.75 (72)	-0.05 0.67 (78)	0.18 0.13 (76)
ph 24 h																	1.0 (119)	0.0001 1.0 (119)	0.15 0.12 (107)	-0.46 0.0001 (101)	-0.35 0.0002 (107)	-0.48 0.0001 (109)
TEMP 24h																		1.0 (118)	-0.09 0.38 (107)	0.06 0.55 (101)	0.11 0.26 (107)	0.01 0.91 (109)
Lean %																			1.0 (107)	-0.23 0.03 (91)	0.003 0.98 (97)	-0.35 0.001 (97)
GP[1]																				1.0 (101)	0.94 0.0001 (101)	0.64 0.0001 (101)
Lactate Content																					1.0 (107)	0.34 0.001 (101)
Glucose Content[2]																						1.0 (109)

[1]Glycolytic Potential = (2 x glucose content) + lactate content; [2]Glucose content = Glucose from the breakdown of glycogen and free glucose

Figure 3.1a. Blood lactate concentration at all sampling points in Experiment 1.

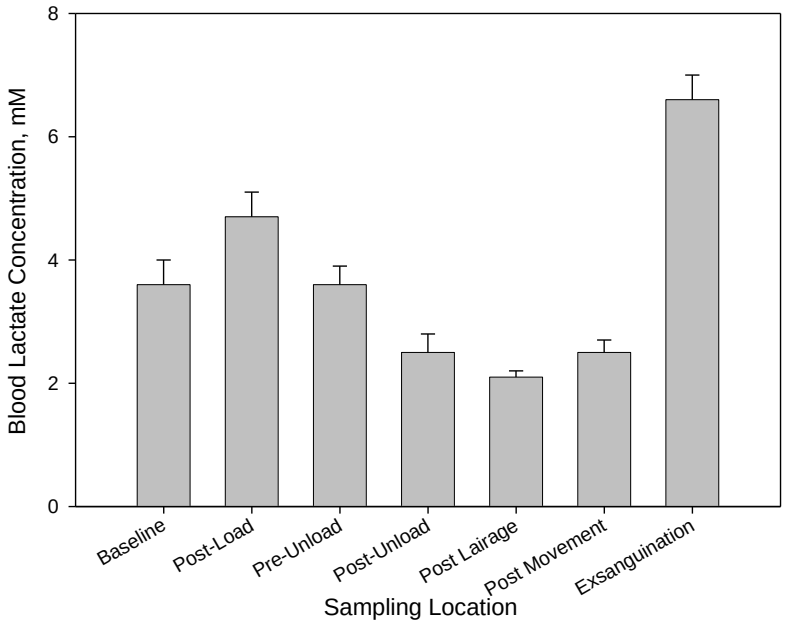

Figure 3.1b. Rectal temperatures across all sampling points in Experiment 1.

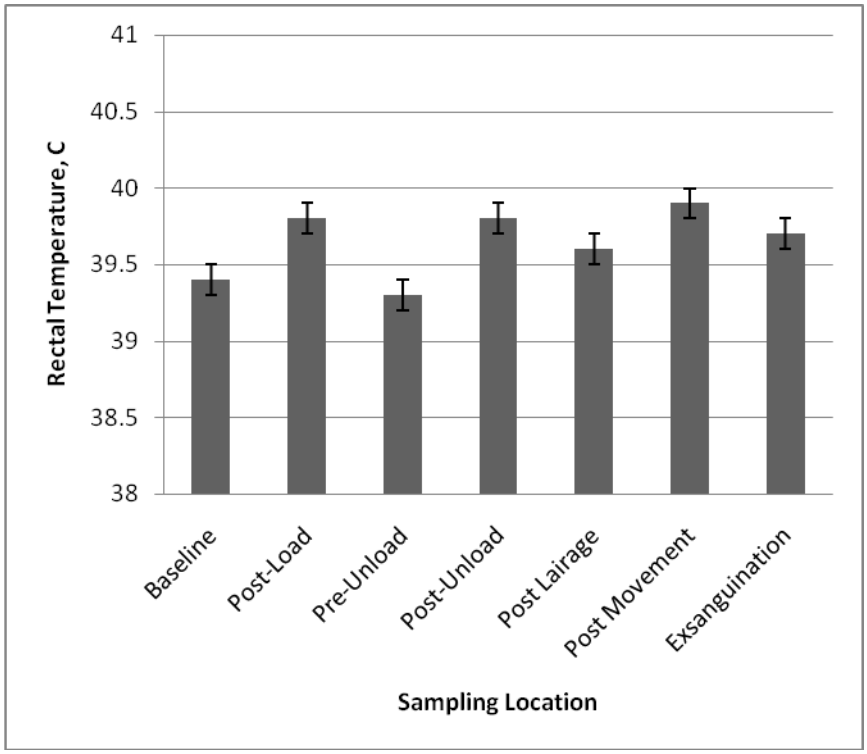

Figure 3.1c. Blood lactate concentration across all sampling points in Experiment 2.

