EVALUATING MITIGATION STRATEGIES TO PROMOTE RECOVERY FROM ACUTE HYPERTHERMIA IN SWINE

by

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A Dissertation

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Doctor of Philosophy

Department of Animal Sciences

West Lafayette, Indiana

December 2019

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For Ayoko, my wife and best friend

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to Dr. Jay Johnson, my graduate mentor for giving me the opportunity to pursue my PhD, for all his teaching and transfer of knowledge, and for spending so much time in helping me.

I would like to thank my committee members, Dr. John Radcliffe, Dr. Brianna Gaskill, Dr. Kimberly Buhman, and Dr. Adeola Olayiwola for serving on my graduate committee, for their additional guidance during my graduate study, and for their open door policy that allowed me to seek help at any time.

Thanks to my fellow graduate students for their assistance. I would also like to thank all the undergraduate assistants for their help during live trials, data entry, and video analyses.

I would not finish without expressing my appreciation to USDA-ARS Livestock Behavior Unit for giving me the opportunity to pursue my PhD.

Finally, thanks to my family and friends whose support and encouragement gave me the strength to finish the program.

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Institution: Purdue University
Degree Received: December 2019
Title: Evaluating Mitigation Strategies to Promote Recovery From Acute Hyperthermia In Swine
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Heat stress (**HS**) is one of the consequential important problems facing the swine industry. The negative effects of HS include reduced growth performance, reproductive efficiency, and carcass quality as well as increased morbidity and mortality. Although, the swine industry has developed several abatement strategies (i.e., fans, cooling pads, sprinklers, etc.), these approaches may be ineffective in the future as global temperatures continue to rise and the frequency of more severe heat waves increases in regions where animal agriculture is prevalent. These extreme heat events put pigs (especially those approaching market weight) at risk for acute hyperthermia that can lead to death unless body temperature is rapidly returned to euthermia and thermoregulatory function is restored. Therefore, evaluating mitigation strategies to promote recovery from acute hyperthermia is of utmost importance for improving pigs' health and well-being and ensuring profitability and food security. In four experiments, the existence of microclimates in grow-finish barns during late summer was ascertained and a rapid cooling technique using cold water dousing and feed removal to promote recovery from acute hyperthermia in pigs was evaluated. In the first study, it was determined that microclimates exist in grow-finish barns and that pigs raised in pens that were not located directly below air inlets and ventilation fans had greater body temperature and reduced feed efficiency despite similarities in the in-barn ambient temperature and relative humidity. These data exemplify the importance of adequate ventilation systems in swine barns and the impact of microclimates on pigs' health and productivity during warm summer months. In the second study, grow-finish pigs that did not have feed access were exposed to acute HS and then rapidly or gradually cooled. Following the acute HS and recovery phase, all pigs were maintained under thermoneutral conditions and then euthanized over three days to determine the temporal effects of the cooling treatment on body temperature and intestinal integrity. The results showed that rapid cooling following acute hyperthermia in pigs was effective in returning body

temperature to euthermia more rapidly compared to gradual cooling and rapid cooling prevented further intestinal damage. Based on these results, it was hypothesized that feed removal may have played a role in the effectiveness of rapid cooling. Therefore, a third experiment was conducted in which grow-finish pigs with or without access to feed were exposed to an acute HS challenge and then rapidly cooled. This study concluded that feed access was a determinant factor in the cooling outcome, as the gastrointestinal temperature returned to euthermia during the rapid cooling period more rapidly when feed was removed. Finally, a fourth study was conducted to evaluate the effects of feed removal in the absence of rapid cooling on the systemic inflammatory response and short-term growth performance of grow-finish pigs. However, it was determined that feed removal alone did not reduce the inflammatory response as expected. Overall, these studies demonstrate the risk for grow-finish pigs during summer heat events and the potential use of rapid cooling in combination with feed removal for promoting recovery from acute hyperthermia in pigs.

CHAPTER 1.   LITERATURE REVIEW

1.1   Introduction

Heat stress (**HS**) negatively affects livestock health and productivity, resulting in substantial economic losses (St-Pierre et al., 2003). Although advances in management practices including environmental and nutritional modifications have been implemented over the years, production remains suboptimal and morbidity and mortality have increased due to extreme heat events (Baumgard and Rhoads, 2013; Nienaber and Hahn, 2007). These negative consequences of HS are expected to increase as global temperature as well as heat wave frequency and intensity continues to rise (NOAA, 2018), and it is likely that this will jeopardize global food security (Baumgard et al., 2015).

Animals modify their physiology to cope with environmental temperature insults; however, the physiological changes elicited in this regard may negatively affect health and reduce production efficiency in pigs (Johnson, 2018). Under HS conditions, feed intake is reduced, and post-absorptive metabolism is shifted toward increased glucose usage and reduced adipose tissue mobilization for energy (Baumgard and Rhoads, 2013), resulting in reduced growth performance and carcass quality. In addition, a HS-induced increase in intestinal permeability is linked to endotoxemia and a greater inflammatory response (Lambert, 2009). These negative impacts of HS are likely to be worsened in the case of acute hyperthermia because current management strategies may not be adequate in an extreme heat event. Therefore, management strategies to promote recovery from acute hyperthermia are necessary for improving pigs' health and reducing economic losses.

1.2   Heat stress impacts on animals

Heat stress negatively affects animal agriculture, creates substantial economic losses (St-Pierre et al., 2003), and jeopardizes global food security (Battisti and Naylor, 2009). In tropical and subtropical regions where the temperature is warm throughout the year, HS is a constant problem as opposed to temperate regions where the summers are occasionally hot (Renaudeau et al., 2010). The negative impacts of HS are seen in different aspects of production across all livestock species

(St-Pierre et al., 2003). In the US, HS is estimated to cause the livestock industry about $2.4 billion annually (St-Pierre et al., 2003). With abatement strategies, the economic losses can only be reduced to $1.7 billion or by 29%. For the swine industry, the economic losses are estimated at $300 million, of which $202 million are from the grow-finish pigs (St-Pierre et al., 2003). Although, grow-finish pigs and lactating sows are at greater risk of HS, it is important to note that significant losses may also occur during gestation and later in life due to *in utero* HS, which has been shown to permanently increase body temperature in pigs (Johnson et al., 2015a).

Economic losses occur through reduced performance, reproductive efficiency, and product quality (Baumgard and Rhoads, 2013). Reduced feed intake, an indirect effect of HS, has been thought to be totally responsible for decreased production performance (as reviewed by Baumgard et al., 2015). Pigs subjected to a constant heat load for 6 h had 80% reduction in feed intake and lost 3 kg body weight compared to thermoneutral-exposed pigs (Pearce et al., 2014). A 3-d diurnal HS exposure reduced feed intake by 30% and produced a 7% weight loss compared with thermoneutral pigs (Gabler et al., 2018). However, recent reports indicate that HS directly shift energy metabolism. This is supported by the fact that under HS conditions, glucose usage is increased while fat mobilization from adipose tissues is decreased (Baumgard and Rhoads, 2013), which may reduce carcass quality due to lower meat yields. Specifically, HS has been shown to increase overall carcass fat in pigs (Heath, 1983; Kouba et al., 2001; Xin et al., 2016), and decrease the lean-to-fat ratio (Johnson et al., 2015b). Another avenue HS causes economic loses is through reduced reproductive efficiency, which is characterized by delayed onset of puberty, decreased farrowing rate, reduced spermatozoid count, abnormal spermatozoids, and intrauterine growth retardation that affects performance later in life (Ross et al., 2017; Johnson, 2018). In addition to the reduction in productivity, carcass quality, and reproductive performance, severe HS often results in death or increased health care costs due to morbidity (Baumgard and Rhoads, 2013).

1.3 Thermoregulation

The thermal strategies used by homeotherms to regulate and maintain internal temperature homeostasis involve non-evaporative heat loss, evaporative heat loss, and heat production (Blatteis, 1998). The non-evaporative heat loss, also referred to as sensible heat loss, is achieved through the channels of conduction, convection and radiation (Blatteis, 1998). Depending on the

environmental temperature, animals can either increase heat production or heat loss to maintain a constant body temperature (Figure 1.1; Mount, 1979). Between C-D (thermoneutral zone; Figure 1.1), animals can maintain thermal homeostasis with minimal metabolic heat production and achieve maximal production performance (Hillman, 2009). In the thermoneutral zone, body temperature is mainly maintained by the non-evaporative sensible heat loss. The thermoneutral zone is delimited at the lower end by the lower critical temperature (**LCT**) and at the upper end by the upper critical temperature (**UCT**). When the ambient temperature ventures above the UCT (Figure 1.1: D), animals increase heat dissipation by evaporative cooling (Figure 1.1: D-E) and decrease metabolic heat production by reducing feed intake to regulate internal body temperature (Hillman, 2009). However, should thermoregulatory mechanisms fail, the body temperature would continue to rise leading to hyperthermia (Figure 1.1: F). Conversely, when the environmental temperature falls below the LCT (Figure 1.1: C), animals reduce heat loss through vasoconstriction and increase metabolic heat production to maintain euthermia (Figure 1.1: B-C), but failure to regulate body temperature in B-C leads to hypothermia (Figure 1.1: A). For most mammals, heat production can be increased by shivering, non-shivering thermogenesis, and metabolic heat. Non-shivering thermogenesis is heat produced above the basal metabolism without any muscular activity by the sympathetic activation of brown fat (Blatteis, 1998). Non-shivering thermogenesis is an important source of energy for thermoregulation in most mammal neonates due to the presence of brown adipose tissue, except in pigs (Blatteis, 1998). This puts piglets at greater risk for hypothermia due to lack of brown fat and inability to use non-shivering thermogenesis. Greater metabolic heat production can be achieved by an increase in feed intake (Westerterp, 2004) and shivering (Blatteis, 1998; DeShazer et al., 2009). Shivering is defined as involuntary skeletal muscle contraction that increases metabolism and generates heat (Blatteis, 1998).
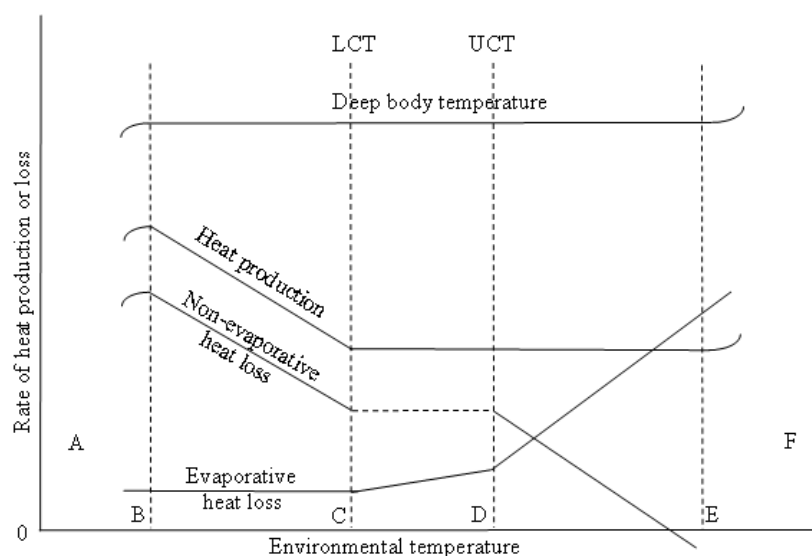
Figure 1.1 Deep body temperature regulation balancing heat production, non-evaporative and evaporative heat loss as a function of environmental temperature.

A, zone of hypothermia; BC cool zone; CD, thermoneutral zone; DE, warm zone; F, zone of hyperthermia; UCT, upper critical temperature; LCT, lower critical temperature. Adapted from Mount, 1979 and Kadzere et al., 2002.

1.3.1   Non-evaporative heat loss

Non-evaporative heat exchange channels (conduction, convection, and radiation) are effective means for dissipating heat when the body temperature is greater than environment temperature. However, these channels become ineffective in dissipating heat when the thermal gradient between the surface and the air is reduced or contribute to heat gain when the thermal gradient is negative (Collier and Gebremedhin, 2015).

1.3.1.1   Radiation

Radiative heat exchange occurs between objects that are not in contact. Radiant energy is emitted in the form of electromagnetic waves through space (Acker and Cunningham, 1991). It involves shortwave radiation from the sun with a wavelength of 0.3-4.0 µm and longwave radiation from the ground, surrounding objects, and the atmosphere with a wavelength between of 4.0-100 µm (DeShazer et al., 2009). The magnitude of the electromagnetic wave emitted or absorbed by an animal depends on emissivity, surface temperature, effective radiative area, and coat color

(Morimoto, 1998; Gebremedhin, 2012). Emissivity is the ratio of the radiant energy emitted by the surface to that emitted by a black body at the same temperature (Gebremedhin, 2012). In an unbuffered outside environment, an animal is exposed to both the shortwave from the sun and the longwave from the surroundings, and the surface color becomes a determinant factor in radiant energy absorption (Mount, 1979). An animal with a dark-colored coat absorbs more radiant energy compared to white coat in the visible portion of the spectrum (Gebremedhin, 2012). However, in confinement or in the absence of sunlight, the radiant energy exchange depends solely on the longwave, the absorption of which is unaffected by surface color (Mount, 1979; DeShazer et al., 2009).

1.3.1.2 Conduction

Conductive heat exchange is based on kinetic energy transfer from warmer to cooler molecules. Heat is transferred from the body to air or other objects in contact with the skin surface (Blatteis, 1998). The magnitude of conductive heat transfer depends on the surface temperature of objects in contact, surface contact area, and thermal conductivity (Blatteis, 1998). For instance, the thermal conductivity of air is 4% of that of water (0.024 $W.m^{-1}.k^{-1}$ compared with 0.561 $W.m^{-1}.k^{-1}$, respectively; Blatteis, 1998). Pigs use conductive heat loss extensively in the barn or outside in the wild. In the wild, pigs increase heat dissipation by lying in wallows (Olsen et al., 2001). They spend more time lying on the cold floor when ambient temperature rises (Aarnink et al., 2006) and adopt positions that increase their surface contact (up to 20% of the total surface area) to maximize conductive heat loss and regulate body temperature (Bruce and Clark, 1979).

1.3.1.3 Convection

Convective heat loss involves the movement of molecules in the air or a liquid medium (Blatteis, 1998). In the air, two types of convection are observed. First, a natural or free convection occurs when the air in contact with the skin surface is heated. Due to reduced density, the heated air rises and is replaced by cooler air (DeShazer et al., 2009). Second, a forced convection is achieved when air is moved by natural or force ventilation over a surface (Mount, 1979; Blatteis, 1998). Within the body, convective heat transfer occurs through blood flow to and from different anatomical regions, and to the thermal shell (Iups, 2001). The increase in blood flow to the skin and mucosal surfaces of the respiratory system through vasodilation supports heat loss to the surroundings

(Blatteis, 1998). Convective heat loss occurring in the respiratory tract constitutes an important avenue of heat loss (Maia and Loureiro, 2005).

1.3.2 Evaporative heat loss

Evaporation is a transition from liquid to gas, a change in physical state that requires energy (Fournel et al., 2017). The energy required to vaporize water, latent heat of vaporization, comes from the skin or the respiratory tract where the process takes place leading to the cooling of the vascular bed underneath them and the surrounding tissues (Blatteis, 1998). The latent heat of vaporization of water, which depends on prevailing temperature, decreases from 598 cal at 0°C to 579 cal at 30°C per gram of water (Curtis, 1983). This energy is taken from the body itself making evaporation a highly effective cooling mechanism (Curtis, 1983). Evaporative cooling is driven by vapor pressure differences and remains the only efficient means for heat loss when thermal gradient is too narrow or negative (Curtis, 1983). Evaporative cooling takes place on the skin and the upper respiratory tract depending on the species. Water for cutaneous heat loss comes through the skin by diffusion, but mostly from the sweat (Mount, 1979).

Active sweat glands are present in most mammals, except in pigs (Hillman, 2009). Sweat glands are limited (Renaudeau et al., 2006) and unresponsive to thermal stimulation in pigs (Ingram, 1967). Consequently, pigs need supplemental wetting of the skin for cutaneous evaporative loss. While the opportunity for supplemental wetting of the skin exists (wallows) in the wild (Olsen et al., 2001), it is generally lacking in modern production housings, except when sprinklers are used. Therefore, pigs may wallow in their urine and feces to increase evaporative heat loss when the temperature rises (Huynh et al., 2005a). In addition to cutaneous evaporative heat loss, respiratory evaporative heat loss is used by all animals. The respiratory evaporative heat loss occurs through air movement in and out of the respiratory tract. During inspiration, air is saturated with water vapor and heated (conduction and convection), and the inspired air temperature is equal to that of the body by the time it reaches the alveoli (Mount, 1979). The vaporization of water from the mucosal lining accounts for the evaporative cooling (Mount, 1979). During expiration, parts of the latent heat (condensation) and sensible heat are returned to the mucosa. As a result, the expired air temperature is lower compared to that of the body, but greater than the inspired air (Mount, 1979). Increased respiration rate (panting) leads to cooling of the mucosa and the underlying vascular bed,

thus removing significant heat from the body (Mount, 1979). In mammals, increased respiration rate above normal serves as an early sign of HS (Nienaber and Hahn, 2007).

1.4 Animal response to heat stress

Animals have developed coping mechanisms to alleviate the negative effects of elevated environmental temperatures (Collier et al., 2018). The most important ones are heat acclimation and heat shock response.

1.4.1 Heat acclimation, acclimatization, and adaptation

Acclimation is the physiological or behavioral changes that are developed by an animal in response to a single environmental stressor (Bernabucci et al., 2010). In response to HS, these changes are aimed at reducing the heat strain. Stress is often confused with strain. Stress is any external force or agent (e.g. heat) that displaces a biological system from its resting state (Yousef, 1985). Strain is the internal displacement from the resting state resulting from the stress or stressor, for example, an increase in body temperature is response to HS (Spiers, 2012). The acclimation response involves the central nervous system, effector cells (Hypothalamic-Pituitary-Adrenal axis), and hormones including thyroid hormones (T3 and T4), prolactin, glucocorticoids, and mineralocorticoids (Bernabucci et al., 2010), and requires days or weeks to develop (Bernabucci et al., 2010; Ely et al., 2014). In swine, acclimation to elevated ambient temperature can occur in approximately 10 days from the initial exposure in some breeds (Renaudeau et al., 2008). Contrary to acclimation, acclimatization involves physiological or behavioral changes in response to several individual stressors simultaneously in the natural environment, either climatic or environmental (Bernabucci et al., 2010). They improve the fitness of an animal to survive stressful conditions (Bernabucci et al., 2010). These physiological or biological changes disappear when the environmental stressors are removed. However, when the stressors remain for prolonged periods of time, animals will adapt to these environmental conditions (Yousef, 1985).

Adaptation is defined as genotypic or phenotypic changes that reduce the physiological strain caused by the prolonged environmental stressors (Bernabucci et al., 2010). Phenotypic adaptations that reduce physiological strain on an animal during its lifetime are not passed on to the next generation. For example, pigs raised in cold weather (5°C) had smaller ears, shorter limbs and tail,

and are hairier compared with littermates reared in warm (35°C) weather (Weaver and Ingram, 1969). Contrary to phenotypic adaptations, genetic adaptations can occur across generations or in one generation and are heritable to ensure the survival of a specie in adverse conditions (Yousef, 1985). For example, cattle and goats (Gaughan et al., 2018) from subtropical regions develop morphological adaptations (i.e. hair coat color and thickness, skin thickness, and limbs conformation) making them more adapted to HS compared with those in temperate regions (Berman, 2011).

1.4.2 Behavioral thermoregulation

Behavioral thermoregulation occurs to increase heat loss and reduce environmental heat load when the ambient temperature approaches the animal's UCT. To maximize heat loss, animals change posture from lying to standing position to increase the surface area for evaporative and convective heat loss, or lying on a cool surface to increase conductive heat loss (Hillman, 2009). Furthermore, animals wet their skin or wallow to increase evaporative heat loss (Hillman, 2009; Olsen et al., 2001). Wallowing in the mud is more effective than just wetting the skin because the mud holds more water for an extended period of time allowing pigs to maximize heat loss (Collier and Gebremedhin, 2015). Additional thermoregulatory behaviors include seeking shade to reduce direct solar radiation (Hillman, 2009; Terrien et al., 2011) and adopting lying or standing position to avoid direct solar and ground radiations (Gaughan et al., 2018). Another thermoregulatory behavior to decrease heat load is the reduction in feed intake (Baumgard and Rhoads, 2012). Feed consumption generates heat due to nutrient processing and fermentation in the gastrointestinal tract (Curtis, 1983). The heat of nutrient processing is generated by the activity of eating (prehension, mastication, ingestion, rumination), digestion, absorption, and utilization of the nutrients (Curtis, 1983; Brown-Brandl et al., 2004). On the other hand, the heat of fermentation is produced by microbial activities in the gastrointestinal tract, and it is most significant in ruminant (Curtis, 1983). Reduced feed intake during HS is well-documented across livestock species (de Souza et al., 2016; Abuajamieh et al., 2018). In the swine industry, the reduction in feed intake is more pronounced because pigs are more susceptible to HS due to the lack of functional sweat glands, the presence of subcutaneous fat that impedes heat dissipation (Hillman, 2009), and genetic selection for leaner meat that increases muscle mass and protein turnover resulting in greater heat production (Renaudeau et al., 2011).

1.4.3 Physiological changes

When the ambient temperature approaches or exceeds the upper limit of the thermoneutral zone, behavioral adjustments become ineffective and heat-stressed animals resort to physiological changes involving evaporative heat loss: sweating and increased respiration rate or panting (Hillman, 2009). All livestock species utilize both sweating and panting to some extent, except poultry and swine. Poultry and swine do not sweat and rely mostly on guttural fluttering or panting for evaporative heat loss (Johnson, 2018). Under commercial production systems where pigs do not have access to a wallow to wet their skin for evaporative cooling, they rely on heat loss from the respiration as the main avenue of evaporative heat loss. Hence, the increase in respiration rate has been used as an initial physiological indicator of HS in pigs (Brown-Brandl et al., 2001; Patience et al., 2005; Pearce et al., 2015; Seibert et al., 2018). Respiration rate has been shown to increase depending on the relative humidity at a given ambient temperature. The upper critical limit, at which respiration rate starts increasing, was about 2°C lower in growing pigs when high humidity increased from 50 to 80% (Huynh et al., 2005b).

Although a rise in respiration rate or panting is an efficient way to increase evaporative heat loss, it is well-known to disrupt the blood acid-base balance and cause respiratory alkalosis (Patience et al., 2005; Collier and Gebremedhin, 2015). Panting causes excessive loss of CO_2 resulting in decreased partial pressure of CO_2 in pigs (Patience et al., 2005; Liu et al., 2018), poultry (Teeter et al., 1985; Borges et al., 2004) and sheep (Chauhan et al., 2015). The changes in partial pressure of CO_2 in combination with the reduction in bicarbonate levels increase pH, and this results in respiratory alkalosis (Hamm et al., 2015). Respiratory alkalosis may have negative consequences on physiological processes, biochemical reactions (Liu et al., 2018), and growth performance as reported in broiler chickens (Teeter et al., 1985). Thus, it is important that respiratory alkalosis be prevented to improve animal health. To alleviate respiratory alkalosis, nutritional supplementations have been investigated. For example, vitamin E (200 IU/kg) supplementation reduced respiratory alkalosis in heat-stressed pigs (Liu et al., 2018).

1.4.4 Heat shock response

The heat shock response is a short and rapid molecular mechanism, at the cellular levels, that is used as the body's response to sublethal temperatures (Horowitz, 2002). The mechanism involves

heat shock proteins (**HSP**), which act primarily as chaperone and cellular repair molecules (Horowitz and Robinson, 2007). They are involved in nascent protein folding, protein trafficking between cellular compartments, and the prevention of protein denaturation and aggregation (Kim et al., 2018). Heat shock proteins are a large family of proteins that are classified based on their molecular weight and include HSP40, HSP60, HSP70, HSP90, 110kDa and small HSP (Kim et al., 2018). However, the most abundant and best characterized are the HSP70 families. Heat shock proteins can be constitutive or inducible. The inducible forms are produced in response to various stressors including heat, hypoxia, energy depletion, and nutritional stress (Kregel, 2002). These stressors evoke a rapid increase in HSP production, which is mediated by heat shock factor (**HSF**) 1. Heat shock factor 1 is bound to cytosolic HSPs in an inactive form. In response to any of the aforementioned stressors, HSF1 is dissociated from the cytosolic HSP and is then phosphorylated for trimerization before its translocation in the nucleus (Kiang and Tsokos, 1998). Once in the nucleus, HSF1 binds to heat shock element in the promoter region and initiates the transcription of several HSP genes mRNA, which are translocated in the cytosol for HSPs synthesis (Kregel, 2002).

Heat shock proteins have been increased in various organs including intestine, kidney, liver etc. where their cytoprotective function has been established following HS (as reviewed by Archana et al., 2017). For example, HSP90 was upregulated in pigs intestine due to HS (Cervantes et al., 2016). Heat stress causes intestinal damage and HSP90 was increases to protect intestinal cells and prevent protein denaturation or aggregation (Kim et al., 2018). Although, HSPs act primarily in the cytosol, they can be released extracellularly in response to stress (Sepponen and Pösö, 2006; Hecker and McGarvey, 2011; Calderwood et al., 2016). The mechanisms of their release from cells are not well understood, but two main hypotheses prevail (Asea, 2007). First, HSPs are passively released from necrotic cells, severe blunt trauma, surgery, and following infections, but not from cells undergoing apoptosis. Second, HSPs can be released via exosomes (Bausero et al., 2005; Asea, 2007). The HSP within exosomes may play an important role in the immune system (Bausero et al., 2005).

1.5   Heat stress and gastrointestinal barrier function

1.5.1   Gastrointestinal barrier function

The gastrointestinal track serves multiple functions including nutrient digestion and absorption, immunity, and as a physical barrier between the luminal content and the general circulation, (Celi et al., 2019). The intestinal epithelium, a monocellular layer, is composed of enterocytes, goblet cells, enteroendocrine cells, and Paneth cells (Sánchez de Medina et al., 2014). It forms a highly selective barrier that allows the absorption of nutrients and ions, but restricts the passage of large molecules and pathogens into the body (Van Spaendonk et al., 2017). Movements across the intestinal epithelium occur through the transcellular and paracellular pathways. The transcellular pathway is achieved via passive and active transport. While the passive transport is a passage of molecules across the enterocyte membrane along their concentration gradient, the active transport requires energy generated by ATP hydrolysis or electro-osmotic gradient by NA^+/K^+-ATPase pump at the basolateral membrane of the enterocytes (Fanning et al., 1999). The paracellular transport is a passive diffusion and is tightly regulated by a tight junction complex that seals the intercellular spaces on the intestinal epithelium (Van Spaendonk et al., 2017).

The tight junction is comprised of transmembrane proteins such as claudins, occludins, junctional adhesion molecules, and scaffolding proteins (e.g., zona occludens: **ZO**), which anchor transmembrane proteins to the actin cytoskeleton (Figure 1.2; Martínez et al., 2012). These proteins define the barrier function of the paracellular space. Claudin, the most apical component of the tight junction, regulates the pore pathway that is selective of the size and charge across the paracelullar space (Raleigh et al., 2011). Zonula occludens regulates the paracelullar passage of uncharged molecules (Raleigh et al., 2011). The roles of occludin is not well-understood, but it is located at the tight junction in its phosphorylated state (Suzuki et al., 2009). The intestinal tight junction is a dynamic structure that responds to multiple factors, and different phosphorylation-based pathways are involved in its regulation. Briefly, in response to stimuli, the myosin light chain kinase (**MLCK**: a Ca $^{2+}$ calmodulin-dependent Ser-Thr kinase) phosphorylates the myosin light chain (**MLC**). Once phosphorylated, MLC undergoes conformational changes that causes the contraction of the actomyosin cytoskeleton and the internalization of occludins resulting in increased permeability (Martínez et al., 2012). Alternatively, when located in the tight junction under normal conditions, occludin is highly phosphorylated at Thr residues (Suzuki et al., 2009).

Upon stimulation, these residues are dephosphorylated resulting in the internalization of occludin (Martínez et al., 2012).
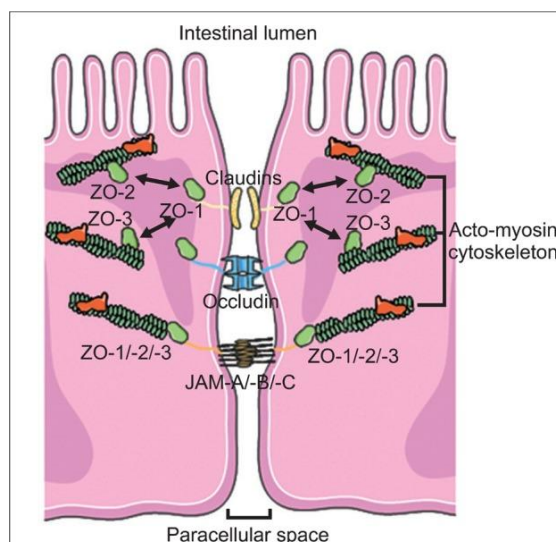
Figure 1.2 Molecular components of the tight junction.

Abbreviations: **ZO,** Zonula occludens; **JAM**, Junctional adhesion molecule (Martínez et al., 2012).

1.5.2 Gastrointestinal barrier dysfunction under heat stress

1.5.2.1 Tight junction

Under normal conditions, the tight junction is selective and generally impermeable to large molecules with molecular weight greater than 150 kDa (Gupta et al., 2017). However, when exposed to heat, the tight junction is disrupted, increasing the intestinal permeability to larger molecules such as food antigens and endotoxins (Lambert, 2009). This disruption is the sum of direct heat toxicity to the enterocytes and a cascade of events resulting from physiological changes in response to hyperthermia (Gupta et al., 2017). To maximize heat loss, hyperthermic mammals redistribute blood to the periphery through subcutaneous vasodilation and gastrointestinal track vasoconstriction (Romanovsky and Blatteis, 1996). This causes ischemia of the intestinal epithelium resulting in hypoxia, free radical production, ATP depletion, and acidosis (Lambert et al., 2002; Gupta et al., 2017). These physiological stimuli may trigger MLC phosphorylation resulting in the tight junction disruption and increased intestinal permeability (Martínez et al., 2012).

The increase in intestinal permeability has been measured by the flux of macromolecules such as fluorescein isothiocyanate-dextran, inulin, etc.) and the transepithelial resistance (**TER**) of the epithelium (Fanning et al., 1999). The intestinal TER was reduced in heat-stressed pigs (Pearce et al., 2012; Pearce et al., 2014; Liu et al., 2016). Dokladny et al., (2008) demonstrated that the TER was inversely correlated with the paracellular transport when caco-2 cells were exposed to elevated temperature. Heat stress increased paracellular transport of fluorescein isothiocyanate-dextran in pigs (Pearce et al., 2012; Pearce et al., 2014; Liu et al., 2016), chickens (Song et al., 2014), and rats (Oliver et al., 2012). Beside the immediate HS-induced disruption of tight junctions, acute HS or long-term HS exposure leads to changes in tight junction protein gene expression (Dokladny et al., 2015). Zonula occludens 1, claudin 1, and occludins are downregulated in both the jejunum and ileum in chickens exposed to cyclical HS for 21 d (Wu et al., 2018). However, exposure to constant HS of 35°C for 24 h increases ileal claudin 3 and occludin gene expression, while claudin 1 is not affected in pigs (Pearce et al., 2013). Therefore, the effect of HS on tight junction protein expression may differ depending on species HS protocol and intensity.

1.5.2.2 Intestinal mucus layer

The intestinal mucus layer plays an important role in intestinal barrier function by preventing pathogen contact with the intestinal epithelium and allowing selective passage of nutrients (Sánchez de Medina et al., 2014). The mucus is formed by mucins (**MUC**), gel-like glycosylated proteins produced by goblet cells. Several MUC ranging from MUC1 to MUC20 have been identified; however, MUC2 is the major one secreted by goblet cells (Kim and Ho, 2010). Goblet cells secrete other products including trefoil peptides involved in epithelium restitution, resisting-like molecule β that plays an immunological function, and fragment crystallizable (Fc)-γ binding protein that stabilizes the mucin network in the mucus layer (Kim and Ho, 2010). The mucus layer also contains antimicrobial peptides (β defensins and lysozymes) secreted by Paneth cells and secretory IgA secreted by enterocytes (Kim and Ho, 2010). The physical characteristics and antimicrobial peptides of the mucus layer make it the first line of defense in the intestine (Capaldo et al., 2017). Unfortunately, several stressors such as weaning, feed restriction, infection, and heat can negatively affect the goblet cells and mucus layer function (Jung and Saif, 2017; Johnson et al., 2018). For example, jejunal and ileal goblet cell surface area was decreased in pigs recovering from a 3 d HS-exposure (Abuajamieh et al., 2018) and ileal goblet cell counts were reduced in

heat-stressed quails (Sandikci et al., 2004). Conversely, Ashraf et al., (2013) reported increased goblet cell counts and increased MUC 2 production in heat-stressed broilers. Similarly, Pearce et al., (2014) reported increased MUC 2 production in pigs exposed to HS for 6 h. This inconsistent goblet cell dynamics may be related to differences in species, intestinal segments, and HS protocols (Abuajamieh et al., 2018).

1.6 Heat stress and the immune system

Heat stress activates the hypothalamic-pituitary-adrenal axis, resulting in the secretion of glucocorticoids that play an important role in the immune system function (Habeeb et al., 2018). The hypothalamus secretes corticotropin releasing hormone, which stimulates the anterior pituitary to release the adrenocorticotropic releasing hormone (Chrousos, 1995). Adrenocorticotropic releasing hormone in turn stimulates the adrenal cortex to secrete corticosterone or cortisol, depending on the species. Corticosterone is predominant in birds, rodents, reptiles, and amphibians, while cortisol is secreted in most mammals and in fish (Cockrem, 2013). These glucocorticoids play a critical role in the regulation of inflammatory responses by inhibiting production of proinflammatory cytokines (IL-12, TNF-α, and IFN-γ), and stimulating anti-inflammatory cytokines (IL-10 and IL-4) production to keep the immune system in check (Elenkov and Chrousos, 2002).

Heat stress is also associated with endotoxemia. Lipopolysaccharide (**LPS**), derived from the membrane of gram-negative bacteria and present in the gut, is a potent stimulator of the immune system (Wyns et al., 2015). Hyperthermia-induced increased intestinal permeability increases LPS translocation in the systemic circulation (Lambert et al., 2002; Pearce et al., 2013; Johnson et al., 2016), which can result in increased inflammtory responses. In addition, hyperthermia has been shown to increase the release of IL-1α and IL-6 from skeletal muscle into the systemic circulation (Bouchama and Knochel, 2002).

1.7 Heat stress and energy partition

The energy consumed by an animal and its repartitioning towards production is affected by the thermal environment (DeShazer et al., 2009). The gross energy of the diet is partitioned into net

energy, waste (i.e. feces, urine, and gases), and heat increment (Figure 1.3; Pond et al., 2004). The net energy is the portion of the energy consumed by the animal that will be used for maintenance and production. Under HS conditions, the heat mitigation efforts increase the maintenance cost (Campos et al., 2014) reducing energy available for production. Heat stress also reduces feed intake thereby decreasing the gross energy consumed by the animal. In addition, HS has been associated with endotoxemia (Gabler et al., 2018) and increased whole-body inflammatory responses (Lambert, 2009). This implies that energy for production will be repartitioned toward activation and maintenance of the immune system (Kvidera et al., 2017). Furthermore, heat shock response developed by animals in response to sublethal temperature is costly (Krebs and Loeschcke, 1994). The production and function of HSPs as chaperone molecule require energy, which is repartitioned from the net energy for production (Baumgard et al., 2013).
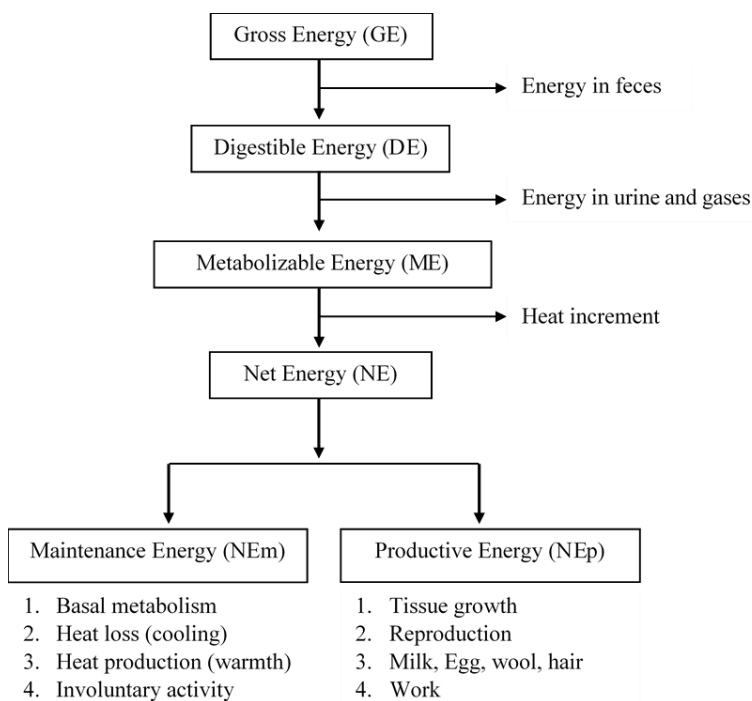
Figure 1.3 Schematic diagram of Energy partition.

Adapted from Pond et al., 2004.

1.8   Heat stress mitigation strategies

Heat stress affects multiple aspects of physiology, immunology, metabolism, and anatomy leading to poor health, welfare, and productivity in livestock. It is important to develop mitigation strategies to reduce the negative impacts of HS on animals and to ensure global food security. Strategies to mitigate the negative impacts of HS can be achieved through nutritional intervention, management, and improvements in genetic selection (Johnson, 2018).

1.8.1   Nutritional strategies

Nutritional management to reduce HS involves manipulation of the energy and protein content of the diet and supplementation with feed additives (Cottrell et al., 2015). First, altering the crude protein and crude fiber contents of the diet is based on the principle that their consumption generates more heat and may increase body temperature during times of HS (Patience et al., 2015). Crude protein and crude fiber are generally reduced while fat content is increased due to its lower heat increment (Mayorga et al., 2018). This practice provides heat-stressed pigs with means to reduce heat production without reducing energy intake, which could compensate for the effects of depressed feed intake on growth performance. Second, the use of feed additives may be beneficial in targeting the negative effects of HS. For example, the physiological changes that occur to support heat dissipation results in oxidative stress that damages intestinal epithelium and increases intestinal permeability to endotoxins (Hall et al., 1999).

Different feed additives including minerals, antioxidants, and amino acids have been investigated to target intestinal health. Zinc is well-known to promote wound healing and intestinal function (Lansdown et al., 2007). Intestinal mucosal damage was reduced in weanling pigs fed a high dose (500 mg/kg) of nano zinc oxide above the NRC (2012) requirements. Zinc has been shown to improve intestinal barrier integrity with zinc supplementation at 220 ppm in in heat-stressed growing pigs (Fernandez et al., 2014). Antioxidants, such as 1.0 ppm Se and 200 IU/kg Vit E supplementation above the NRC (2012) recommendations, have been shown to reduce gut leakiness in heat-stressed pigs (Liu et al., 2016). Dietary supplementation with betaine, a methylated amino acid, has been shown to improve intestinal health. Betaine mainly functions to donate methyl groups for several methylation reactions in the body (Kettunen et al., 2001). However, it also serves as osmolyte in cells subjected to osmotic pressure (Cronje, 2005). Dietary

betaine accumulation in epithelial cells makes them more resistant to osmotic pressure and to heat-induced intestinal damage (Cronje, 2005). Another amino acid, glutamine, a primary source of energy for enterocytes, may be used to improve intestinal health under HS conditions. Dietary glutamine prevented the loss of intestinal barrier function in heat-stressed mice (Soares et al., 2014) and broilers (Wu et al., 2018). Furthermore, although glutamine has been shown to improve overall health and productivity following stressors, such as weaning and transport (Duttlinger et al., 2019) or transport and HS in pigs (Johnson and Lay Jr, 2017), its impact on the intestinal health under HS in grow-finish pigs is yet to be investigated.

1.8.2 Management strategies

Heat stress management strategies constitute a more direct way to reduce heat load in livestock. Mitigation systems based on evaporative heat loss, conductive and convective heat transfer have been demonstrated to be effective.

1.8.2.1 Air conditioning

The air conditioning system circulates refrigerated air through the barn to decrease air temperature. It is the most effective method for mitigating HS in livestock (Fournel et al., 2017). The use of air conditioning improved milk yield and conception rates in dairy cows (Thatcher et al., 1974). Compared with shading, air conditioning improved pregnancy rates in cows (Wise et al., 1988). Although, air conditioning can be an effective heat mitigation strategy in livestock, it is costly to operate and to maintain and may not be economically viable (Armstrong, 1994).

1.8.2.2 Shade systems

Providing shade is the simplest and the most economical way to reduce direct solar radiation (Fournel et al., 2017). Shading systems are effective in any climate and can be provided naturally by trees or by artificial roofs. The use of shading decreased respiration rate and rectal temperature in dairy cows (Brown-Brandl et al., 2005; Veissier et al., 2018). Shading has been used to provide a microclimate in crop-livestock systems and reduce the temperature and humidity index by 3.7% compared to unshaded areas (Karvatte et al., 2016). Furthermore, the respiration rate was reduced in shaded cows compared to unshaded ones (Veissier et al., 2018). In pigs, Blackshaw and Blackshaw, (1994) showed that shade-seeking behavior was greater than 87% in boars, sows, and growing pigs when the ambient temperature increased from 26 to 30°C, and was 85% in weanling

pigs at ambient temperature above 35°C. This indicates that when the ambient temperature is above the UCT of their thermoneutral zone, outdoor pigs would seek shade to reduce environmental heat load.

1.8.2.3 Ventilation

Ventilation plays a major role in controlling the environmental conditions in livestock buildings by removing heat and moisture, limiting the buildup of noxious gases (ammonia, carbon dioxide etc.), and minimizing odor and dust (Rong et al., 2016). Livestock buildings are mechanically or naturally ventilated (Mossad, 2009). Natural ventilation is powered by wind and thermal buoyancy and does not require energy compared with mechanical ventilation (Brockett and Albright, 1987; Zhang et al., 1989). It is most effective in sidewall and ridge opening buildings, which are well oriented to avoid direct sunlight (Renaudeau et al., 2012). Although natural ventilation lower energy costs compared with forced ventilation, it cannot provide a complete control over airflow within livestock buildings because it depends on the temperature gradient between inside and outside air and wind speed and direction (Ecim-Djuric and Topisirovic, 2010; Jones et al., 2015a).

Contrary to natural ventilation, mechanical or forced ventilation gives more control over the environmental conditions inside animal facilities but requires energy. Mechanical ventilation includes positive, negative, and neutral pressure systems (Jones et al., 2015b). For the negative pressure system, exhaust fans expel air out creating a vacuum that pulls fresh air into the building through inlets. Positive pressure system forces outside air into the building with fans to replace warmer air. The third mechanical ventilation system is the neutral pressure system, which combine both the negative and positive pressure systems. The neutral pressure system uses fans to push air in and out of the building (Jones et al., 2015b). Different forms of mechanical ventilation including low-profile cross ventilation and tunnel ventilation were developed to increase airflow in livestock buildings. Low-profile cross ventilation system moves air parallel to animals or across the buildings, whereas tunnel ventilation moves air down the length of the building (Fournel et al., 2017). In the tunnel ventilation, air inlets and exhaust fans are located at opposite ends of the building (Smith and Harner, 2012). Regardless of the ventilation type, ventilation must provide adequate air movement for effective evaporative and convective heat loss (Renaudeau et al., 2012).