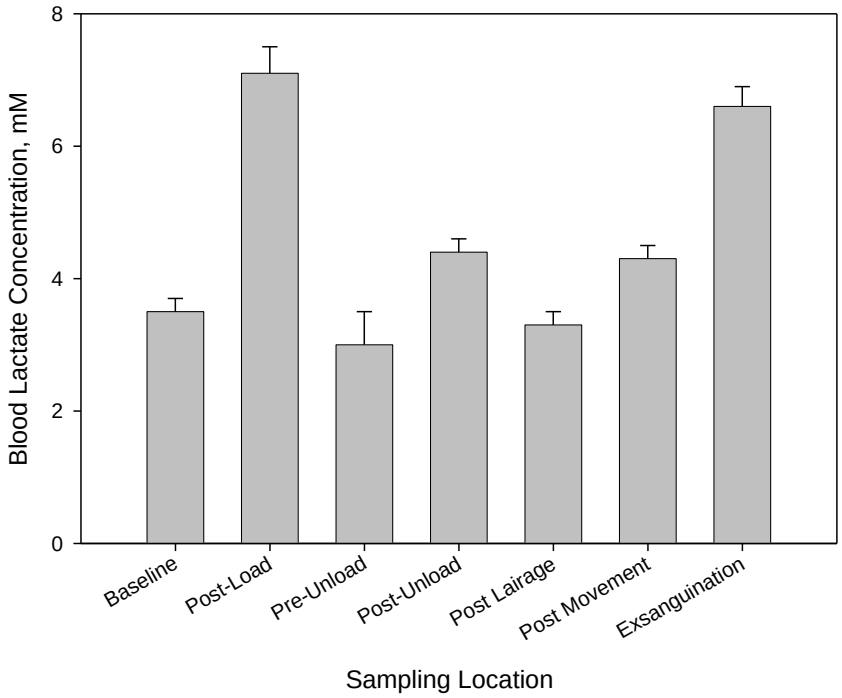

Figure 3.1d. Rectal temperature across all sampling points in Experiment 2.

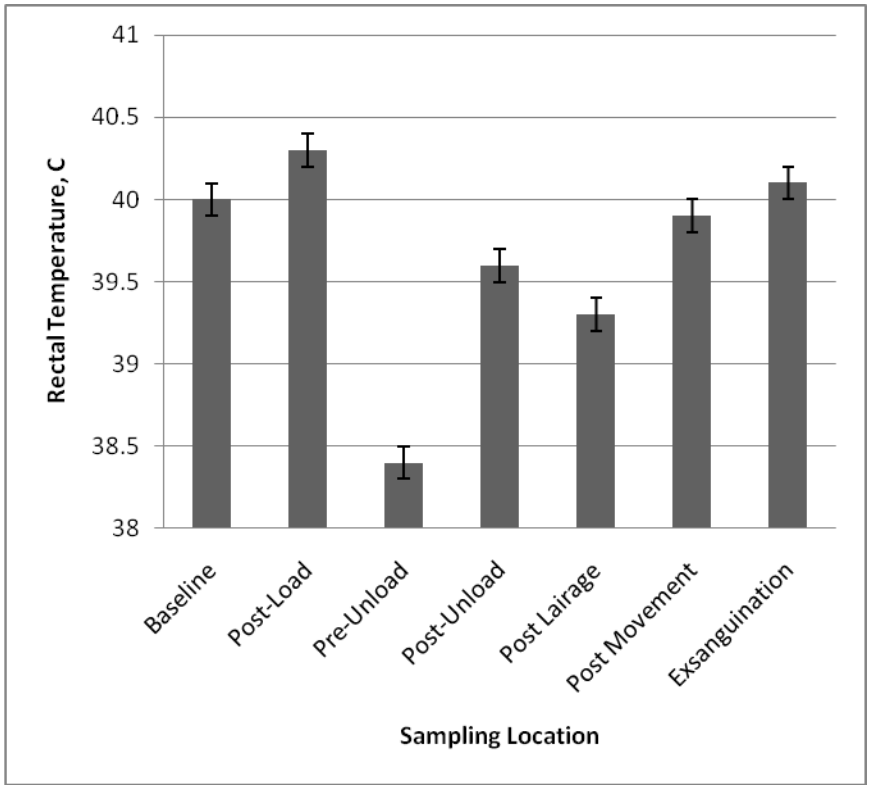

Figure 4.1. Pre-slaughter handling area (image is not to scale).