1.8.2.4   Sprinklers

Sprinkler systems allow supplemental wetting of the animal's hair coat or skin, which in combination with air movement constitutes one of the most effective means for removing excessive body heat in animals (Renaudeau et al., 2012). Sprinkler systems are generally combined with ventilation and are used worldwide across species (Renaudeau et al., 2012; Fournel et al., 2017). Sprinklers reduced the temperature humidity index by 6 units (Fournel et al., 2017). When using sprinklers, heat load was reduced and milk yield was improved in dairy cattle (Turner et al., 1992). Sprinkler systems with high air velocity of tunnel ventilation reduced HS in broiler chickens (Liang et al., 2014). In pigs, sprinkler systems decreased respiration rate and skin temperature, and increased average daily gain by 19% compared to water bath and non-cooled controls (Huynh et al., 2006). Although, sprinklers are effective in removing excessive body heat, they are less efficient on heavier animals due to lower surface area-to-mass ratio (Renaudeau et al., 2012). Additionally, sprinkler efficiency is improved when larger water droplets are applied intermittently to allow time for water to evaporate from the skin (Renaudeau et al., 2012).

1.8.2.5   Evaporative pads

The evaporative pad cooling consists of moving air into a building through a wet pad. The incoming air transfers energy to the pad, which in turn loses it in the form of latent heat. As a result, the ambient temperature in the building is lowered while its relative humidity is increased (Fournel et al., 2017). Evaporative pad cooling is extensively used in farrowing houses. It has been shown to reduce respiration rate and skin temperature and increase conductive heat loss in sows (Justino et al., 2014). However, due to the risk of increased relative humidity, evaporative pad cooling may not be effective in high humid regions (Renaudeau et al., 2012).

1.8.2.6   Fogging/misting

Fogging and misting are based on the evaporative cooling principle. While misting injects larger droplets under high pressure (> 5 MPa), fogging generates finer water molecules under lower pressure ($\leq$ 5 MPa) into the air (Haeussermann et al., 2007). As the water droplets evaporate, heat is removed from the air, and the relative humidity and water vapor increases inside the building (Fournel et al., 2017).

1.8.2.7 Conductive cooling

Conductive cooling is based on heat transfer down the thermal gradient between an animal and a cooler surface, such as water mattress, heat exchanger underneath the bedding, circulating cooled water through the floor or cooling pad (Ortiz et al., 2015; Riskowski et al., 2018). In dairy cows, embedded heat exchangers have been used to increase conductive heat loss (Gebremedhin et al., 2016). Moreover, cooled perches have been shown to delay panting in hens (Hu et al., 2016). In pigs, flood cooling by circulating cold water through pipes embedded in concrete floors has improved thermal comfort and growth performance in grow-finish pigs (Huynh et al., 2004). Furthermore Cabezón et al., (2017) demonstrated that cooling pads in farrowing crates can reduce the negative effects of HS on lactating sows.

1.8.2.8 Rapid cooling

Rapid cooling is important for alleviating the negative impacts of acute hyperthermia. Acute hyperthermia is the most severe form of heat related illnesses. It occurs when the body's heat dissipation mechanisms are unable to balance heat loss and gain resulting in a rapid increase in core body temperature (Bouchama et al., 2007). Acute hyperthermia leads to multiorgan injury and death or neurological damage in survivors without prompt return of body temperature to euthermia (Bouchama and Knochel, 2002; Armstrong et al., 2007; Leon and Helwig, 2010). The prognosis of acute hyperthermia depends on the duration of heat exposure, how fast cooling started, and prior medical conditions such as cardiovascular diseases (Hifumi et al., 2018). Several rapid cooling techniques exist and include immersion in cold water, application of ice pack or cooling blanket on the body, and wetting the body surface with water in combination with fans (Armstrong et al., 1996; Smith, 2005; Gaudio and Grissom, 2016). Among these cooling techniques, cold water immersion has been shown to be the most effective treatment for acute hyperthermia (Casa et al., 2007).

Animals are at risk for acute hyperthermia during extreme heat events, and rapid cooling methods could be a viable method for alleviating its negative effects. Rapid cooling by cold water (4 to 6°C) dousing has been evaluated in different species including horses for exercise-induced hyperthermia (Marlin et al., 1998), antelopes for capture-induced hyperthermia (Sawicka et al., 2015), and pigs for classic hyperthermia (Johnson et al., 2016). In the first two studies (Marlin et al., 1998; Sawicka

et al., 2015), rapid cooling was effective in reducing body temperature (based on rectal temperature) compared with the study conducted in pigs (Johnson et al., 2016). The differences between these three studies may be due to feed access. In the study by Johnson et al., (2016), pigs were allowed access to feed during the cooling procedure as opposed to the other studies (Marlin et al., 1998; Sawicka et al., 2015), and feed access may have caused a delay in the return to euthermia since feed intake increases body temperature (Cervantes et al., 2018).

1.9   Summary

Despite the management strategies used by the swine industry, production remains suboptimal, and morbidity and mortality are increased due to extreme heat events during the summer. In the case of extreme heat events, grow-finish pigs are at greater risk of acute hyperthermia due to low surface area-to-mass ratio and the presence of subcutaneous fat layer that impedes heat dissipation. Therefore, evaluating and developing strategies to mitigate the negative impacts of acute hyperthermia in pigs is warranted. Because acute hyperthermia results from the inability of the body to maintain temperature homeostasis, a rapid return of body temperature to euthermia is key for recovery and favorable prognosis. Feed removal and rapid cooling have been evaluated in poultry and humans, respectively, and constitute possible avenues to promote recovery from acute hyperthermia in pigs. However, the modality and conditions of their applications for effective results in pigs are unknown. Additionally, variation in environmental conditions within swine facilities may affect pigs' thermoregulation during warm summer months. Therefore, the aims of the studies conducted for this dissertation were to evaluate the effects of HS conditions on swine physiology and assess the effectiveness of rapid cooling and feed removal as management strategies to promote recovery from acute hyperthermia in pigs.

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CHAPTER 2.   EFFECTS OF PEN LOCATION ON THERMOREGULATION AND GROWTH PERFORMANCE IN GROW-FINISH PIGS DURING LATE SUMMER

2.1   Abstract

The effects of pen location on swine thermoregulation and growth performance were determined over 6 weeks during late summer. A total of 128 mixed sex pigs [Duroc x (Landrace x Yorkshire)] were randomly assigned to 16 pens in two grow-finish barns (n = 8 pens/barn; 57.43 ± 1.33 kg initial (**BW**)). Pen locations were determined based on orientation to ventilation fans and air inlets. Internal pens (**IP**; n = 4/barn) were in direct line of sight between the fans and air inlets while peripheral pens (**PP**; n = 4/barn) were located 0.70 ± 0.29 m to either side of a fan. Two sentinel gilts per pen were selected and vaginal temperature (T_V) was measured in 10-min intervals using T_V data loggers. Additionally, trunk skin temperature (T_S) was measured with an infrared camera and respiration rate (**RR**) was measured by counting flank movements of the sentinel gilts twice daily (0800 and 1500 hours). Pen airspeed was measured twice daily (0800 and 1500 hours) at pig level with an anemometer. Individual pen ambient temperature (T_A) and relative humidity (**RH**) were recorded daily in 10-min intervals. Feed consumption and BW were determined every 2 weeks. Data were analyzed using PROC MIXED in SAS 9.4. Although airspeed was reduced overall ($P = 0.01$; 11%) in PP compared to IP, no differences ($P > 0.10$) in T_A (27.53 ± 1.73°C) or RH (68.47 ± 5.92%) were detected. An overall increase ($P \leq 0.02$) in T_V (0.23°C), minimum T_V (0.18°C), and maximum T_V (0.29°C) was detected in PP versus IP housed pigs. Similarly, from 0800 to 1900 hours and 2000 to 0700 hours, T_V was greater overall ($P \leq 0.01$; 0.22 and 0.25°C, respectively) in PP compared with IP housed pigs. An overall decrease in T_S ($P = 0.04$) was observed in PP (37.39 ± 0.14°C) compared with IP (37.61 ± 0.14°C) housed pigs. No RR differences ($P > 0.10$; 76 ± 4 breaths per minute) were detected with any comparison. While no average daily gain (**ADG**) and average daily feed intake (**ADFI**) differences were detected ($P > 0.10$; 0.74 ± 0.03 kg/d and 2.26 ± 0.08 kg/d, respectively), gain-to-feed ratio (**G:F**) was decreased

($P = 0.02$; 6%) in PP compared to IP housed pigs. In summary, pigs located in PP had greater body temperature and reduced G:F despite similarities in T_A and RH between all pens.

Keywords: feed efficiency, pen location, pigs, productivity, thermoregulation

2.2   Introduction

In intensive production systems, pigs are reared in confinement to provide optimal environmental conditions and maximize welfare and productivity. However, higher summer temperatures can overwhelm cooling systems in swine facilities (i.e., fans, evaporative coolers), thus subjecting pigs to temperature conditions above their thermal comfort zone. Exposure to ambient temperature (**T_A**) above the thermal comfort zone (i.e., heat stress) can negatively impact reproductive efficiency, growth rate, and health in pigs resulting in economic losses despite advances in barn cooling technologies (Axaopoulos et al., 1992; St-Pierre et al., 2003). In addition, variation in either T_A, relative humidity (**RH**), or airspeed creates microenvironments in swine barns (Costa et al., 2014; Massari et al., 2016). Although numerous reports have evaluated the direct effects of heat stress on production losses (as reviewed by Johnson et al., 2015a) and thermoregulation (Huynh et al., 2007), few have investigated the impact of microenvironments on swine thermoregulation and productivity.

Previous reports demonstrated that in-barn environmental variability affected pig behavior (Geers et al., 1986; Costa et al., 2014). Furthermore, the existence of microclimates within farrowing barns negatively impacts sow productivity (Morello et al., 2018). However, few studies have investigated the effects of microclimates on thermoregulation and productivity in grow-to-finish facilities. Therefore, the study objective was to ascertain the existence of microclimates in grow-finish barns and characterize their impacts on swine productivity and thermoregulation during late summer.

2.3   Materials and methods

2.3.1   Animals and experimental design

All procedures involving pigs were approved by the Purdue University Animal Care and Use Committee (protocol #1603001380). Animal care and use standards were based upon the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (Federation of Animal Science Societies, 2010). The study was conducted from mid-July to mid-August 2016 over a 6-week period at the Purdue Animal Sciences Research and Education Center Swine Farm, in West Lafayette, IN. Data loggers (HOBO; accuracy $\pm$ 0.2°C; data logger temp/RH; Onset; Bourne, MA) were used to monitor average daily T_A (21.79 to 29.96°C range) and RH (65.16 to 92.60% range) outside the barns. A total of 128 mixed sex (50% barrows and 50% gilts) crossbred pigs [Duroc x (Landrace x Yorkshire); 57.43 $\pm$ 1.33 kg initial (**BW**)] were randomly assigned to 16 pens in two grow-finish barns (n = 8 pens/barn)] and pens were balanced across treatments by sex and BW. Both barns (72.2 m x 10.1 m) were identical and had concrete side walls, concrete slatted floors and were side ventilated with 13 hood fans (TURBO 0.10 static pressure; 61 cm diameter; 117.5 m^3/min per fan) on 1 wall, and 13 air inlets on the opposite wall. Throughout the study, ventilation fans were set based on in-barn T_A and pig level airspeed ranged from 0.10 to 0.60 m/s, which was within the recommended cold to hot weather ranges for finishing pigs, respectively (Midwest Plan Service, 1972). All pens (4.27 m x 1.68 m) were separated by 0.91-m high panels with vertical bars. Each pen was equipped with adjustable nipple type drinker and two-hole dry feeder. Pigs were housed in pens located in two distinct locations within each barn, based on the orientation of the pens to ventilation fans and air inlets. Internal pens (**IP**; n = 4/barn) were directly in between the fans and the air inlets, while the peripheral pens (**PP**; n = 4/barn) were located 0.70 $\pm$ 0.29 m to either side of the closest ventilation fan. All pigs had ad libitum access to feed and water and were fed standard commercial corn-soybean meal based diets for two phases of 21 d each to meet nutrient requirements (NRC, 2012) based on age per standard swine industry practice (phase 3: 88% dry matter (**DM**), 3351.2 kcal/kg metabolizable energy (**ME**), 16% crude protein (**CP**) and 0.85% standardized ileal digestible (**SID**) lysine; phase 4: 88% DM, 3356.6 kcal/kg ME, 14.2% CP and 0.73% SID lysine).

2.3.2 Measurements

Each individual pen was equipped with one data logger mounted at pig height to record pig level T_A and RH in 10-min intervals throughout the entire experiment. Individual pen airspeed (m/s) was measured with an anemometer (Testo Model 425; Sparta, NJ) at the pig level (approximately 0.50 m above the slatted floor) twice daily (0800 and 1500 hours) during the thermal measurement periods.

Two sentinel gilts were randomly selected per pen for vaginal temperature (T_V), trunk region skin temperature (T_S), and respiration rate (RR) measurements within three periods (P) lasting two weeks each ($P1$ = week 1 and 2; $P2$ = week 3 and 4; $P3$ = week 5 and 6) for 9 d per period (27 days in total). The same two sentinel gilts per pen were monitored throughout the entirety of the experiment and their data were averaged for the entire pen. Calibrated thermochron temperature recorders (iButton, accuracy $\pm$ 0.1°C; Dallas Semi-conductor, Maxim, Irving, TX) were attached to blank controlled internal drug releasing devices (Eazi-Breed CIDR; Zoetis, New York, NY), and inserted intravaginally into the two sentinel gilts selected per pen to record T_V in 10-min intervals 24 h per day throughout the entire 27-d monitoring period. T_V monitors were constructed in accordance with a previous report by Johnson and Shade (2017). RR and T_S were assessed in the sentinel gilts twice daily (0800 and 1500 hours) throughout the entire 27-d monitoring period. RR (breaths per min; **bpm**) was determined by counting flank movements for 15 s and then multiplying by 4. Trunk T_S was measured by taking a broadside photo of individual pigs from a distance of approximately 1.5 m using an infrared camera (FLIR Model T440, accuracy $\pm$ 0.1°C; emissivity = 0.95; FLIR Systems Inc, USA). Care was taken to ensure that the side of the pig was dry during thermal imaging so that T_S was not influenced by previous contact with the ground that could leave excess moisture on the skin. Because of this, the side of the pig in which the thermal image was taken was not always consistent. Infrared photos were analyzed with the FLIR Tools software (version 2.1). For image analysis, the minimum, maximum, and mean temperature of the trunk region of the pig (i.e., all skin caudal to the neck and dorsal to the elbow and stifle) was measured. For RR and T_S, an average daily value and an average value for the morning (0800-1000 hours) and for the afternoon (1500-1700 hours) were calculated and used in the final analysis. For T_V, an average daily value, a value for the daytime (0800-1900 hours) and nighttime (2000-0700 hours), a daily maximum T_V, and daily minimum T_V were calculated and used in the final

analysis. Feed consumption and BW on a per pen basis were measured at the end of P1, P2 and P3 and were used to determine ADFI, ADG, and G:F for each period.

A thermal circulation index (**TCI**) was calculated using T_S, T_A and T_V in the following equation as described by Curtis (1983): $TCI = (T_S - T_A) / (T_V - T_S)$. The TCI was used to determine the pig capacity to dissipate heat from the core to the skin and subsequently to its surroundings under steady state thermal conditions. T_V and T_A were averaged from 0800-1000 hours and 1500-1700 hours to correspond to the timeline of T_S measurements and used in the TCI calculation.

2.3.3   Statistics

Data were analyzed using the PROC MIXED procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). Because pigs were group housed during all measures, pen was considered the experimental unit for all analyses. Barn was included in the model as a random effect, while pen location (IP, PP), day of measurement (1-27) or period (P1, P2, P3), and their interaction were considered fixed effects. A two-sample t-test was performed to compare the initial BW and final BW between PP and IP housed pigs. Day of measurement effects are only presented and discussed when there is an interaction with pen location because it was expected that overall day differences would be observed due to natural daily variation in environmental conditions and only the effects of pen location were of interest in the present study. All thermal indices data were analyzed using repeated measures and the covariance structure was determined based on goodness of fit criteria (Littell et al., 1998) with day as the repeated effect. Performance data were analyzed using repeated measures and covariance structure was selected based on goodness of fit criteria (Littell et al., 1998) with period as the repeated effect. Pen initial BW was used as covariate, but it was not significant for any of the performance parameters and was dropped from the final analysis. Values are reported as least square means ± SE, statistical differences were considered at $P \leq 0.05$ and tendencies were considered at $0.05 < P \leq 0.10$.

2.4   Results

2.4.1   Environmental conditions

No pen location by day interaction was detected ($P > 0.10$) for T_A, maximum daily T_A, minimum daily T_A, the maximum T_A vs minimum T_A difference, RH, maximum daily RH, minimum daily

RH, the maximum RH vs minimum RH difference, and airspeed (Table 2.1; Figure 2.1). Overall, no differences were detected ($P > 0.10$) in T_A, maximum daily T_A, minimum daily T_A, the maximum T_A vs minimum T_A difference, RH, maximum daily RH, minimum daily RH, and the maximum RH vs minimum RH difference between PP and IP (Table 2.1). However, airspeed was reduced overall ($P = 0.01$; 11%) in PP compared with IP (Table 2.1). Overall, day of measurement had an effect on T_A ($P < 0.01$), where T_A ranged from 24.60 ± 0.30°C on day 9 to 30.50 ± 0.30°C on day 25 (Figure 2.1A). Similarly, day of measurement had an effect on RH ($P < 0.01$), which ranged from 58.30 ± 0.80 % on day 22 to 80.03 ± 0.80 % on day 6 (Figure 2.1B). airspeed tended to be greater ($P = 0.06$) on days 5, 6 and 12 compared with days 10, 13, 16, 17, 21 and 23 (Figure 2.1C). Day of measurement differed overall ($P < 0.01$) for maximum, minimum, and their difference for RH and T_A (data not presented).

2.4.2 Vaginal temperature

No pen location by day interaction was detected ($P > 0.10$) for any T_V comparison (Table 2.1; Figure 2.2). T_V was greater overall ($P < 0.01$; 0.23°C) in PP compared with IP housed pigs (Table 2.1). Overall, minimum and maximum T_V were greater ($P \leq 0.02$; 0.18 and 0.29°C, respectively) in PP compared to IP housed pigs (Table 2.1). From 0800-1900 hours and 2000-0700 hours, T_V was greater ($P \leq 0.01$; 0.22 and 0.25°C, respectively) in PP compared with IP housed pigs (Table 2.1). Day of measurement had an overall effect on T_V ($P < 0.01$), which ranged from 39.53 ± 0.09°C on d 27 to 39.99 ± 0.09°C on d 12 (Figure 2.2).

2.4.3 Skin temperature

No pen location by day interaction was detected ($P > 0.10$) for any T_S comparison (Table 2.1; Figure 2.3). Skin temperature was decreased overall ($P = 0.04$; 0.22°C) in PP compared with IP housed pigs (Table 2.1). Day of measurement had an overall effect on T_S ($P < 0.01$), which ranged from 36.37 ± 0.20°C on day 9 to 38.25 ± 0.20°C on day 11 (Figure 2.3). In PP compared with IP housed pigs, T_S was reduced ($P = 0.03$; 0.30°C) from 0800-1000 hours and tended to be reduced ($P = 0.09$; 0.17°C) from 1500-1700 hours (Table 2.1).

2.4.4 Thermal circulation index

The TCI tended to be reduced ($P = 0.08$) in PP compared with IP housed pigs on days 15, 16, 20, 24, 25 and 26 (Figure 2.4). The TCI was decreased overall ($P < 0.01$; 21%) in PP compared with

IP housed pigs (Table 2.1). Day of measurement had an overall effect on TCI ($P = 0.01$), which ranged from 3.44 ± 0.49 on day 10 to 5.80 ± 0.58 on day 20 (Figure 2.4).

2.4.5   Respiration rate

There was no pen location by day of measurement interaction effect ($P > 0.10$) for RR, RR from 0800-1000 hours, and RR from 1500-1700 hours (Table 2.1). RR was similar ($P = 0.87$; 76 ± 4 bpm) for PP and IP housed pigs (Table 2.1). Similarly, no pen location differences were observed ($P > 0.10$) for RR from 0800-1000 hours (64 ± 3 bpm) and 1500-1700 hours (88 ± 5 bpm; Table 2.1). However, day of measurement had an effect ($P < 0.01$) on RR, which ranged from $65 \pm$ bpm on day 9 to 90 ± 5 bpm on day 25 (data not presented).

2.4.6   Growth performance

No pen location by period interaction effect was detected ($P > 0.10$) for ADG, ADFI, and G:F (Table 2.2). No overall ADG or ADFI differences were detected ($P > 0.10$; 0.74 ± 0.03 kg/d and 2.26 ± 0.08 kg/d, respectively) between PP and IP housed pigs (Table 2.2). However, G:F was reduced overall ($P = 0.02$; 6%) in PP compared with IP pens (Table 2.2). Average daily feed intake was greater overall ($P < 0.01$; 15.7%) during P3 compared with P1 and P2 (Table 2.2). Similarly, ADG was greater ($P < 0.01$; 14.8%) during P3 compared with P1 and P2 (Table 2.2). There was no period effect detected for G:F (Table 2.2). When comparing PP to IP housed pigs, no differences ($P > 0.10$) in initial BW (57.75 ± 1.93 kg and 57.12 ± 1.97 kg, respectively) and final BW (89.72 ± 1.83 kg and 87.82 ± 1.23 kg, respectively) were detected (data not presented).

2.5   Discussion

To mitigate the negative impacts of heat stress on swine, the in-barn environmental conditions may be improved through building design, ventilation systems, and the use of evaporative cooling techniques (Renaudeau et al., 2012). Despite these improvements, variation in environmental conditions (i.e., T_A, RH, and airspeed) within facilities may occur and this can negatively affect swine productivity (Morello et al., 2018). In the present study, pigs located in PP had a reduction in G:F compared with those housed in IP, but no differences in ADG or ADFI were detected. While the lack of ADG differences are surprising considering previous research describing reduced ADG in pigs reared in pens located away from fans and air inlets (Kluzáková et al., 2013),

the reduced G:F indicates that swine performance may be influenced by in-barn location. Although the specific mechanism for the reduction in G:F is currently unknown, it may be due to thermoregulatory differences between pigs located in PP versus IP since increased body temperature can reduce G:F in growing pigs (as reviewed by Johnson, 2018).

A reduced ability to dissipate body heat can result in elevated core body temperature and subsequently reduced productivity in swine (Renaudeau et al., 2008; Johnson et al., 2015a). In the present study, PP housed pigs had overall increased T_V, minimum T_V and maximum T_V compared with those in IP, and this may have resulted in the aforementioned reduction in G:F of PP housed pigs. Several studies have reported that heat stress negatively impacts G:F in swine (Kerr et al., 2003; Renaudeau et al., 2008; Johnson et al., 2015a), and it has been suggested that this may be due to the physiological strain caused by increased body temperature. Increasing body temperature causes morphological changes to the intestine indicative of damage (Pearce et al., 2012), and because PP housed pigs has a greater body temperature, they may have had more intestinal damage compared with IP housed pigs. As a result, the absorptive capacity of the intestine may have been reduced, resulting in a decrease in digestible energy gained from the feed for PP compared with IP housed pigs. Furthermore, an increase in body temperature can increase intestinal permeability to pathogens, which activates the immune system in pigs (Baumgard et al., 2015), and this is an energetically costly process (Kvidera et al., 2017) that may re-partition energy away from growth and reduce G:F. Regardless of the mechanism, it appears that pen location may influence body temperature in pigs and negatively affect performance.

Microclimate variation within swine facilities (which can be caused by spatial differences in RH, T_A, and airspeed) exists, and can affect pigs' thermoregulation (Sällvik and Walberg, 1984; Costa et al., 2014). In the present study, no pen location differences were detected in RH and T_A, but T_A varied by day from thermoneutral conditions to a maximum of approximately 2.5°C below the upper temperature extreme for grow-finish pigs (Federation of Animal Science Societies, 2010). Despite the lack of pen location T_A and RH differences, pig level airspeed was significantly reduced overall in PP compared with IP. Airspeed plays an important role in convective heat loss (Curtis, 1983), and its reduction can decrease heat dissipation capacities (Bond et al., 1965; Close et al., 1981), resulting in elevated body temperature (Mitchell, 1985). In the present study, despite

similarities in T_A and RH between PP and IP, an 11% decrease in pig-level airspeed was detected and it is possible that this difference influenced the increase in T_V for PP housed pigs. This is because heat loss is partially dependent on the movement of air across the skin (i.e., convection), and with a reduction in pig-level airspeed, it is likely that heat loss by the body would be reduced (Johnson et al., 2018). As such, T_S in the present study was reduced in PP compared with IP housed pigs. Because an increase in T_S is a general indicator of greater heat loss (Johnson et al., 2015b), this could help explain the increased T_V in PP compared with IP housed pigs. In addition, a decrease in heat dissipation may be explained by a 21% reduction in the TCI of PP compared with IP housed pigs, which further suggests a reduced ability to dissipate body heat (Close et al., 1981).

Although statistically significant pen location pig-level airspeed differences were detected that could help explain the thermoregulatory differences, it should be mentioned that the absolute difference was relatively small (i.e., only a 0.03 m/s difference). Therefore, although specific reasons are currently unknown, it is possible that other factors that were not measured in the present study may have influenced the body temperature and production differences observed between pen locations. For example, IP housed pigs may have utilized behavioral thermoregulation (i.e., wetting of the skin with waterers, use of concrete flooring for convective cooling, etc.) more extensively than PP housed pigs, which could explain the reduced T_V. Alternatively, PP housed pigs may have had a greater rate of illness compared with IP housed pigs during the study, which could explain the elevated body temperatures (i.e., pyretic response) and reduced productivity because mounting an immune response diverts nutrients away from growth and towards the immune system (Kvidera et al., 2017). Therefore, these factors should be taken into consideration in future studies on the effects of pen location on pig thermoregulation and productivity.

While PP housed pigs had an increase in body temperature compared with IP housed pigs, no RR differences were detected. This was surprising considering that RR is a sensitive indicator of heat stress in pigs (Lucy and Safranski, 2017). However, this may be because RR was not monitored during the hottest period of the day (1400 hours), and it is possible that differences would have been detected if RR measures were taken more often. Nevertheless, because the overall RR for all pigs in the present study was approximately 49% greater than levels previously reported in

thermoneutral housed pigs (Johnson et al., 2015b), it is likely that both PP and IP housed pigs were suffering from heat stress due to warm summer environmental conditions (Becker et al., 1992).

Though these data may provide valuable information on the effects of microclimate variation on pig thermoregulation and productivity, some limitations are worth mentioning. Temperature measurements were only performed on gilts and may not reflect the thermal status of the barrows. However, because G:F was reduced overall for PP housed pigs and growth performance measures were taken on a per pen basis, this may suggest that the gilts and barrows have a similar growth performance response to pen location. Additionally, the experiment was conducted during a short time period (6 weeks from mid-July to mid-August) and the decrease in G:F might not reflect the entire grow-finish period. Furthermore, the study was conducted in side ventilated barns and the findings may not be applicable to tunnel ventilated barns. Nonetheless, these data illustrate the importance of the microclimate variability in grow-finish barns and its potential impact on thermoregulation and performance of pigs during summer months.

2.6 Conclusions

Variable thermal conditions in swine facilities can negatively affect the welfare and overall productive capacity of pigs. It was determined that pen location differences in pig thermoregulation and performance existed and that these differences may have been associated with pen-to-pen microclimate variation. While this study has furthered our understanding of the impact of microclimates within grow-finish facilities, future work should be conducted to evaluate these effects over a longer period, in different barn types, and consider other variables such as illness rate and pig thermoregulatory behavior.

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Table 2.1 Effects of pen location (**L**) and day of measurement (**D**) on environmental conditions and thermal indices in grow-to-finish barn and pigs during late summer.

Parameters	Pen Location			*P*-value		
	PP[a]	IP[b]	SEM	L	D	L x D
Environmental conditions						
Airspeed[c], m/s	0.24	0.27	0.01	0.01	0.06	0.56
RH, %	68.46	68.47	0.70	1.00	<0.01	1.00
Max RH, %	83.75	83.52	0.66	0.69	<0.01	0.86
Min RH, %	54.69	54.28	0.91	0.33	<0.01	0.99
Max-Min RH, %	27.92	28.24	0.48	0.58	<0.01	0.50
T_A, °C	27.63	27.43	0.31	0.53	<0.01	0.67
Max T_A, °C	31.65	31.53	0.35	0.52	<0.01	0.84
Min T_A, °C	23.96	23.66	0.22	0.34	<0.01	0.99
Max-Min T_A, °C	7.68	7.87	0.22	0.23	<0.01	0.90
Pig thermal indices						
RR, bpm	76	76	4	0.87	<0.01	0.41
RR (0800-1000 h)	64	63	3	0.75	<0.01	0.75
RR (1500-1700 h)	87	88	5	0.66	<0.01	0.67
T_V, °C	39.90	39.67	0.06	<0.01	<0.01	0.60
Max T_V, °C	40.34	40.05	0.07	<0.01	<0.01	0.73
Min T_V, °C	39.54	39.36	0.06	0.02	0.06	0.44
T_V (0800-1900 h)	40.02	39.80	0.07	<0.01	<0.01	0.83
T_V (2000-0700 h)	39.79	39.54	0.07	0.01	0.07	0.30
T_S, °C	37.39	37.61	0.14	0.04	<0.01	0.48
T_S (0800-1000 h)	36.23	36.52	0.13	0.03	<0.01	0.77
T_S (1500-1700 h)	38.55	38.72	0.25	0.09	<0.01	0.30
TCI	4.06	5.16	0.23	<0.01	0.01	0.08

Max, Maximum; Min, Minimum.
[a]Peripheral pens, located 0.70 ± 0.29 m to either side of the closest fan.
[b]Internal pens, located in direct line of sight between the fans and the air inlets.
[c]Airspeed measured at pig level.

Table 2.2 Effects of pen location (**L**) and recording period (**P**)[a] on growth parameters in grow-to-finish pigs during late summer.

Parameters	P1		P2		P3		SEM	*P*-value		
	PP[b]	IP[c]	PP	IP	PP	IP		L	P	L x P
ADFI, kg	2.12	2.11	2.21	2.17	2.50	2.45	0.08	0.71	<0.01	0.96
ADG, kg	0.68	0.69	0.68	0.74	0.80	0.81	0.03	0.28	<0.01	0.78
G:F, kg/kg	0.32	0.33	0.32	0.35	0.32	0.33	0.01	0.02	0.40	0.18

[a]Measurement once every 2 weeks.

[b]Peripheral pens, located 0.70 ± 0.29 m to either side of the closest fan.

[c]Internal pens, located in direct line of sight between the fans and the air inlets.
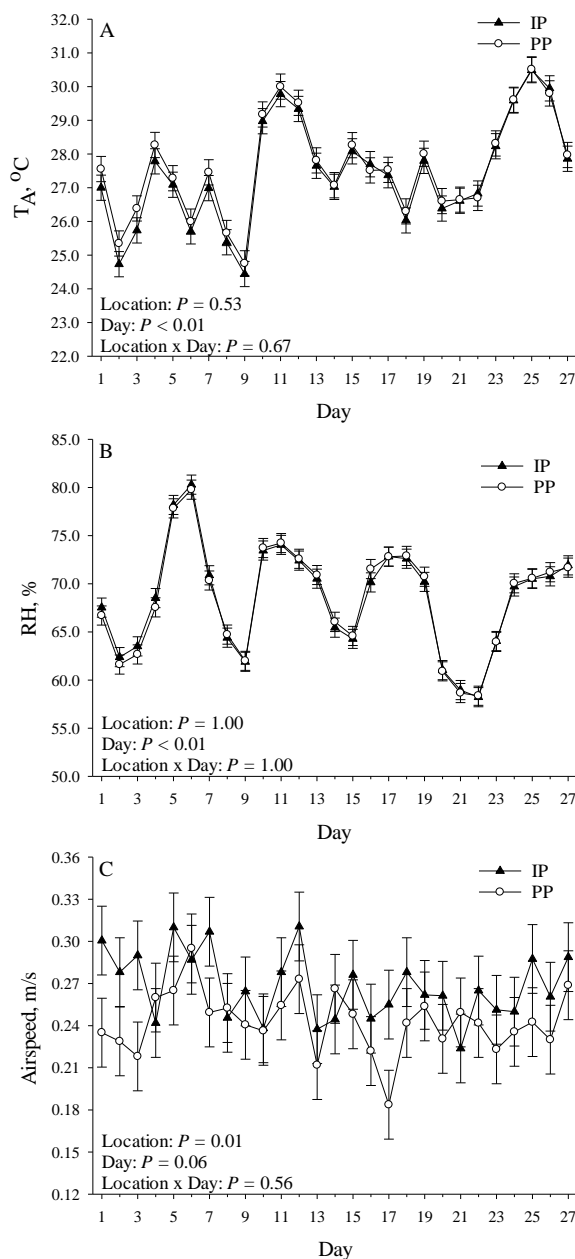
Figure 2.1 Effects of pen location on ambient temperature, relative humidity and airspeed.

Effects of pen location on ambient temperature, relative humidity and airspeed (**IP** = internal pens, located in direct line of sight between the fans and the air inlets; **PP** = peripheral pens, located 0.70 ± 0.29 m to either side of the closest fan) on (A) average daily ambient temperature ($\mathbf{T_A}$), (B) average daily relative humidity (**RH**) and (C) average daily airspeed by day of temperature measurement. Error bars indicate ± 1 SEM.
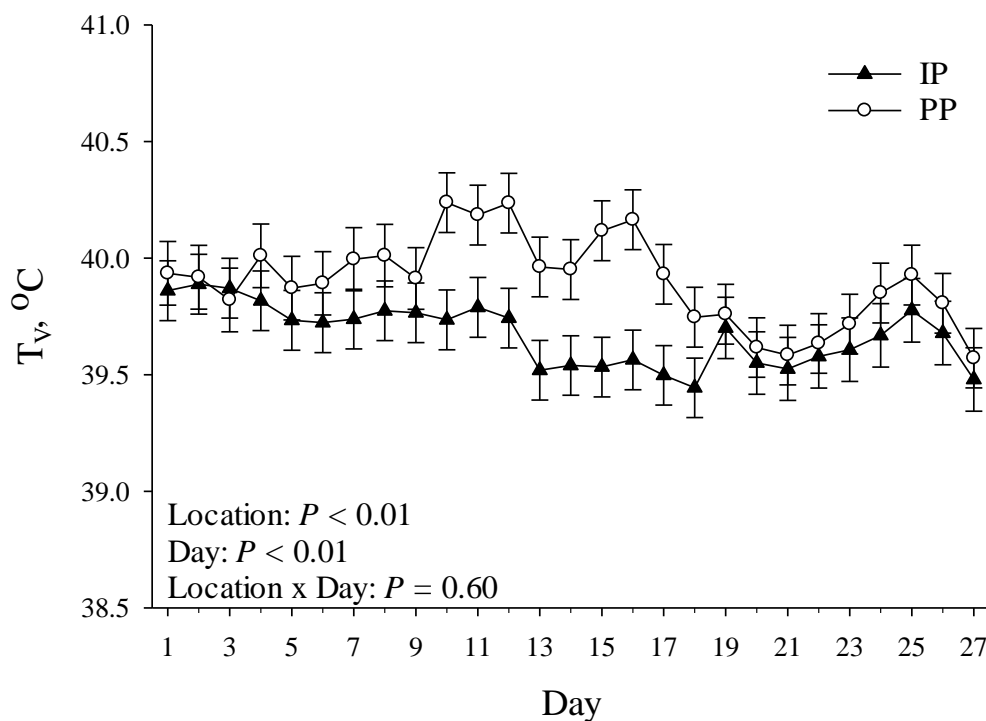
Figure 2.2 The effects of pen location vaginal temperature.

The effects of pen location vaginal temperature (**IP** = internal pens, located in direct line of sight between the fans and the air inlets; **PP =** peripheral pens, located 0.70 ± 0.29 m to either side of the closest fan) on average daily vaginal temperature (**T**$_V$) by day of temperature measurement. Error bars indicate ± 1 SEM.
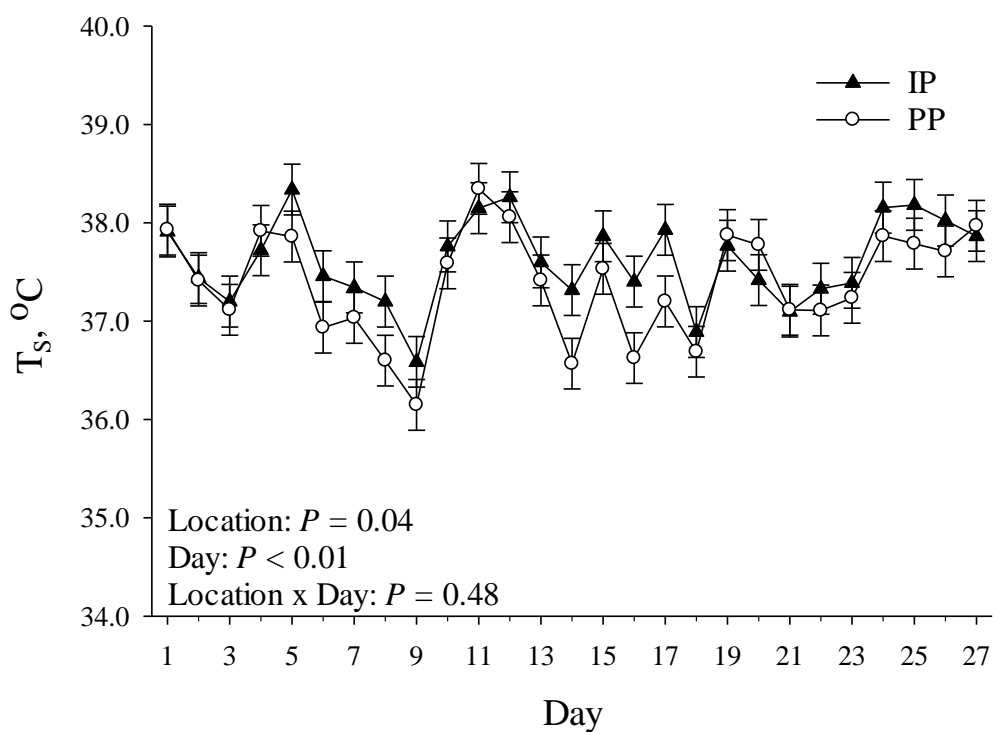
Figure 2.3 Effects of pen location on skin temperature.

Effects of pen location on skin temperature (**IP** = internal pens, located in direct line of sight between the fans and the air inlets; **PP** = peripheral pens, located 0.70 ± 0.29 m to either side of the closest fan) on average daily skin temperature (T_S) by day of temperature measurement. Error bars indicate $\pm$ 1 SEM.
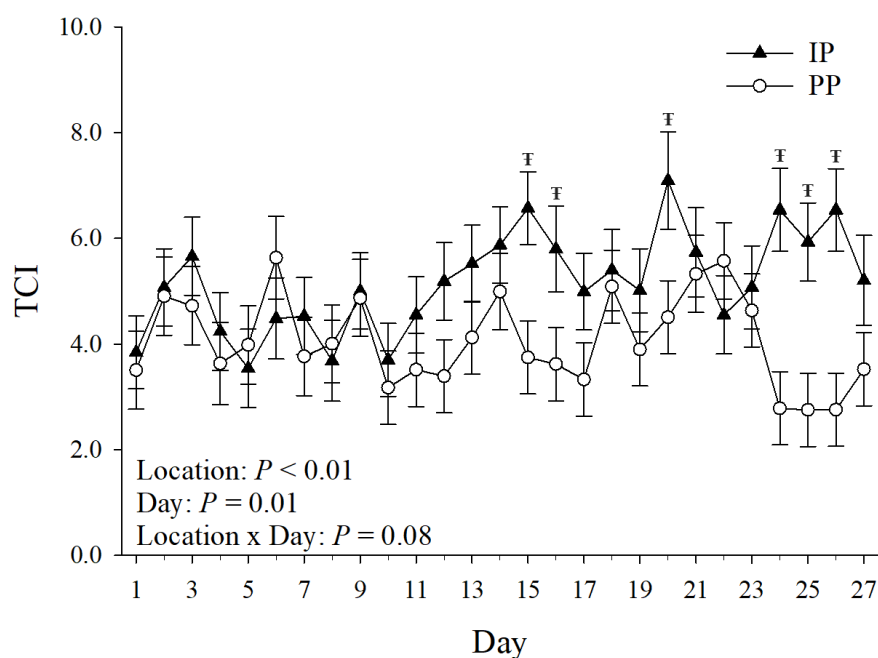
Figure 2.4 Effects of pen location on thermal circulation index.

Effects of pen location on TCI (**IP** = internal pens, located in direct line of sight between the fans and the air inlets; **PP** = peripheral pens, located 0.70 ± 0.29 m to either side of the closest fan) on average daily thermal circulation index (**TCI**) by day of temperature measurement. Error bars indicate ± 1 SEM. [F]Symbol indicates location by day of measurement interaction effect.

CHAPTER 3.   TIME COURSE DETERMINATION OF THE EFFECTS OF RAPID AND GRADUAL COOLING AFTER ACUTE HYPERTHERMIA ON BODY TEMPERATURE AND INTESTINAL INTEGRITY IN PIGS

3.1   Abstract

Rapid cooling after acute hyperthermia may cause a sustained increase in body temperature and exacerbate intestinal damage in pigs. Therefore, the study objective was to evaluate the temporal effects of rapid and gradual cooling on body temperature response and intestinal integrity after acute hyperthermia in pigs. In three repetitions, 54 pigs [83.3 ± 6.7 kg initial body weight (**BW**)], balanced by sex were exposed to thermoneutral conditions for 6h (**TN**; n = 6 pigs/repetition; 21.1 ± 2.0°C), or heat stress conditions (**HS**; 39.3 ± 1.6°C) for 3h, followed by a 3h recovery period of gradual cooling [**HSGC**; n = 6 pigs/repetition; gradual decrease from HS to TN conditions] or rapid cooling [**HSRC**; n = 6 pigs/repetition; rapid TN exposure and cold water (4.0°C) dousing every 30 min for 1.5h]. Feed was withheld throughout the entire 6h period, but water was provided *ad libitum*. Gastrointestinal (T_{GI}) and rectal (T_R) temperatures were recorded every 15 min during the HS and recovery periods. Six pigs per repetition (n = 2/treatment) were euthanized and jejunal and ileal samples were collected for histology immediately after (d0), 2d after, and 4d after the recovery period. Data were analyzed using PROC MIXED in SAS 9.4. Overall, rapid cooling reduced T_R and T_{GI} ($P < 0.01$; 0.95°C and 0.74°C, respectively) compared to gradual cooling. Jejunal villus height was reduced overall ($P = 0.02$; 14.01%) in HSGC compared to HSRC and TN pigs. Jejunal villus height-to-crypt depth ratio was reduced overall ($P = 0.05$; 16.76%) in HSGC compared to TN pigs. Ileal villus height was reduced overall ($P < 0.01$; 16.95%) in HSGC compared to HSRC and TN pigs. No other intestinal morphology differences were detected. In summary, HSRC did not cause a sustained increase in body temperature and did not negatively impact biomarkers of intestinal integrity in pigs.

3.2   Introduction

Acute hyperthermia negatively affects the health and well-being of humans and animals and results in economic losses due to increased health care costs and mortality (St-Pierre et al., 2003; Baumgard and Rhoads, 2013; Kjellstrom et al., 2016). During hyperthermia, blood is diverted from splanchnic tissues to the periphery to increase heat dissipation; however, this causes hypoxia primarily in the intestinal mucosa (Hall et al., 1999), resulting in intestinal damage, increased intestinal permeability, and a greater inflammatory response (Lambert, 2009). These negative impacts are exacerbated during acute hyperthermia, which is characterized by thermoregulatory failure and a sustained increase in body temperature (Bouchama and Knochel, 2002).

In animal agriculture, swine (particularly market weight pigs and lactating sows) are more susceptible to acute hyperthermia in tropical regions and during warm summer months in temperate climates due to the lack of functional sweat glands and presence of subcutaneous fat that impedes heat dissipation (Renaudeau et al., 2012; Baumgard and Rhoads, 2013). To alleviate the negative impacts of heat stress (**HS**) in pigs, different cooling methods (e.g., cooling pads, ventilation, evaporative cooling, etc.) are used; however, these methods may not be adequate during acute hyperthermia (Baumgard and Rhoads, 2013; Nienaber and Hahn, 2007). Therefore, it is important to develop mitigation strategies to combat acute hyperthermia and promote recovery.