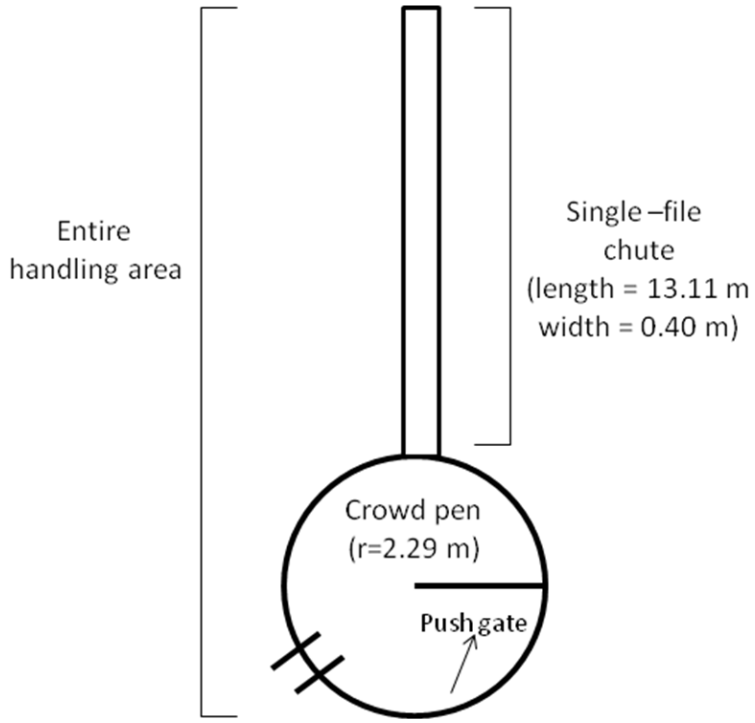

Figure 7.1. Regression of blood lactate concentration (mM) and 60 min pH. One hundred eighteen animals were used in this analysis.

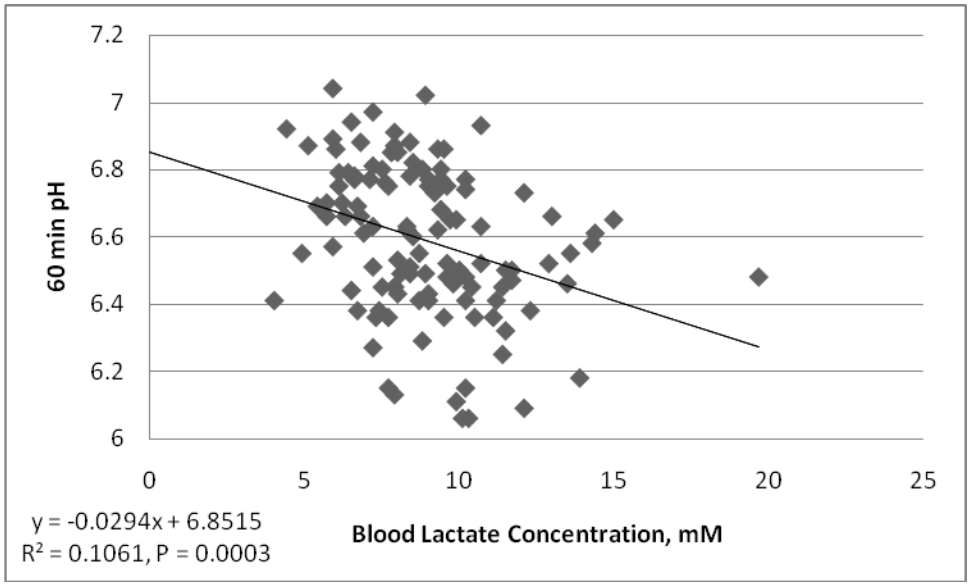

Figure 7.2. Mean drip loss of animals with blood lactate concentrations (mM) less than and greater than 10 mM separated by glycolytic potential value (< or > 100 umol/g of muscle).