Rapid cooling (i.e. ice water immersion) is widely used for heat stroke recovery in humans to quickly reduce body temperature to euthermia (Clapp et al., 2001; Casa et al., 2007). However, previous research in pigs demonstrated that gastrointestinal temperature (T_{GI}) remains elevated when rapid cooling is applied after acute hyperthermia, and this may contribute to greater intestinal damage and a whole-body inflammatory response (Johnson et al., 2016a). Therefore, the study objective was to evaluate the temporal effects of rapid cooling on body temperature, intestinal morphology and integrity, and systemic inflammatory response after acute hyperthermia in pigs. We hypothesized that rapid cooling after acute hyperthermia would prolong the T_{GI} increase, thereby exacerbating hyperthermia-induced intestinal damage and inflammatory responses with long-lasting effects post-recovery.

3.3   Materials and methods

3.3.1   Animals and experimental design

The experiment was approved by the Purdue University Animal Care and Use Committee (protocol no. 609001471). In three repetitions, a total of 54 crossbred [Duroc x (Landrace x Yorkshire)] pigs [n = 9 barrows/repetition and 9 gilts/repetition; 83.3 ± 6.7 kg initial body weight (**BW**)] were transported (1.6 km) from the Purdue University Animal Science and Education Center to the USDA-ARS Food Animal Behavior Research Laboratory, both in West Lafayette, IN. Pigs were allowed a 3-d acclimation period in their new environment and had *ad libitum* access to feed and water. The diet, based on corn and soybean meal, was formulated to meet or exceed the NRC (2012) requirements for grow-finish pigs. One day prior to the thermal challenge at 1500 h, each pig was weighed and orally administered a CorTemp temperature sensor (model HT150002, manufacturer calibrated accuracy ± 0.1°C; resolution = 0.01°C; HQ, Inc, Palmetto, FL). It was previously determined that this bolus administration process would result in the temperature sensor being located between the duodenum and the jejunum at the time the experiment was conducted the following day (Johnson et al., 2016a).

Two experimental rooms [thermoneutral (**TN**) and HS] were each equipped with two data loggers (HOBO, data logger temp/RH; Onset, Bourne, MA) to record ambient temperature (**T_A**) and relative humidity (**RH**) in 10-min intervals. Pigs were blocked by sex and subjected to TN conditions for 6 h (n = 6 pigs/repetition; 21.1 ± 2.0°C, 29.4 ± 1.6% RH), or HS conditions (39.3 ± 1.6°C, 15.9 ± 0.7% RH) for 3 h, followed by a 3-h recovery period of either gradual cooling (**HSGC**; n = 6 pigs/repetition) or rapid cooling (**HSRC**; n = 6 pigs/repetition). For the HS treatment, HSRC and HSGC pigs were moved from the TN room into a pre-heated room (approximately 3-m walking distance and 2 min). During the experiment, all pigs were individually housed in a 1.91 m x 1.58 m pen with a concrete floor. Each pen was equipped with a nipple drinker. All procedures began and ended at the same time each day within each repetition to reduce the influence of circadian rhythm on body temperature measures. Rapid and gradual cooling were performed as previously described by Johnson et al. (2016a, 2016b). Briefly, to achieve rapid cooling, pigs were moved from the HS room into the TN room (approximately 3-m walking distance and 2 min), and then 37.9 L of cold water (4.0°C) was poured over the back of each HSRC pig every 30 min for 1.5 h (total of 4 times). The water was poured over the back of each pig between the shoulder

blades and moving down to the rump and this process took approximately 10 s. For the gradual cooling, HSGC pigs were kept in the HS room and the ambient temperature was reduced by 5°C every 30 min until it returned to TN conditions (22.6 ± 2.3°C, 30.2 ± 4.4% RH). Contrary to the previous study (Johnson et al., 2016a), pigs did not have access to feed but had *ad libitum* water access during the HS and recovery periods. Throughout the HS and recovery periods, T_R was measured using a thermistor thermometer (Cooper Atkin model TM99A, manufacturer calibrated accuracy ± 0.2°C; resolution = 0.1°C Middlefield, CT) and the T_{GI} was recorded through the CorTemp temperature sensors in 15-min intervals.

Immediately following the HS and recovery periods (d 0), two pigs/treatment (n = 1 barrow and 1 gilt) were euthanized, and intestinal tissues were collected. This procedure was repeated again on d 2 and 4 post-recovery. From d 0 to 4 post-recovery, pigs had *ad libitum* access to feed and water, and were maintained under TN conditions with a 12-h light and dark cycle starting at 0700 h.

3.3.2   Sample collection

3.3.2.1   Blood samples

Two blood tubes (serum and EDTA; 5 mL) were obtained from all pigs via jugular venipuncture (BD® vacutainers; Franklin Lakes, NJ; K_3EDTA; serum) on d -1 prior to the HS challenge, at 180 min of the HS period, and at 30 and 90 min during the recovery period. In addition, blood samples were collected at 0800 h on d 2 and 4 of the post-recovery period on all remaining pigs. All blood samples were centrifuged at 1900 x *g* for 15 min at 4.0°C. Plasma and serum were aliquoted and stored at -80.0°C until analysis.

3.3.2.2   Intestinal samples

Six pigs (n = 2/treatment balanced by sex) per tissue collection day (d 0, 2, and 4) within each repetition were euthanized and intestinal samples were immediately collected. Jejunal (2.5 m posterior to the stomach) and ileal (1 m anterior to the ileocecal junction) tissues were collected, snap frozen in liquid nitrogen, and stored at -80.0°C for later analyses. In addition, jejunal and ileal tissue samples were embedded in optimal cutting temperature (OCT) compound (Tissue-Tek; Sakura Finetek Inc., Torrance, CA) within a cryomold and immediately submerged in 2-methylbutane. The 2-methylbutane container was placed in liquid nitrogen, allowing the OCT

compound to solidify without direct contact with the liquid nitrogen. The tissue samples were stored at -80.0°C for later histology. In addition, fresh jejunal samples were collected as previously mentioned, placed in Krebs-Henseleit buffer (5 mM KCl, 124 mM NaCl, 1.2 mM CaCl$_2$, 26 mM NaHCO$_3$, 1.2 mM MgSO$_4$ and 5 mM glucose; pH 7.4) and transported on ice under constant aeration to the laboratory for ex-vivo intestinal permeability measurements with modified Ussing chambers (Physiologic Instruments, San Diego, CA).

3.3.3   Sample analyses

3.3.3.1   Blood analyses

Commercial kits were used to determine plasma tumor necrosis factor alpha (**TNFα** swine Elisa kit, Life Technologies, Frederick, MD), cardiac troponin I (**cTnI**; Ultra-sensitive pig cardiac troponin-I Elisa, Life Diagnostics, Inc, West Chester, PA) and serum lipopolysaccharide (**LPS**, Pierce LAL Chromogenic Endotoxin Quantification Kit, Thermo Fisher Scientific, Rockford, IL) concentrations following the manufacturer's instructions. The chromogenic endotoxin assay was performed with 1/500, rather than the typical 1/1000 serum sample dilution due to low LPS concentrations. The intra-assay coefficients of variation were 10.36%, 11.70%, and 4.51% for TNFα, LPS and cTnI, respectively. Plate was included in the statistical model to account for inter-plate differences.

3.3.3.2   Histology

Jejunal and ileal tissues were referred to the Purdue University Histology and Phenotyping Laboratory for sectioning (2 sections of 5-µm thickness on each slide per pig) and staining in toluidine blue with fast green counter stain. Each section was imaged three times at 10x using Q-capture Pro 6.0 software (Qimaging, Surrey, British Colombia, Canada), and villus height and crypt depth were measured using ImageJ 1.52b software (National Institute of Health; Bethesda, MD). Mast cells were counted directly on six images per slide, taken at 40x by a single trained individual who was blind to the treatments. The mast cells were counted in a surface area delineated by 9 squares of 40 µm x 40 µm dimensions each (total surface = 0.0144 mm^2), which were determined based on the condition that the first square had at least one mast cell present.

3.3.3.3 Active ion transport and intestinal integrity

Jejunal integrity was measured as previously described by Walsh et al. (2012). Briefly, the serosa layer was stripped from the jejunal segments, and the tissues (1.0 cm^2 surface area) were mounted in duplicates into modified Ussing chambers. Tissues were bathed on each side with 4 mL Krebs-Henseleit buffer maintained at 37.0°C using a circulating water bath and were continuously aerated using carbogen gas (95% O_2 and 5% CO_2). The chambers were connected to a dual channel voltage/current clamp by 3% noble agar/3 M KCl salt bridges. Tissues were voltage clamped, short-circuit current (**I$_{sc}$**) was measured, and transepithelial resistance (**TER**) was calculated from the I$_{sc}$ and the potential difference.

The paracellular transport of the jejunal segments were measured using 4000 Da fluorescein isothiocyanate-labeled dextran (**FITC**: Glucose, Sigma-Aldrich, St. Louis, MO). Following active transport measurements, the serosa side chamber was filled with fresh Krebs-Henseleit buffer, whereas the buffer in the mucosa side chamber was replaced by a 2.2 mg/mL of FITC-labeled dextran solution (Pearce et al., 2013a). After a 1-h incubation period, samples from the mucosa and the serosa chambers were plated in duplicate and analyzed in a fluorescence plate reader at excitation and emission wavelengths of 485 nm and 520 nm, respectively.

3.3.3.4 Gene expression

Gene expression of claudin 1 and zonula occludens 1 (**ZO 1**) in the jejunum and ileum were analyzed by real-time polymerase chain reaction (**PCR**) as previously described (Eicher et al., 2017). Briefly, jejunal and ileal tissues were homogenized, and total mRNA was extracted using the RNeasy Mini Kit (Qiagen, Inc., Valencia, CA). After quantification of the extracted mRNA, complementary DNA (**cDNA**) was synthesized using the TaqMan Reverse Transcription Reagents (Applied Biosystems Inc., Foster city, CA). The real-time PCR was performed using the TaqMan Gene Expression Assays (ZO 1: Ss03373514_m1; Claudin 1: Ss04246284_s; Eukaryotic 18S rRNA: Hs03003631_g1), which are combined forward primer, reverse primer, and TaqMan probe (Applied Biosystems Inc., Foster City, CA). The reactions were performed using 22.5 µL of the combined primers and the probe, and 2.5 µL of the cDNA sample. The gene expression results were quantified using the standard curve method, and data were expressed as relative abundance

to 18S, the endogenous control. Real-time PCR was performed in duplicate with a coefficient of variation less than 10%.

3.4   Statistics

Data were analyzed using the PROC MIXED procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). Temperature data were analyzed separately within the HS and recovery periods using repeated measures with an appropriate covariance structure and time (15-min intervals from 0 to 180 min) as the repeated effect as previously described (Johnson et al., 2016a, 2016b). The statistical model for the thermal variables within HS and recovery periods was $Y_{ijk} = \mu + R_i + T_j + K_k + R*T_{ij} + e_{ijk}$, where Y = dependent variable of interest, μ = mean, R = recovery treatment, T = time point within each period, K = replication, and e = error term. Blood data were analyzed within HS, recovery and post-recovery periods. For recovery and post-recovery periods, data were analyzed using an appropriate covariance structure with time (30 and 90 min) and day (d 2 and 4) as repeated effects, respectively. The statistical model for blood data during the recovery period is described as follows: $Y_{ijk} = \mu + R_i + T_j + K_k + R*T_{ij} + e_{ijk}$, where Y= dependent variable of interest, μ = overall mean, R = recovery treatment, T = time point (30 and 90 min), K = replication, and e = error term. The statistical model for blood data in the post-recovery period was $Y_{ijk} = \mu + R_i + D_j + K_k + R*D_{ij} + e_{ijk}$, where Y= dependent variable of interest, μ = overall mean, R = recovery treatment, D = days (d 2 and 4), K = replication, and e = error term. Assay plate was included as a random factor for all blood analyses, and d -1 blood data were used as a covariate when significant. For other variables (HS period blood data, histology, intestinal permeability, gene expression), the statistical model was $Y_{ik} = \mu + R_i + K_k + e_{ik}$, where Y= dependent variable of interest, R = recovery treatment, K = replication, and e = error term. Blood and intestinal permeability data were log- or sqrt-transformed to meet normality assumptions when needed, and back-transformed LSmeans are reported. Individual pigs were the experimental unit, and repetition was included as a random effect in all analyses. Day and sex were included in each analysis as fixed effects; however, the sex effect is reported only when significant. Time effects for body temperature measures (T_R, T_{GI}) and blood parameters (TNFα, cTnI, LPS) are only discussed when they interact with the recovery treatments. Data are reported as LSmeans, and statistical significance was considered at $P \leq 0.05$, and a tendency was defined as $0.05 < P \leq 0.10$.

3.5   Results

3.5.1   Rectal and gastrointestinal temperature

3.5.1.1   Heat stress period

During the HS period, T_R was greater overall ($P < 0.01$; 1.75°C) in HSRC and HSGC pigs compared to TN control pigs (Figure 3.1A; Table 3.1). Minimum and maximum T_R were greater overall ($P < 0.01$; 0.76 and 2.17°C, respectively) in HSRC and HSGC pigs compared to TN pigs, but no differences were detected between HSRC and HSGC pigs (Table 3.1). Overall, T_{GI} was increased ($P < 0.01$; 1.71°C) in HSRC and HSGC pigs compared to TN pigs (Figure 3.1B; Table 3.1). Minimum and maximum T_{GI} were greater overall ($P < 0.01$; 0.90 and 1.07°C, respectively) in HSRC and HSGC pigs compared to TN pigs, but no differences were detected between HSRC and HSGC pigs (Table 3.1).

3.5.1.2   Recovery period

During the recovery period, T_R was greater ($P < 0.01$; 0.88°C) in HSGC pigs compared to HSRC and TN pigs; however, no difference was detected between HSRC and TN pigs (Table 3.1). Minimum T_R was greater ($P < 0.01$) in HSGC and TN pigs by 0.74 and 0.47°C, respectively, compared to HSRC pigs, and greater in HSGC pigs by 0.27°C compared to TN pigs (Table 3.1). Maximum T_R was greater ($P < 0.01$) in HSGC and HSRC pigs (1.47 and 0.74°C, respectively), compared to TN pigs, and greater in HSGC pigs (0.73°C) compared to HSRC pigs (Table 3.1). A treatment by time effect was observed ($P < 0.01$) where T_R was greater at each 15-min time point in HSGC pigs compared to HSRC and TN pigs from 0 to 135 min, but similar between HSGC and TN pigs from 150 to 180 min (Figure 3.1A). Rectal temperature was greater from 0 to 15 min, similar from 30 to 105 min, and reduced from 120 to 180 min in HSRC compared to TN pigs (Figure 3.1A).

During the recovery period, T_{GI} was greater overall ($P < 0.01$; 0.92°C) in HSGC pigs compared to HSRC and TN pigs; however, no differences were detected between HSRC and TN pigs (Table 3.1). Minimum T_{GI} was greater in HSGC pigs compared to HSRC and TN pigs ($P < 0.01$; 0.57°C); however, no differences were detected between HSRC and TN pigs (Table 3.1). Maximum T_{GI} was greater ($P < 0.01$; 1.78°C) in HSRC and HSGC compared to TN pigs, but no difference was detected between HSRC and HSGC pigs (Table 3.1). During the recovery period, a treatment by

time interaction was detected ($P < 0.01$), where T_{GI} was greater at each 15-min time point in HSGC compared to TN pigs from 0 to 180 min, similar between HSGC and HSRC at 0 min, and greater in HSGC compared to HSRC from 15 to 180 min (Figure 3.1B). In addition, T_{GI} was greater from 0 to 15 min, but similar from 30 to 180 min in HSRC compared to TN pigs (Figure 3.1B).

3.5.1.3   Post-recovery period

On d 1 and 3 of the post-recovery period, T_R was reduced overall ($P < 0.01$; 0.24°C) in HSGC and HSRC pigs compared to TN pigs; however, no differences were detected between HSGC and HSRC pigs (39.31 ± 0.08°C; Figure 3.2). No day differences were detected ($P = 0.59$; 39.39 ± 0.08°C) for T_R (Figure 3.2), but there was a treatment by day interaction tendency ($P = 0.10$), due to T_R being similar in all treatments on d 1 but decreasing by 0.51°C in HSGC compared to TN pigs on d 3 post-recovery (Figure 3.2). Sex differences were observed where T_R was greater ($P < 0.01$) in barrows (39.52 ± 0.07°C) compared to gilts, regardless of post-recovery day (39.26 ± 0.07°C; Figure 3.3). In addition, a sex by treatment effect was detected ($P < 0.01$) where TN-barrows had greater T_R (39.88 ± 0.09°C) compared to all other pigs, regardless of post-recovery day (39.29 ± 0.09°C; Figure 3.3). No other differences were detected ($P \geq 0.14$) for T_R or T_{GI} during the post-recovery period (Figure 3.2; 3.3).

3.5.2   Blood parameters

3.5.2.1   Heat stress period

During the HS period, plasma TNFα was reduced overall ($P = 0.02$; 28.01%) in HSRC and HSGC pigs compared to TN pigs, but no differences were detected between HSRC and HSGC pigs (Table 3.2). Plasma cTnI was greater ($P = 0.01$; 708.60%) in HSRC and HSGC pigs compared to TN pigs; however, no differences were detected between HSRC and HSGC pigs (Table 3.2). No other blood parameter differences were detected ($P \geq 0.12$) during the HS period (Table 3.2).

3.5.2.2   Recovery period

During the recovery period, plasma TNFα was reduced overall ($P = 0.01$; 28.35%) in HSGC compared to TN pigs, but no differences were observed between HSGC and HSRC pigs or HSRC and TN pigs (Table 3.2). An increase in cTnI was observed ($P = 0.04$; 552.56%) in HSGC pigs compared to TN and HSRC pigs, but no cTnI differences were detected between HSRC and TN

pigs (Table 3.2). No other blood parameter differences related to the recovery treatment were detected ($P \geq 0.11$) during the recovery period (Table 3.2).

3.5.2.3 Post-recovery period

No blood parameter differences related to the recovery treatment were detected ($P \geq 0.26$) during the post-recovery period (Table 3.3).

3.5.3 Histology

In the jejunum, villus height was reduced ($P = 0.02$; 14.01%) in HSGC pigs compared to HSRC and TN pigs; however, no differences were detected between HSRC and TN pigs (392.27 ± 48.46 µm; Figure 3.4A). An overall day effect was detected where villus height tended to be increased ($P = 0.09$; 13.61%) on d 4 compared to d 0, regardless of recovery treatment (Figure 3.4A). Crypt depth tended to be increased ($P = 0.09$; 15.15%) on d 4 compared to d 0, regardless of recovery treatment (Figure 3.4B). Jejunal villus height-to-crypt depth ratio was reduced overall ($P = 0.05$; 16.76%) in HSGC compared to TN pigs, but no differences were detected between HSRC and TN pigs or HSRC and HSGC pigs (Figure 3.4C). There was a tendency for a treatment by day interaction ($P = 0.10$) on mast cell count in the jejunum, where mast cell count was similar in HSGC, HSRC and TN pigs on d 0 and d 4, but greater (37.55%) in HSGC pigs compared to HSRC and TN pigs on d 2 (Table 3.4). No other jejunal histology differences were detected ($P \geq 0.22$; Table 3.4; Figure 3.4A, B, C).

Ileal villus height was reduced ($P < 0.01$; 16.95%) in HSGC pigs compared to HSRC and TN pigs, but no differences were detected between HSRC (314.00 ± 17.08 µm) and TN pigs (322.55 ± 17.31 µm; Figure 3.4A). Crypt depth tended to be reduced ($P = 0.10$) in HSGC pigs (17.64%) compared to HSRC and TN pigs on d 0, but was similar in HSGC, HSRC and TN pigs on d 2 and d 4 (Figure 3.4B). Villus height-to-crypt depth ratio tended to be decreased ($P = 0.06$; 15.19%) in HSGC compared to TN pigs, but no differences were detected between HSRC and TN or HSGC pigs (Figure 3.4C). No other histology differences were observed in the ileum ($P \geq 0.64$; Table 3.4; Figure 3.4A, B, C).

3.5.4   Intestinal integrity

Regardless of recovery treatment, FITC-labeled dextran transport was increased overall ($P = 0.02$; 278.64%) on d 4 compared to d 0, but no differences were detected between d 0 and d 2 or d 2 and d 4 (Table 3.4). No other intestinal integrity differences were detected ($P \geq 0.13$; Table 3.4).

3.5.5   Gene expression

In the jejunum, claudin 1 gene expression was increased overall ($P = 0.02$; 336.37%) on d 0 compared to d 4, but no differences were detected between d 0 and d 2 or d 2 and d 4 post-recovery (Table 3.4). A sex by treatment interaction was detected ($P = 0.02$) for ZO 1 gene expression, where it was greater in HSGC gilts (0.38 ± 0.06 fold change) compared to TN gilts (0.20 ± 0.03 fold change), HSRC barrows (0.22 ± 0.04 fold change), and HSGC barrows (0.24 ± 0.04 fold change); however, no differences were detected between TN gilts, HSRC barrows, and HSGC barrows (Figure 3.5). No other jejunal gene expression differences were detected ($P \geq 0.11$; Table 3.4; Figure 3.5).

Ileal claudin 1 gene expression was reduced ($P < 0.01$; 72.31%) in HSGC compared to TN pigs on d 2 and was increased (409.68%) in HSGC compared to HSRC and TN pigs on d 4 (Table 3.4). A sex by treatment interaction was detected ($P < 0.01$) for claudin 1 gene expression in the ileum, with reduced expression in HSRC barrows (0.30 ± 0.15 fold change) compared to HSGC barrows (0.82 ± 0.38 fold change), TN barrows (0.89 ± 0.42 fold change), HSGC gilts (0.81 ± 0.38 fold change), and HSRC gilts (1.23 ± 059 fold change). In addition, claudin 1 gene expression was reduced in TN gilts (0.39 ± 0.19 fold change) compared to HSRC gilts and TN barrows, respectively (Figure 3.6). No other ileal gene expression sex differences were detected ($P > 0.05$; Table 3.4; Figure 3.6).

3.6   Discussion

Acute hyperthermia represents a growing challenge for humans and livestock due to increasing heat wave intensity and frequency (Nardone et al., 2010). To combat acute hyperthermia, rapid cooling techniques are used to accelerate heat dissipation by increasing the thermal gradient or water-vapor pressure between the skin and the environment (Bouchama and Knochel, 2002). Although these techniques are effective in returning the T_R to euthermia, previous research in pigs

demonstrates that rapid cooling after acute hyperthermia can prevent T_{GI} from returning to euthermia (Johnson et al., 2016a, 2016b). Contrary to the previous results (Johnson et al., 2016a, 2016b), rapid cooling in the current study was effective in returning T_{GI} to euthermia following acute hyperthermia. Although reasons for the discrepancies between the previous T_{GI} results (Johnson et al., 2016a, 2016b) and T_{GI} results in the present study are unknown, it may be related to differences in feed availability. In the current study, feed was withheld throughout the entire HS and recovery periods whereas feed was available *ad libitum* in the previous studies (Johnson et al., 2016a, 2016b). Therefore, it is possible that the previously observed increase in T_{GI} during the recovery period for HSRC pigs was related to the rapid increase in feeding behavior despite the previous hypothesis that feed removal would exacerbate the rapid cooling-induced increase in T_{GI} (Johnson et al., 2016a). This is because re-feeding can increase the heat of nutrient processing (and subsequently body temperature) in pigs (Cervantes et al., 2018). Because T_{GI} was effectively reduced in the HSRC pigs when feed was withheld in the present study, this may suggest that feed withdrawal should be combined with rapid cooling to more effectively reduce body temperature following acute hyperthermia in pigs.

Although rapid cooling was effective in reducing T_{GI} to euthermia in pigs in the present study, T_R in HSRC pigs was reduced compared to TN pigs during the start of the HS period and at the end of the recovery period, and was reduced compared to previously published rectal temperatures in similar sized pigs housed in TN conditions [Johnson et al., 2015 ($39.30 \pm 0.10°C$); Seibert et al., 2018 ($39.03 \pm 0.03°C$)]. This may indicate that HSRC pigs in the present study became hypothermic at the end of the recovery period and is contrary to previous data in acutely hyperthermic pigs that were rapidly cooled (Johnson et al., 2016a). Although reasons are currently unknown, this discrepency is likely due to feed availability whereby the thermal effect of feeding during the recovery period may have delayed the return of T_R to euthermia and prevented pigs from becoming hypothermic as previously hypothesized (Johnson et al., 2016a). Thus, in the present study it is possible that rapid cooling after acute hyperthermia may have caused excessive heat dissipation that the HSRC pigs were not able to compensate for due to feed withdrawal and the associated decrease in metabolic heat production and body temperature (Romanovsky and Blatteis, 1996; Cervantes et al., 2018). Furthermore, differences in the overall T_{GI} and the T_R response to cooling and the response to cooling method (e.g., rapid versus gradual cooling) are

likely related to the thermal gradient whereby the T_R measured closer to the surface would be more sensitive environmental changes relative to T_{GI} measured at the interior of the body (Blatteis, 1998).

While HSGC pigs did not become hypothermic similar to HSRC pigs during the recovery period, a decrease in T_R was observed for both HSRC and HSGC pigs during the post-recovery period when compared to TN pigs. Previous reports indicate that acutely hyperthermic pigs return to euthermia in the days following a HS challenge (Abuajamieh et al., 2018; Mayorga et al., 2018). However, despite this, data in the current study are consistent with literature in rodent models in which mice became hypothermic in the days following acute hyperthermia (Wilkinson et al., 1988; Leon et al., 2005). It is hypothesized that the hypothermic response is an adaptive thermoregulatory survival mechanism that functions as a protective response of the body to the damage induced by acute hyperthermia (Leon et al., 2005). This hypothesis is supported by observations that mice who become hypothermic following acute hyperthermia have reduced intestinal damage and increased survivability (Wilkinson et al., 1988). Alternatively, this response may simply be an un-regulated event due to the thermal damage of homeostatic mechanisms (Leon et al., 2005). It is important to note however that although the T_R of HSGC and HSRC pigs were reduced relative to TN pigs, the absolute T_R values were similar to previously reported euthermic T_R in similar sized pigs (Johnson et al., 2015; Seibert et al., 2018). Therefore, these data should be interpreted with caution and future research should investigate the mechanism(s) by which reduced T_R occurs in pigs following acute hyperthermia.

In addition to the thermoregulatory effects, acute hyperthermia causes intestinal damage in pigs (Pearce et al., 2013b, Gabler and Pearce 2015). To maximize heat dissipation, hyperthermic animals increase blood flow to the periphery, thereby reducing nutrient and oxygen supply to the intestine (Hall et al., 1999; Lambert, 2009). The ensuing morphological changes (e.g., reduced villus height, crypt depth and villus height-to-crypt depth ratio) are indicative of intestinal damage and are well-documented (Pearce et al., 2013b; Kumar et al., 2017; Abuajamieh et al., 2018). In the present study, rapid cooling during the recovery period prevented, whereas gradual cooling increased morphological changes indicative of intestinal damage relative to TN pigs. These results are contrary to previous findings that HSRC increases morphological changes indicative of intestinal damage compared to HSGC and TN pigs (Johnson et al., 2016a). While the lack of

morphological changes indicative of intestinal damage for HSRC pigs is surprising based on previous results (Johnson et al., 2016a), it was not unexpected since rapid cooling in the present study was effective in returning T_{GI} to euthermia during the recovery period. Furthermore, the T_R of HSRC pigs became hypothermic during the recovery period (versus later in the post-recovery period for HSGC pigs) and this is associated with reduced intestinal damage following acute hyperthermia in mice (Wilkinson et al., 1988). As a result, the total amount of time HSRC pigs had elevated body temperature relative to HSGC pigs was reduced, which may have mitigated the aforementioned hyperthermia-induced increase in intestinal hypoxia and damage (Hall et al., 1999; Lambert, 2009). Therefore, it is likely that heat exposure duration and intensity played a role in preventing intestinal morphological damage for HSRC pigs in the present study.

In addition to the deleterious impact of acute hyperthermia on intestinal morphology, intestinal integrity and permeability may be negatively affected. Previous studies in swine demonstrate that acute hyperthermia (from 2 h up to 7 d) reduces intestinal integrity and increases permeability as shown by a decrease in TER and an increase in FITC transport, respectively (Pearce et al., 2013a, 2013b, 2014; Liu et al., 2016; Gabler et al., 2018). However, contrary to the aforementioned reports, no TER or FITC recovery treatment differences related to recovery treatment were observed in the present study. While reasons for this discrepency are currently unknown, it may be partially related to hypothermia observed in both HSRC and HSGC pigs following acute hyperthermia. As previously mentioned, hypothermia after acute hyperthermia prevents intestinal damage in mice (Wilkinson et al., 1988). Therefore, it is possible that this effect may have protected the intestinal integrity of HSRC and HSGC pigs in the present study. Furthermore, differences between the current study and previous studies (Pearce et al., 2014; Gabler et al., 2018) may be related to the intestinal section analyzed because studies in mice have shown that hyperthermia impacts intestinal sections differently (Novosad et al., 2013), and the aforementioned studies (Pearce et al., 2014; Gabler et al., 2018) used the ileum as opposed to the jejunum for TER and FITC analyses. Regardless of the reason, it appears that the effects of acute hyperthermia on intestinal integrity and permeability are not always consistant and factors such as exposure length, intensity, and intestinal section should be taken into consideration when conducting experiments.

The lack of jejunal integrity differences as indicated by the TER and FITC results was reflected in jejunal tight junction protein gene expression. The tight junction is comprised of transmembrane proteins (e.g., claudins and occludins) and scaffolding proteins (e.g., zonula occludens), which anchor transmembrane proteins to the actin cytoskeleton (Dokladny et al., 2015). These proteins modulate paracellular transport across the intestinal mucosa and can be disrupted under HS conditions (Pearce et al., 2013a; Dokladny et al., 2015). In the present study, no ZO 1 gene expression differences were observed in the jejunum and ileum, and this is likely due to the lack of intestinal integrity and permeability differences. This jejunal result is similar to a previous study, where pigs were subjected to HS for 24 h (Pearce et al., 2013b). Furthermore, in the current study, ileal claudin 1 gene expression was decreased on d 2 compared to d 0 and then increased on d 4 compared to d 2 in HSGC pigs. This pattern may suggest that acute hyperthermia in HSGC pigs initially disrupted the tight junction function in the ileum on d 2 and that claudin 1 gene expression was subsequently upregulated on d 4 to enhance intestinal barrier integrity.

Disruption in tight junction function is associated with increased LPS translocation across the intestinal barrier (Goo et al., 2018; Shukla et al., 2019). Lipopolysaccharide, derived from the membrane of gram-negative bacteria and present in the gut, is a potent stimulator of the immune system (Wyns et al., 2015). Under normal conditions, it is translocated into the bloodstream at low levels (Erridge et al., 2007; Guerville and Boudry, 2016); however, circulating LPS concentrations may be increased when animals are suffering from hyperthermia (Lambert et al., 2002; Pearce et al., 2013a; Johnson et al., 2016a). Contrary to these reports, no LPS differences were observed in the present study among the recovery treatment groups. Because increased LPS translocation during HS is related to reduced intestinal integrity and increased permeability (Dokladny et al., 2015), the lack LPS differences in the present study are not surprising since no intestinal integrity or permeability differences were detected between recovery treatments. Although no LPS differences were observed, TNFα was reduced in HSGC compared to HSRC and TN pigs during the HS and recovery periods. While reduced circulating TNFα for HSGC pigs in the present study is contrary to a previous report in acutely hyperthermic pigs (Johnson et al., 2016a), it is consistent with other reports (Pearce et al., 2015; Abuajamieh et al., 2018) and may be related to heat shock protein (HSP) activity. Although not measured in the current study, HSPs are increased during hyperthermia (Dokladny et al., 2006; Dangi et al., 2015) and have been shown to inhibit NF-kB

and reduce pro-inflammatory cytokines (Chen et al., 2006). Therefore, because HSGC pigs were exposed to elevated ambient temperatures for a longer period of time relative to HSRC pigs, HSP activity may have been increased resulting in a greater decrease in TNFα following HS exposure as previously hypothesized (Pearce et al., 2015). However, because HSP activity was not measured in the present study this hypothesis would have to be confirmed in subsequent experiments.

Cardiac troponin I (cTnI), a regulatory protein of cardiac muscle contraction, is released into circulation when myocardial damage occurs (Kenney et al., 2014), and is often elevated in dogs (Mellor et al., 2006), rats (Quinn et al., 2014) and humans (Hausfater et al., 2010) suffering from acute hyperthermia. In agreement with the aforementioned reports, HSRC and HSGC pigs had overall greater cTnI concentrations compared to TN pigs during the HS period. However, during the recovery period, cTnI concentrations for HSRC pigs returned to TN pig concentrations while cTnI concentrations for HSGC pigs remained elevated. This is likely because rapid cooling quickly returned T_{GI} to euthermia and T_R to hypothermia, thereby reducing the cardiovascular strain caused by acute hyperthermia (Simmons et al., 2008). Conversely, because HSGC pigs were exposed to elevated ambient temperatures for a longer period of time, cardiac output to support increased blood flow and redistribution to the periphery to maximize heat dissipation was likely prolonged (Quinn et al., 2015), which may have caused more cardiovascular strain in HSGC pigs. These data provide evidence that a rapid return of body temperature to euthermia may prevent further cardiovascular damage after acute hyperthermia in pigs, and to our knowledge this is the first study to report the effects of HSRC on cTnI in pigs.

3.7   Conclusions

Based on previous research, we hypothesized that rapid cooling after acute hyperthermia would prolong the body temperature increase and exacerbate hyperthermia-induced intestinal damage and inflammatory responses post-recovery when compared to gradual cooling. However, this hypothesis was not proven as we determined that rapid cooling was more effective than gradual cooling in returning body temperature to euthermia (T_{GI}), making T_R hypothermic, and reducing the negative effects of acute hyperthermia on intestinal health. It is likely that this effect was partially driven by feed withdrawal and its impact on the heat of nutrient processing as previous studies provided pigs with *ad libitum* feed. However, because feed was withheld for all pigs in the

present study, it is unclear whether feed removal alone played a critical role in altering the thermoregulatory response and further research is needed to elucidate this effect. Nevertheless, this study provides more insight and expands our understanding of the effects of rapid cooling on the treatment of acute hyperthermia in pigs.

3.8   References

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Table 3.1 Body temperature differences in pigs rapidly or gradually cooled after acute hyperthermia.

| Parameter | Recovery treatments | | | SEM | P-value |
	TN[1]	HSRC[2]	HSGC[3]		R[4]
Heat stress period					
T_{GI}[5], °C	39.79[a]	41.48[b]	41.51[b]	0.13	<0.01
Min[6] T_{GI}, °C	39.42[a]	40.39[b]	40.24[b]	0.16	<0.01
Max[7] T_{GI}, °C	41.14[a]	42.19[b]	42.23[b]	0.16	<0.01
T_R[8], °C	39.01[a]	40.71[b]	40.80[b]	0.14	<0.01
Min T_R, °C	38.74[a]	39.49[b]	39.51[b]	0.08	<0.01
Max T_R, °C	39.28[a]	41.37[b]	41.53[b]	0.24	<0.01
Recovery period					
T_{GI}, °C	39.80[a]	40.16[a]	40.90[b]	0.14	<0.01
Min T_{GI}, °C	39.52[a]	39.46[a]	40.06[b]	0.16	<0.01
Max T_{GI}, °C	40.03[a]	41.90[b]	41.71[b]	0.17	<0.01
T_R, °C	39.07[a]	38.93[a]	39.88[b]	0.11	<0.01
Min T_R, °C	38.80[a]	38.33[b]	39.07[c]	0.09	<0.01
Max T_R, °C	39.32[a]	40.06[b]	40.79[c]	0.22	<0.01

[1]Thermoneutral.

[2]Heat stress and rapid cooling.

[3]Heat stress and gradual cooling.

[4]Recovery treatments (TN, HSRC and HSGC).

[5]Gastrointestinal tract temperature.

[6]Minimum.

[7]Maximum.

[8]Rectal temperature.

[a,b,c]Letters within a row indicate recovery treatment differences during the heat stress and recovery periods ($P \leq 0.05$).

Table 3.2 Effects of rapid and gradual cooling after acute hyperthermia on blood parameters during the heat stress and recovery periods in pigs.

Parameter	Recovery treatment			SEM	P-value
	TN[1]	HSRC[2]	HSGC[3]		R[4]
Heat stress period[5]					
TNFα[6], pg/mL	71.68[a]	50.93[b]	52.27[b]	5.55	0.02
LPS[7], EU/mL	5.43	8.04	6.45	1.79	0.12
cTnI[8], pg/mL	0.93[b]	5.89[a]	9.15[a]	2.49	0.01
Recovery period[9]					
TNFα, pg/mL	62.97[a]	53.59[ab]	45.12[b]	5.28	0.01
LPS, EU/mL	5.89	6.80	6.20	1.96	0.77
cTnI, pg/mL	5.52[b]	5.17[b]	34.90[a]	9.40	0.04

[1]Thermoneutral.

[2]Heat stress and rapid cooling.

[3]Heat stress and gradual cooing.

[4]Recovery treatment (TN, HSRC, HSGC).

[5]Blood samples taken at 180 min of the heat stress period.

[6]Tumor necrosis factor alpha.

[7]Lipopolysaccharide.

[8]Cardiac troponin I.

[9]Blood samples taken at 30 and 90 min of the recovery period.

[a,b]Letters within a row indicate recovery treatment differences ($P \leq 0.05$).

Table 3.3 Effects of rapid and gradual cooling after acute hyperthermia on blood parameters on day 2 and day 4 during the post-recovery period in pigs.

Parameter	Day 2			Day 4				P-value		
	TN[1]	HSRC[2]	HSGC[3]	TN	HSRC	HSGC	SEM	R[4]	D[5]	R x D
TNFα[6], pg/mL	60.49	81.90	59.89	65.16	62.68	65.95	11.05	0.74	0.70	0.26
LPS[7], EU/mL	5.96	4.89	6.37	5.05	7.79	10.32	2.59	0.54	0.27	0.39
cTnI[8], pg/mL	0.87	0.40	0.53	0.01	0.00	0.01	0.32	0.91	0.03	0.96

[1]Thermoneutral.

[2]Heat stress and rapid cooling.

[3]Heat stress and gradual cooing.

[4]Recovery treatment (TN, HSRC, HSGC).

[5]Day (Day 0 = heat stress and recovery treatment day; Day 2 = two days post-recovery; Day 4 = four days post-recovery).

[6]Tumor necrosis factor alpha.

[7]Lipopolysaccharide.

[8]Cardiac troponin I.

Significant differences ($P \leq 0.05$).

Table 3.4 Intestinal mast cell count, gene expression, and integrity on day 0, day 2, and day 4 in pigs that were rapidly or gradually cooled after acute hyperthermia.

Parameter	Day 0			Day 2			Day 4			SEM	P-value		
	TN[1]	HSRC[2]	HSGC[3]	TN	HSRC	HSGC	TN	HSRC	HSGC		R[4]	D[5]	R x D
Jejunum													
Mast cell/mm^2	518.9^y	642.29^{xy}	497.69^y	595.29^y	577.09^y	806.33^x	653.87^{xy}	563.20^y	588.35^y	102.34	0.75	0.22	0.10
Claudin 1, fold change	0.05	0.09	0.34	0.10	0.07	0.06	0.02	0.04	0.05	0.04	0.11	0.02	0.14
ZO[6] 1, fold change	0.25	0.26	0.43	0.25	0.28	0.25	0.20	0.22	0.26	0.05	0.17	0.17	0.45
Ileum													
Mast cell/mm^2	558.90	580.07	516.98	505.40	635.05	667.44	595.51	611.90	574.85	88.07	0.66	0.67	0.64
Claudin 1, fold change	0.63^{abcd}	0.74^{abc}	0.94^{ab}	1.30^a	0.81^{abc}	0.36^{cd}	0.25^d	0.37^{bcd}	1.58^a	0.40	0.45	0.40	<0.01
ZO 1, fold change	0.71	0.99	1.73	0.99	1.10	1.00	0.64	0.66	1.05	0.34	0.25	0.44	0.73
Jejunal integrity													
Basal TER[7], Ω.cm^2	77.13	64.01	69.02	84.95	66.41	64.88	54.25	69.63	77.81	8.32	0.73	0.78	0.13
FITC[8], µg/mL	0.59	0.23	0.21	0.99	0.61	0.67	1.53	1.23	1.14	0.39	0.44	0.02	1.00

[1]Thermoneutral.

[2]Heat stress and rapid cooling.

[3]Heat stress and gradual cooing.

[4]Recovery treatment (TN, HSRC, HSGC).

[5]Day (Day 0 = heat stress and recovery treatment day; Day 2 = two days post-recovery; Day 4 = four days post-recovery).

[6]Zonula occludens.

[7]Transepithelial resistance.

[8]Fluorescein isothiocyanate.

[a,b,c]Letters within a row indicate recovery treatment differences ($P \leq 0.05$).

[x,y]Letters within a row indicate recovery treatment tendencies ($0.05 < P \leq 0.10$).
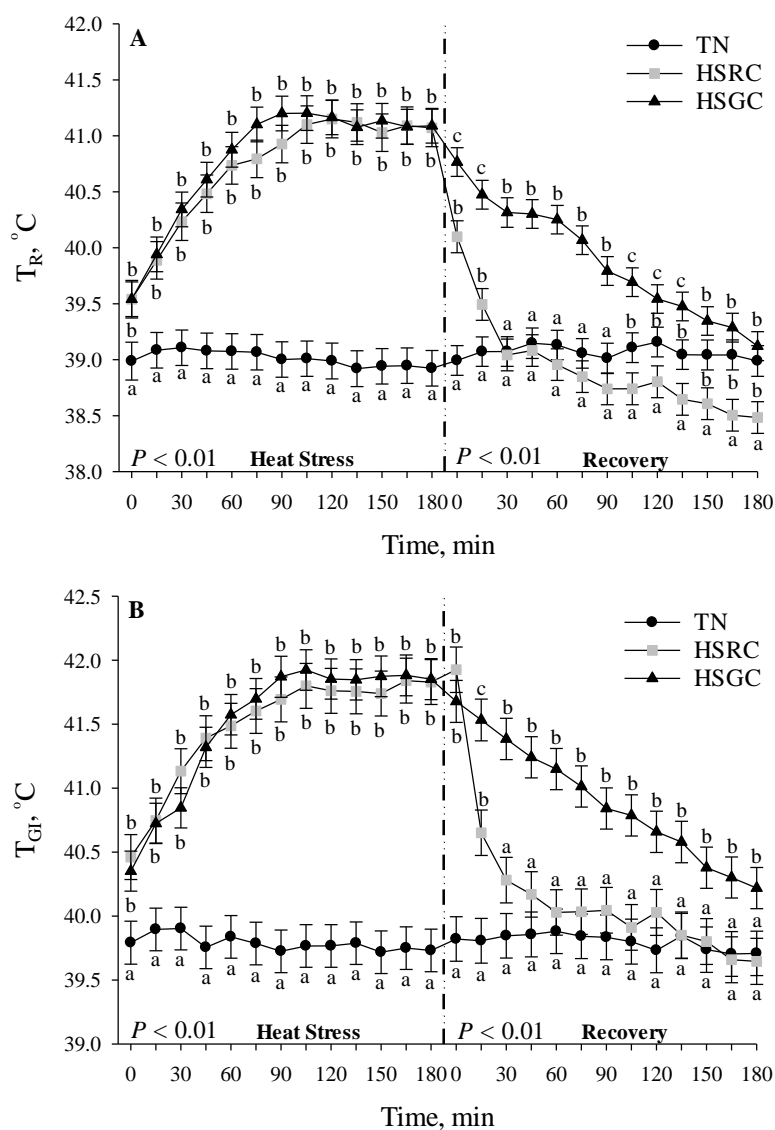
Figure 3.1 Effect of recovery treatment on rectal and gastrointestinal temperatures.

Effect of recovery treatment on (A) rectal temperature (T_R) recorded in 15-min intervals during the heat stress and recovery periods, and (B) gastrointestinal temperature (T_{GI}) recorded in 15-min intervals during the heat stress and recovery periods in pigs. TN, thermoneutral; HSRC, heat stress followed by rapid cooling; HSGC, heat stress followed by gradual cooling. Error bars indicate ± 1 SE. [a,b,c]Letters indicate recovery treatment differences at each 15-min time point during the heat stress and recovery periods ($P \leq 0.05$).
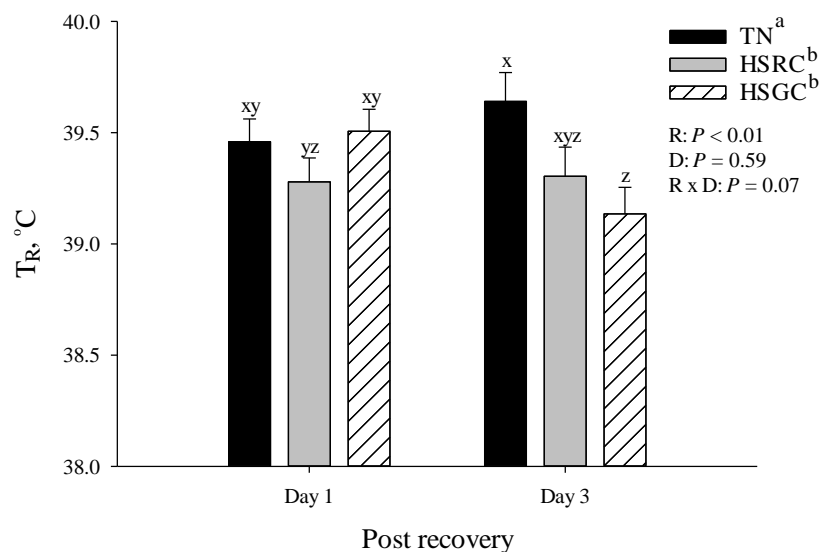
Figure 3.2 Effect of recovery treatments after acute hyperthermia on rectal temperature.

Effect of recovery treatments (R) after acute hyperthermia on rectal temperature (T_R) recorded during the post-recovery period (Day 1 and 3). D, Day; TN, thermoneutral; HSRC, heat stress followed by rapid cooling; HSGC, heat stress followed by gradual cooling. Error bars indicate ± 1 SE. [a,b]Letters indicate differences between treatments ($P \leq 0.05$). [x,z,y]Letters indicate recovery treatment by day tendencies ($0.05 < P \leq 0.10$).
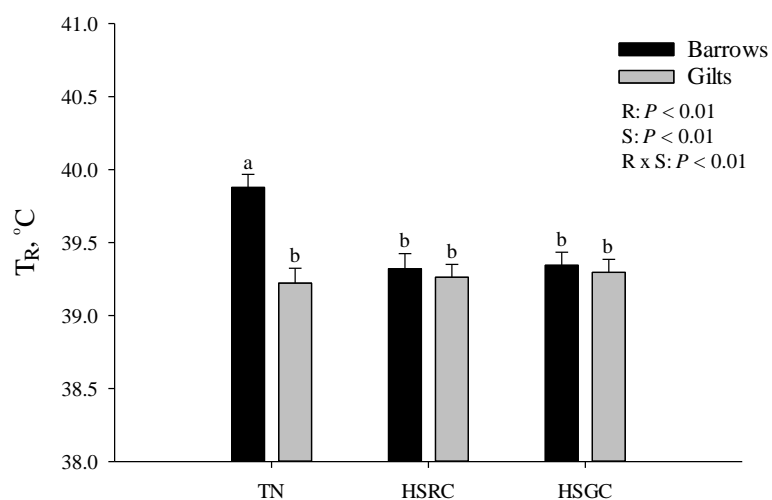
Figure 3.3 Effect of recovery treatments and sex after acute hyperthermia on overall rectal temperature

Effect of recovery treatments (R) and sex (S) after acute hyperthermia on overall rectal temperature (T_R) recorded during the post-recovery period (Day 1 and 3). TN, thermoneutral; HSRC, heat stress followed by rapid cooling; HSGC, heat stress followed by gradual cooling. Error bars indicate ± 1 SE. [a,b]Letters indicate recovery treatments by sex differences ($P \leq 0.05$).
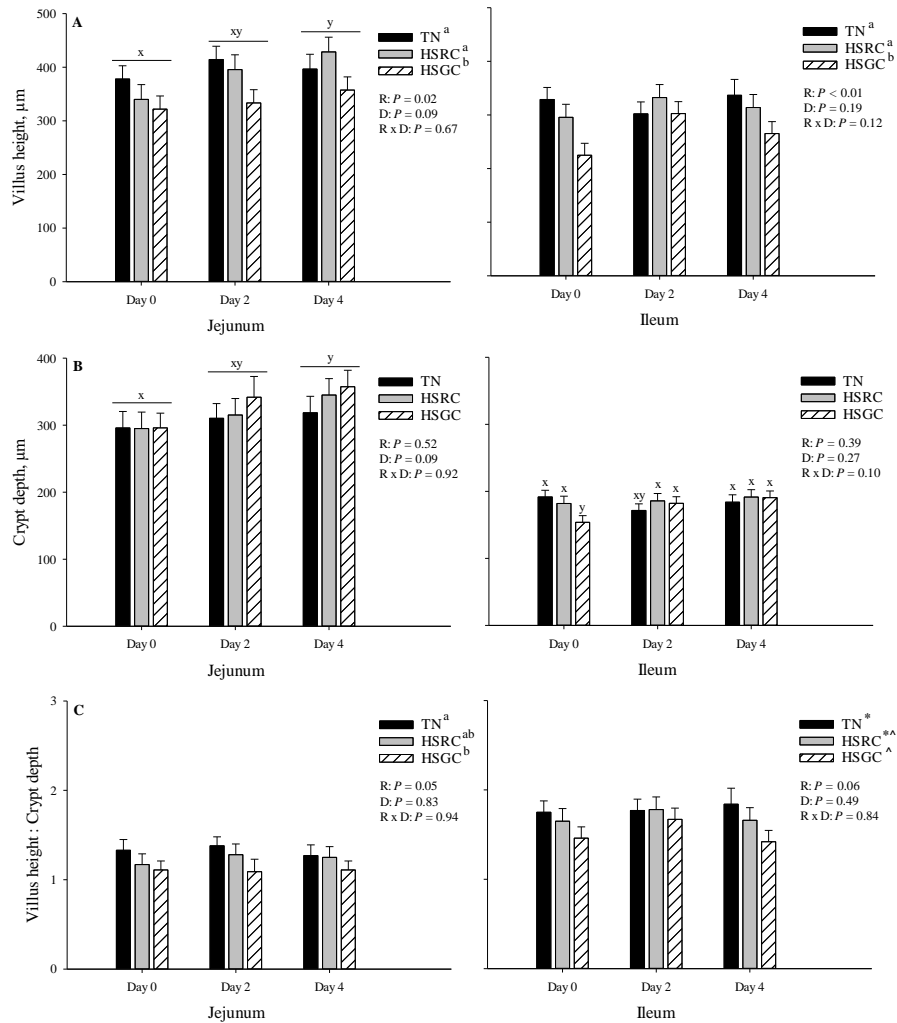
Figure 3.4 Effect of recovery treatments after acute hyperthermia on intestinal histology.

Effect of recovery treatments (R) after acute hyperthermia on (A) villus height in the jejunum and ileum, (B) crypt depth in the jejunum and ileum, and (C) villus height-to-crypt depth ratio in the jejunum and ileum in pigs, immediately at the end of the recovery (Day 0) and during the post-recovery period (Day 2 and 4). D, Day; TN, thermoneutral; HSRC, heat stress followed by rapid cooling; HSGC, heat stress followed by gradual cooling. Error bars indicate ± 1 SE. [a,b] Letters indicate recovery treatment differences ($P \leq 0.05$). *,^Symbols indicate recovery treatment tendencies ($0.05 < P \leq 0.10$). [x,y]Letters indicate overall day tendencies ($0.05 < P \leq 0.10$).
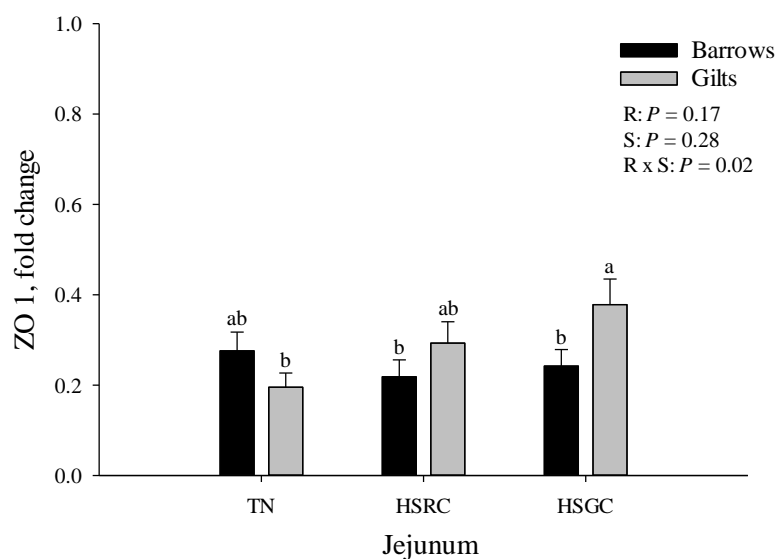
Figure 3.5 Effect of recovery treatments and sex after acute hyperthermia on overall jejunal zonula occludens 1 gene expression.

Effect of recovery treatments (R) and sex (S) after acute hyperthermia on overall jejunal zonula occludens (ZO) 1. TN, thermoneutral; HSRC, heat stress followed by rapid cooling; HSGC, heat stress followed by gradual cooling. Error bars indicate ± 1 SE. [a,b]Letters indicate recovery treatments by sex differences ($P \leq 0.05$).
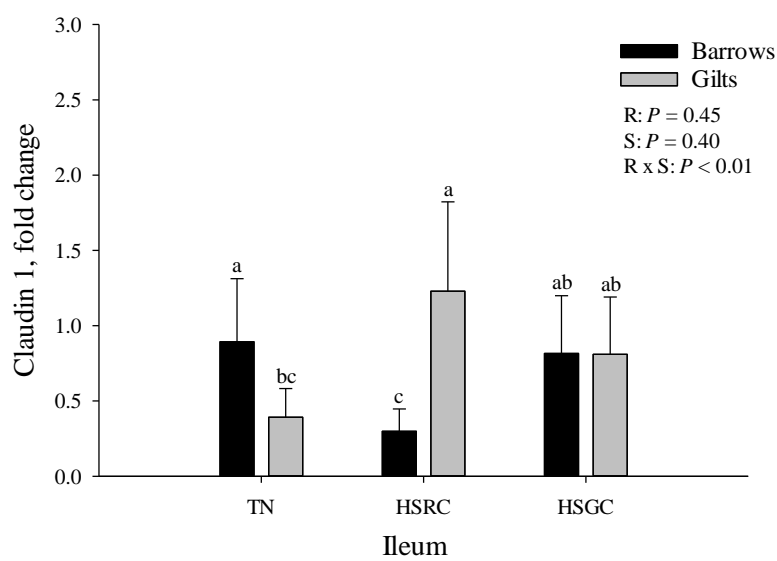
Figure 3.6 Effect of recovery treatments and sex after acute hyperthermia on overall ileal
Claudin 1 gene expression.

Effect of recovery treatments (R) and sex (S) after acute hyperthermia on overall ileal Claudin 1
gene expression. TN, thermoneutral; HSRC, heat stress followed by rapid cooling; HSGC, heat
stress followed by gradual cooling. Error bars indicate ± 1 SE. [a,b]Letters indicate recovery
treatments by sex differences ($P \leq 0.05$).

CHAPTER 4.   EFFECTS OF FEED REMOVAL ON THERMOREGULATION AND INTESTINAL MORPHOLOGY IN PIGS RECOVERING FROM ACUTE HYPERTHERMIA

4.1   Abstract

Feed consumption increases body temperature and may delay a return to euthermia and exacerbate intestinal injury following acute hyperthermia recovery in pigs. Therefore, the study objective was to evaluate the effects of feed removal on body temperature, feeding behavior, and intestinal morphology in pigs exposed to acute hyperthermia and then rapidly cooled. Twenty-four gilts (78.53 ± 5.46 kg) were exposed to thermoneutrality (**TN**; n = 12 pigs; 21.21 ± 0.31°C; 61.88 ± 6.93% RH) for 6 h, or heat stress (**HS**; 38.51 ± 0.60°C; 36.38 ± 3.40% RH) for 3 h followed by a 3-h recovery period of rapid cooling (**HSC;** n = 12 pigs; TN conditions and cold water dousing). Within each recovery treatment, one-half of the pigs were provided feed *ad libitum* (**AF**; n = 6 pigs/recovery treatment) and one-half of the pigs were not provided feed (**NF**; n = 6 pigs/recovery treatment). Gastrointestinal (T_{GI}), vaginal (T_V), and skin (T_{sk}) temperatures, and respiration rate (**RR**) were recorded every 15 min. Pigs were video-recorded to assess feeding attempts. Immediately following the 6-h thermal stress period pigs were euthanized, and intestinal samples were collected to assess morphology. During the HS period, Tv, T_{GI}, T_{sk}, and RR were increased ($P < 0.01$; 1.63°C, 2.05°C, 8.32°C, and 89 breaths per minute, respectively) in HSC versus TN pigs, regardless of feeding treatment. Gastrointestinal temperature was greater ($P = 0.03$; 0.97°C) in HSC+AF versus HSC+NF pigs from 45-180 min of the recovery period. During the recovery period, feeding attempts were greater ($P = 0.02$; 197.67%) in AF versus NF pigs. A decrease ($P < 0.01$) in jejunum and ileum villus height (24.72% and 26.11%, respectively) and villus height-to-crypt depth ratio (24.35% and 25.29%, respectively) was observed in HSC versus TN pigs, regardless of feeding treatment. Ileum goblet cells were reduced ($P = 0.01$; 37.87%) in HSC versus TN pigs, regardless of feeding treatment. In summary, T_{GI} decreased more rapidly following acute hyperthermia when feed was removed, and this has implications towards using feed removal as a strategy to promote acute hyperthermia recovery in pigs.

4.2   Introduction

Heat stress (**HS**) represents a growing challenge for livestock health and productivity. Pigs are particularly affected by HS due to limited evaporative heat loss capacity resulting from non-functional sweat glands (Turnpenny et al., 2000), which is compounded by a lack of wallowing access in commercial facilities. Furthermore, HS susceptibility is exacerbated in heavier pigs (e.g., market weight pigs, sows, and boars) due to a low surface area-to-mass ratio and greater subcutaneous fat depths that can limit heat dissipation capacity (Renaudeau et al., 2012) and in lactating sows due to increased metabolic heat production (Cabezon et al., 2016; Johnson et al., 2019). As a result, HS can reduce production efficiency and product quality for the swine industry (as reviewed by Johnson, 2018). Although management strategies (i.e., shading, cooling pads, floor cooling, evaporative cooling, etc.) can be effective in reducing the negative impacts of HS (Renaudeau et al., 2012; Parois et al., 2018), production still remains suboptimal, and morbidity and mortality may be increased in the case of acute hyperthermia (as reviewed by Baumgard and Rhoads, 2013 and Johnson, 2018). Therefore, it is necessary to develop mitigation strategies to combat acute hyperthermia and promote recovery.

Several recent studies have evaluated the effects of cooling methods on acute hyperthermia recovery in swine (Johnson et al., 2016a,b; Sapkota et al., 2016; Kpodo et al., 2018). However, while some reports have determined that rapid cooling (e.g., return to thermoneutral environment and dousing with cold water) can be an effective method of quickly returning pigs to euthermia and preventing intestinal damage (Kpodo et al., 2018), others have shown that rapid cooling prevents the return of body temperature to euthermia, exacerbates intestinal damage, and increases the whole-body inflammatory response (Johnson et al., 2016a,b; Sapkota et al., 2016). While reasons for these discrepancies are currently unknown, it may be due to study design differences and the effects of feed access (i.e., rapid cooling was only effective in studies where feed was withdrawn) as previously suggested (Kpodo et al., 2018). Therefore, the study objective was to determine the effects of feed removal on thermoregulation and intestinal morphology in pigs that were rapidly cooled following acute hyperthermia. We hypothesized that feed removal during acute hyperthermia and rapid cooling would hasten the return of body temperature to euthermia and reduce morphological indicators of intestinal damage in pigs relative to those that had *ad libitum* feed access.

4.3   Materials and methods

4.3.1   Animals and experimental design

All animal procedures were approved by the Purdue University Animal Care and Use Committee (no. 1802001689). Animal care and use standards were based on the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (Federation of Animal Science Societies, 2010). The study was conducted in May 2018 at the Purdue University Swine Farm. Twenty-four crossbred gilts [Duroc x (Landrace x Yorkshire); n = 12/repetition; 78.53 ± 5.46 kg initial body weight (**BW**)] were selected and housed in individual pens (1.22 m x 2.01 m pen). Pigs were given 1 d to acclimate to their new environment. On the day prior to the experiment at 1500 h, a calibrated thermochron temperature recorder (iButton, accuracy ± 0.1°C; Dallas Semi-conductor, Maxim, Irving, TX) attached to a blank controlled internal drug releasing device (CIDR; Eazi-Breed; Zoetis, New York, NY) was inserted intra-vaginally into each pig to record vaginal temperature (T_V) as previously described by Johnson and Shade (2017). In addition, each pig was administered a CorTemp temperature sensor (model HT150002, accuracy ± 0.1°C; HQ, Inc, Palmetto, FL) to monitor gastrointestinal temperature (T_{GI}). The temperature sensor was expected to be located between the duodenum and the jejunum at the time of the experiment as previously determined (Johnson et al., 2016a).

On the following day, pigs were housed in thermoneutral conditions [**TN**; n = 6 pigs/repetition; 21.21 ± 0.31°C; 61.88 ± 6.93% relative humidity (**RH**)] for 6 h (Figure 4.1), or constant HS conditions for 3 h (38.51 ± 0.60°C; 36.38 ± 3.40% RH), followed by a 3 h cooling period in which T_A was rapidly reduced to TN conditions (25.10 ± 3.71°C; 58.4% RH; Figure 4.1) and 37.9 L gallons of cold water (4.0°C) was poured over each pigs back every 30 min for 1.5 h (**HSC;** n = 6 pigs/repetition). Each room was equipped with two data loggers (HOBO, data logger temp/RH; accuracy ± 0.2°C; Onset, Bourne, MA) to record ambient temperature (T_A) and RH in 15-min intervals. Within each recovery treatment, although feeders were present in all pens, one-half of the pigs had *ad libitum* access to feed (**AF**; n = 3 pigs/recovery treatment/repetition) and one-half of the pigs were not provided feed (**NF**; n = 3 pigs/recovery treatment/repetition), but all pigs had *ad libitum* water access. Pigs with feed access were fed a standard corn and soybean meal diet, which was formulated to meet or exceed the requirements for grow-finish pigs (NRC, 2012).

4.3.2 Body temperature and respiration rate

During the experiment, T_{GI}, T_V, skin temperature (**T_{sk}**), and respiration rate (**RR**) were measured in all pigs in 15-min intervals. Gastrointestinal temperature was measured through the CorTemp temperature sensors, and T_V was recorded by the pre-programmed iButtons of the vaginal implants. Skin temperature was measured by taking a broad side photo of each pig using an infrared camera (FLIR Model T440, accuracy ± 0.1°C; emissivity = 0.95; FLIR Systems Inc., USA), and photos were analyzed with FLIR Tools Software (Version 5.13) by one individual blind to the treatments. Briefly, T_{Sk} was determined by drawing a circle on the trunk area (all skin caudal to the neck and dorsal to the elbow and stifle) and the mean, maximum, and minimum temperatures were recorded. Respiration rate (breaths per min; **bpm**) was determined by counting flank movements for 15 s and then multiplying by 4.

4.3.3 Consumption attempt recording and analyses

Pigs were video-recorded during the experiment using ceiling mounted cameras (Panasonic WV-CP254H, Matsushita Electric Industrial Co. Ltd.., Osaka, Japan). Each camera was oriented to capture two pens (1 AF and 1 NF pig). The video data were analyzed in Observer XT 11.5 (Noldus; The Netherlands) using a continuous sampling technique, and consumption attempts [feeding attempt (head in feeder) and drinking attempts (snout in contact with nipple drinker)], were determined by two trained individuals who were blind to the treatments and maintained an agreement of 90% or greater. A percent of total observations per hour was determined for each pig and used in the analyses.

4.3.4 Histology

Intestinal samples were collected at the end of the recovery period immediately following euthanasia. Proximal jejunal (2.5 m posterior to the stomach) and ileal (1 m anterior to the ileocecal junction) sections were flushed with phosphate buffer solution and stored in 10% formalin. Tissues were later submitted to the Purdue University Histology and Phenotyping Laboratory for sectioning (5-µm thickness, 2 sections/slide) and staining in Alcian blue and Giemsa. Three images per section (6 images per pig) were taken using a Q-capture Pro 6.0 software (Qimaging, Survey, British Columbia, Canada). Villus height and crypt depth were measured, and goblet cells were counted by a trained individual using ImageJ 1.52b software (National Institute of Health;

Bethesda, MD). Mast cells were counted directly on six images per slide at 40X under the microscope field of view (Total surface = 0.047 mm^2) by a single trained individual. Mean villus height, crypt depth, villus height-to-crypt depth ratio, goblet cell count, and mast cell count per pig were used in the final analyses.

4.3.5   Statistics

Data were analyzed as a 2 x 2 factorial arrangement [recovery treatment (TN and HSC) and feeding treatment (AF and NF)] using the PROC MIXED procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). The linear additive model used for all data was: $Y_{ijk} = \mu + R_i + F_j + K_k + R*F_{ij} + e_{ijk}$, where Y = dependent variable of interest, μ = mean, R = recovery treatment, F = feeding treatment, and e = error term, K = replication. Temperature data were analyzed separately within the HS and recovery periods using repeated measures with an appropriate covariance structure and time (15-min intervals from 0 to 180 min) as the repeated effect (Johnson et al., 2016a,b; Kpodo et al., 2018). Consumption attempt data were analyzed separately within the HS and recovery periods using repeated measures with an appropriate covariance structure and hour (1-3) as the repeated effect. Individual pigs were considered the experimental unit, and repetition was included as a random factor in all analyses. Consumption attempt data were log-transformed to meet normality assumption, and back-transformed LSmeans are reported for ease of interpretation. Statistical significance was considered at $P \leq 0.05$, and a tendency was defined as $0.05 < P \leq 0.10$.

4.4   Results

4.4.1   Gastrointestinal temperature

4.4.1.1   Heat stress period

During the HS period, T_{GI}, minimum T_{GI}, and maximum T_{GI} were increased ($P < 0.01$; 2.05, 0.76, and 2.69°C, respectively) in HSC compared to TN pigs (Table 4.1). A recovery treatment by time interaction was detected ($P < 0.01$) where T_{GI} was greater in HSC compared to TN pigs at every 15-min time point from 30 to 180 min, but no differences were detected between HSC and TN pigs at 0 and 15 min (Figure 4.2A). No other T_{GI} differences were detected ($P \geq 0.47$) during the HS period (Table 4.1).

4.4.1.2   Recovery period

During the recovery period, T_{GI} and maximum T_{GI} were increased ($P < 0.01$; 0.86 and 2.66°C, respectively) in HSC compared to TN pigs, regardless of feeding treatment (Table 4.1). Overall, T_{GI} and minimum T_{GI} were greater ($P = 0.01$; 0.51 and 0.56°C, respectively) in AF compared to NF pigs (Table 4.1). Minimum T_{GI} tended to be greater ($P = 0.10$; 0.79°C) in HSC+AF compared to HSC+NF and TN+NF pigs, but no differences were detected between any other treatment combination (Table 4.1). Gastrointestinal temperature was greater overall ($P = 0.03$; 2.41°C) from 0 to 30 min in HSC compared to TN pigs, regardless of feeding treatment (Figure 4.2A). An increase in T_{GI} was detected ($P = 0.03$; 0.97°C) in HSC+AF compared to HSC+NF pigs from 45 to 180 min, and in HSC+AF compared to TN+AF and TN+NF pigs (1.36 and 1.64°C, respectively) from 45 to 75 min (Figure 4.2A). Gastrointestinal temperature was greater ($P = 0.03$; 0.84°C) in HSC+AF compared to TN+NF pigs from 90 to 135 min (Figure 4.2A). No other T_{GI} differences were detected ($P > 0.05$) during the recovery period (Table 4.1; Figure 4.2A).

4.4.2   Vaginal temperature

4.4.2.1   Heat stress period

During the HS period, T_V, minimum T_V, and maximum T_V were greater ($P \leq 0.03$; 1.63, 0.14, and 2.47°C, respectively) in HSC compared to TN pigs, regardless of feeding treatment (Table 4.1). Vaginal temperature was greater overall ($P < 0.01$; 1.85°C) in HSC compared to TN pigs at every time point except 0 and 15 min, regardless of feeding treatment (Figure 4.2B). No other T_V differences were detected ($P \geq 0.27$) during the HS period (Table 4.1).

4.4.2.2   Recovery period

During the recovery period, T_V and maximum T_V were greater ($P \leq 0.04$; 0.39 and 2.35°C, respectively) in HSC pigs compared to TN pigs, regardless of feeding treatment (Table 4.1). Vaginal temperature was greater ($P = 0.02$; 1.53°C) from 0 to 30 min in HSC compared to TN pigs, regardless of feeding treatment. At 45 min of the recovery period, T_V was greater ($P = 0.02$; 0.56°C) in HSC+AF compared to TN+AF pigs (Figure 4.2B). No other T_V differences were detected ($P \geq 0.21$) during the recovery period (Table 4.1; Figure 4.2B).

4.4.3 Skin temperature

4.4.3.1 Heat stress period

During the HS period, T_{sk} was greater ($P < 0.01$; 8.32°C) in HSC compared to TN pigs, regardless of feeding treatment (Table 4.1; Figure 4.2C). Minimum T_{sk} and maximum T_{sk} were increased ($P < 0.01$; 6.72 and 8.63°C, respectively) in HSC compared to TN pigs, regardless of feeding treatment (Table 4.1). No other T_{sk} differences were detected ($P \geq 0.13$) during the HS period (Table 4.1).

4.4.3.2 Recovery period

During the recovery period, overall T_{sk} and minimum T_{sk} were reduced ($P < 0.01$; 0.90 and 3.68°C, respectively) in HSC compared to TN pigs, regardless of feeding treatment (Table 4.1). Overall, maximum T_{sk} was greater ($P < 0.01$; 4.87°C) in HSC compared to TN pigs, regardless of feeding treatment (Table 4.1). Skin temperature was greater ($P < 0.01$; 3.72°C) from 0 to 30 min but reduced (2.71°C) from 60 to 165 min in HSC compared to TN pigs, regardless of feeding treatment (Figure 4.2C). No other T_{sk} differences were observed ($P \geq 0.16$) during the recovery period (Table 4.1; Figure 4.2C).

4.4.4 Respiration rate

4.4.4.1 Heat stress period

During the HS period, RR was increased ($P < 0.01$; 89 bpm) in HSC compared to TN pigs, regardless of feeding treatment (Table 4.1; Figure 4.2D). No other RR differences were observed ($P \geq 0.12$) during the HS period (Table 4.1; Figure 4.2D).

4.4.4.2 Recovery period

During the recovery period, RR was greater overall ($P < 0.01$; 21 bpm) in HSC compared to TN pigs, regardless of feeding treatment (Table 4.1). Respiration rate was greater ($P < 0.01$; 33 bpm), from 0 to 105 min in HSC compared to TN pigs, regardless of feeding treatment (Figure 4.2D). No other RR differences were observed ($P \geq 0.28$) with during the recovery period (Table 4.1).

4.4.5   Feeding and drinking attempts

4.4.5.1   Heat stress period

During the HS period, feeding attempts were reduced overall ($P < 0.01$; 90.68%) in HSC compared to TN pigs, regardless of feeding treatment (Figure 4.3A). Drinking attempts tended to be increased overall ($P = 0.07$; 69.50%) in TN+AF compared to TN+NF pigs (Table 4.2). No other feeding and drinking attempt differences were detected ($P \geq 0.17$) during the HS period (Table 4.2; Figure 4.3A, B).

4.4.5.2   Recovery period

During the recovery period, feeding attempts were greater ($P = 0.02$; 197.74%) in AF compared to NF pigs, regardless of recovery treatment (Figure 4.3A). No other feeding or drinking attempt differences were detected ($P \geq 0.13$) during the recovery period (Table 4.2; Figure 4.3A, B).

4.4.6   Histology

Jejunal villus height and villus height-to-crypt depth ratio were reduced overall ($P < 0.01$; 24.72 and 24.35%, respectively) in HSC compared to TN pigs, regardless of feeding treatment (Table 4.3). Jejunal goblet cell count tended to be decreased ($P = 0.08$; 30.10%) in AF compared to NF pigs, regardless of recovery treatment (Table 4.3). No other jejunal histology differences were observed ($P \geq 0.41$; Table 4.3).

Ileal villus height and villus height-to-crypt depth ratio were reduced ($P < 0.01$; 26.10 and, 25.29%, respectively) in HSC pigs compared to TN pigs, regardless of feeding treatment (Table 4.3). Ileal goblet cell count was greater overall ($P = 0.01$; 37.87%) in HSC compared to TN pigs, regardless of feeding treatment (Table 4.3). No other ileal histology differences were observed ($P \geq 0.21$; Table 4.3).

4.5   Discussion

Acute hyperthermia is characterized by an uncontrolled increase in body temperature when thermoregulatory mechanisms are overwhelmed by metabolic and environmental heat loads (Bouchama and Knochel, 2002; Smith, 2005). Without a rapid return of body temperature to euthermia, acute hyperthermia can lead to organ damage and increased rates of morbidity and

mortality (Gaudio and Grissom, 2016). As such, rapid cooling has been evaluated as a management tool to alleviate the negative impacts of acute hyperthermia in pigs (Johnson et al., 2016a,b; Sapkota et al., 2016; Kpodo et al., 2018), but results are conflicting depending on whether pigs have feed access or do not have feed access during acute hyperthermia recovery. Specifically, in studies where feed access was allowed (Johnson et al., 2016a,b; Sapkota et al., 2016), the return of T_{GI} to euthermia was delayed during rapid cooling whereas in studies where feed access was not allowed, T_{GI} quickly returned to euthermia during rapid cooling (Kpodo et al., 2018). In the current study, in agreement with the hypothesis, the return of T_{GI} to euthermia during the recovery period was more rapid in HSC+NF versus HSC+AF pigs. However, no other body temperature indices (i.e., RR, T_{SK}, T_R) differences were detected for recovery treatment by feeding treatment interactions. Because T_{GI} was reduced more rapidly in HSC+NF compared to HSC+AF pigs in the absence of increased heat dissipation capacity (i.e., increased RR or T_{SK}; Blatteis, 1998), this likely indicates that the feeding treatment alone was responsible for the more rapid decrease in T_{GI} for HSC+NF pigs as previously suggested (Kpodo et al., 2018).`

Pigs reduce voluntary feed intake to decrease metabolic heat load during times of acute hyperthermia (Pearce et al., 2014; Rauw et al., 2017). However, upon removal of the environmental insult (i.e., return of T_A to TN conditions and/or rapid cooling), voluntary feed intake (Xin and DeShazer, 1992) and feeding behavior (Johnson et al., 2016a) immediately returns to normal levels. Unfortunately, a rapid increase in feed intake has the potential to add metabolic heat to the body (i.e., heat of nutrient processing; Cervantes et al., 2018) and delay the return of body temperature to euthermia after acute hyperthermia. In the current study, there was an overall increase in feeding attempts for AF compared to NF pigs during the recovery period. When considering the lack of thermoregulatory differences (e.g., RR and T_{SK}), the increase in feeding attempts (and likely feed intake) may explain the decreased rate of reduction in T_{GI} for HSC+AF versus HSC+NF pigs during the recovery period. However, although an overall increase in feeding attempts during the recovery period was observed in AF versus NF pigs, no feeding attempt differences were observed between HSC+AF and HSC+NF pigs, which is surprising considering the numerical increase in feeding attempts during the recovery period for HSC+AF pigs. While reasons for the lack of feeding attempt differences are currently unclear, it is possible that HSC+AF pigs may have had fewer feeding attempts but consumed more feed at each attempt leading to the

increase in metabolic heat production and ultimately T_{GI} compared to HSC+NF pigs during the recovery period. However, due to study design this hypothesis cannot be confirmed in the present experiment. Alternatively, discrepancies between the current study and previous results (Johnson et al., 2016a) may be due to experimental design differences. Pigs in the present study remained in the HS room and T_A was decreased over 1 h to TN conditions whereas pigs in the previous study (Johnson et al., 2016a) were immediately moved from HS to TN conditions before water dousing. This means that T_A remained elevated for pigs in the present study for a longer period of time when compared to the previous study (Johnson et al., 2016a), and this may have initially reduced their feeding attempts because feeding behavior is influenced by T_A (Xin and DeShazer, 1992). Regardless of the reason, the delayed return of T_{GI} to euthermia in HSC+AF compared to HSC+NF pigs independent of thermoregulatory differences may indicate that feed removal could be combined with rapid cooling for a faster return of T_{GI} to euthermia as previously hypothesized (Kpodo et al., 2018).

A swift return of body temperature to euthermia is important to restore thermoregulatory function and reduce the negative effects of acute hyperthermia on organs (Pease et al., 2009). Acute hyperthermia results from the inability of the body to maintain its internal temperature homeostasis due unbalanced heat dissipation and heat loads (Bouchama and Knochel, 2002). In the current study, regardless of feeding treatment, exposure to HS conditions resulted in marked increases in all body temperature and thermoregulatory measures (e.g., T_V, T_{sk}, T_{GI}, and RR) when compared to TN conditions, suggesting that HSC pigs were suffering from hyperthermia during the HS period. The lack of recovery treatment by feeding treatment differences on body temperature is not surprising, since heat-stressed animals decrease feed intake as a strategy to reduce metabolic heat load and combat hyperthermia (Pearce et al..2014; Ma et al., 2019). Although, feed intake was not measured in the present study, the similar feeding attempts between HSC+AF and HSC+NF pigs suggests that HSC+AF pigs may have reduced their feed intake during the HS challenge thereby reducing metabolic heat production and limiting a feed intake-induced body temperature increase.

While no feeding and recovery treatment interactions were observed for thermoregulatory measures during the HS period, all body temperature measures were reduced over time from 0 to

180 min for HSC pigs during the recovery period. These results agree with previous reports in acutely hyperthermic pigs that are rapidly cooled (Johnson et al., 2016a,b; Kpodo et al., 2018) and were expected because rapid cooling increases the temperature gradient between the core and the skin (Casa et al., 2007) leading to a decrease in body temperature over time in multiple species (Vaile et al., 2011; Walker et al., 2014; Sawicka et al., 2015). Although body temperature was reduced in both the current and previous studies (Johnson et al., 2016a,b; Kpodo et al., 2018), HSC pigs in the current study had a more rapid reduction in body temperature to euthermia over time during the recovery period when compared to studies where pigs were provided feed *ad libitum* (Johnson et al., 2016a,b). While reasons for this discrepancy cannot be completely explained by this experiment, it may be due to body mass differences in which pigs in the current study were approximately 10.1 kg lighter compared to the Johnson et al. (2016a) study and approximately 59.3 kg lighter compared to pigs in the Johnson et al. (2016b) study. This is because the surface area to mass ratio is greater in younger and/or smaller pigs (Renaudeau et al., 2012), which can allow for improved heat dissipation capacity (Blatties, 1998). In addition, although body temperature measures were reduced in HSC pigs over time during the recovery period, these measures remained elevated overall when compared to TN-exposed pigs despite the fact that HSC pigs returned to euthermia by the end of the recovery period. It is likely that the greater body temperature for HSC pigs at the beginning of the recovery period was the driver for this observed increase. An alternative explanation may be that the overall increase in body temperature indices for HSC compared to TN pigs may have been due to a decrease in heat dissipation capacity through the skin due to cold water dousing as previously observed (Marlin et al., 1998; Johnson et al., 2016a,b; Kpodo et al., 2018). Because an increase in T_{sk} is generally associated with greater blood flow to the skin and heat dissipation to the environment (Blatteis, 1998), the observed reduction in T_{sk} may suggest that vasoconstriction occurred leading to a decrease in heat transfer and a maintenance in body temperature above that of TN pigs during the recovery period as a whole.

To maximize heat dissipation, hyperthermic mammals divert blood to the periphery by a combination of subcutaneous vasodilation and gastrointestinal tract vasoconstriction (Hales et al., 1979; Romanovsky and Blatteis, 1996). However, these physiological changes deprive enterocytes of oxygen and nutrients, resulting in increased intestinal permeability and morphological indicators of intestinal damage (Hall et al., 1999; Lambert, 2009). Morphological indicators of

intestinal damage resulting from HS exposure such as reduced villus height, crypt depth, villus height-to-crypt depth ratio are well-documented (Johnson et al., 2016a; Kumar et al., 2017; Abuajamieh et al., 2018). In accordance with these reports, villus height and villus height-to-crypt depth ratio were reduced in both the jejunum and ileum of HSC compared TN pigs, likely indicating that HSC pigs had compromised intestinal function relative to TN exposed pigs. However, despite the recovery treatment differences observed, no intestinal morphology differences were detected between HSC+AF and HSC+NF pigs. This is contrary to previous study in which rapidly cooled pigs having access to feed had increased intestinal damage (Johnson et al., 2016a). However, this discrepancy may be explained by the almost 5-fold reduction in feeding attempts during the first hour of recovery for pigs in the current study when compared to the previous study (Johnson et al., 2016a) since rapid re-feeding after fasting is associated with greater intestinal damage in pigs (Lallès and David, 2011). While reasons for this discrepancy are currently unclear, they may be due to sex differences since pigs in the present study were all gilts and the previous study only used barrows (Johnson et al., 2016a). Alternatively, because pigs in the previous study were larger, they may have been more motivated to consume feed because larger pigs have greater ADFI (Duttlinger et al., 2019). Regardless of the reason, these results may imply that the rate of re-feeding after acute hyperthermia could be a more important determinant of intestinal damage than the rate of body temperature reduction during recovery. However, this hypothesis would have to be confirmed in subsequent studies.

In addition to its negative effects on villus height and crypt depth, acute hyperthermia reduces goblet cell count and activity (Ashraf et al., 2013; Abuajamieh et al., 2018). Goblet cells are epithelial cells that produce mucins, the principal component of the protective mucus layers of the intestine (Birchenough et al., 2015). The roles of the mucus are to lubricate the intestine and prevent bacteria adhesion to the epithelium (Kim and Ho, 2010; Broom, 2018). Decreased goblet cell count in response to stressors or disease states is well-documented (Jung and Saif, 2017; Johnson et al., 2018). In the present study, although no differences were observed in the jejunum, ileal goblet cell count was reduced in HSC compared to TN pigs, and this is consistent with previous reports in heat-stressed pigs (Johnson et al., 2018) and quails (Sandikci et al., 2004). The decrease in goblet cell count suggests reduced mucin production and potentially compromised intestinal function that could lead to greater susceptibility to infection. Despite the fact that overall

recovery treatment differences were observed for goblet cell counts, no feeding treatment-related differences were detected. This is consistent with the aforementioned lack of villus height and crypt depth differences and may be due to the decrease in re-feeding attempts during the recovery phase in the current study compared with previous reports (Johnson et al., 2016a).

4.6   Conclusions

We hypothesized that feed withdrawal would contribute to a more rapid return of body temperature to euthermia and reduce hyperthermia-induced intestinal damage in pigs. In agreement with the hypothesis, it was determined that feed removal hastened the return of T_{GI} to euthermia. However, contrary to the hypothesis, feed removal did not prevent intestinal damage as indicated by morphological measures. Regardless, these data suggest that feed removal may accelerate the return of T_{GI} to euthermia when pigs are rapidly cooled after acute hyperthermia, which is a key element for favorable prognosis. Furthermore, these data may have positive implications towards using feed removal in combination with rapid cooling as a strategy to promote acute hyperthermia recovery in pigs.

4.7   References

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Table 4.1 Effects of rapid cooling after acute hyperthermia on thermoregulation parameters in pigs with or without access to feed.

Parameter	Recovery treatment + Feeding treatment					P-value		
	TN[1]+AF[2]	TN+NF[3]	HSC[4]+AF	HSC+NF	SEM	R[5]	F[6]	R x F
Heat stress period								
T_{GI}[7], °C	40	39.77	41.96	41.91	0.21	<0.01	0.51	0.66
Min[8] T_{GI}, °C	39.65	39.51	40.33	40.35	0.15	<0.01	0.68	0.56
Max[9] T_{GI}, °C	40.34	40.18	42.82	43.07	0.30	<0.01	0.87	0.48
T_V[10], °C	38.89	38.92	40.5	40.57	0.15	<0.01	0.76	0.93
Min T_V, °C	38.76	38.75	38.96	38.83	0.09	0.03	0.21	0.27
Max T_V, °C	38.99	39.07	41.33	41.66	0.22	<0.01	0.42	0.62
T_{sk}[11], °C	32.24	32.61	40.69	40.8	0.15	<0.01	0.13	0.39
Min T_{sk}, °C	31.15	31.52	37.88	38.23	0.28	<0.01	0.26	0.98
Max T_{sk}, °C	33.1	33.42	41.70	42.07	0.23	<0.01	0.15	0.91
RR[12], bpm	40	39	123	133	4	<0.01	0.17	0.12
Recovery period								
T_{GI}, °C	40.04	39.76	41.13	40.39	0.19	<0.01	0.01	0.27
Min T_{GI}, °C	39.69[xy]	39.48[y]	40.16[x]	39.26[y]	0.20	0.54	0.01	0.10
Max T_{GI}, °C	40.32	40.08	42.73	42.99	0.28	<0.01	0.98	0.41
T_V, °C	38.82	38.85	39.26	39.18	0.17	0.04	0.90	0.76
Min T_V, °C	38.66	38.72	38.54	38.52	0.15	0.21	0.87	0.75
Max T_V, °C	38.97	38.99	41.13	41.52	0.25	<0.01	0.47	0.51
T_{sk}, °C	32.09	32.57	31.28	31.58	0.25	<0.01	0.16	0.74
Min T_{sk}, °C	31.08	31.78	27.82	27.68	0.27	<0.01	0.33	0.16
Max T_{sk}, °C	32.95	32.3	37.1	37.88	0.33	<0.01	0.17	0.58
RR, bpm	31	34	52	54	2	<0.01	0.21	0.72

[1]TN, thermoneutral.

[2]AF, access to feed.

[3]NF, no feed access.

[4]HSC, heat stress followed by rapid cooling ·

[5]Recovery treatment.

[6]Feeding treatment.

[7]T_{GI}, gastrointestinal temperature.

[8]Min, minimum.

[9]Max, maximum.

[10]T_V, vaginal temperature.

[11]T_{sk}, trunk skin temperature.

[12]RR, respiration rate.

[xyz]Letters indicate tendencies $0.05 < P \leq 0.10$.

Table 4.2 Effects of rapid cooling after acute hyperthermia on feeding attempts and drinking attempts in pigs with or without access to feed.

Parameter	Recovery treatment + Feeding treatment				SEM	P-value		
	TN^1+AF^2	$TN+NF^3$	HSC^4+AF	HSC+NF		R^5	F^6	R x F
Heat stress period								
Feeding, %	1.91	1.2	0.16	0.13	0.44	<0.01	0.56	0.63
Drinking, %	0.43^y	1.41^x	1.20^{xy}	0.69^{xy}	0.42	0.83	0.55	0.07
Recovery period								
Feeding, %	1.51	0.84	2.33	0.45	0.56	0.99	0.02	0.28
Drinking, %	0.60	1.10	1.49	1.69	0.50	0.13	0.43	0.64

[1]TN, thermoneutral.

[2]AF, access to feed.

[3]NF, no feed access.

[2]HSC, heat stress followed by rapid cooling.

[5]Recovery treatment.

[6]Feeding treatment.

Differences at $P \leq 0.05$.

[xyz]Letters indicate tendencies $0.05 < P \leq 0.10$.

Table 4.3 Effects of rapid cooling after acute hyperthermia on intestinal morphology and mast cell density in pigs with or without access to feed.

	Recovery treatment + Feeding treatment					P-value		
	TN[1]+AF[2]	TN+NF[3]	HSC[4]+AF	HSC+NF	SEM	R[5]	F[6]	R x F
Jejunum								
Villus height, μm	447.64	437.57	326.37	340.03	27.28	<0.01	0.95	0.69
Crypt depth, μm	303.66	298.90	297.78	289.72	17.56	0.70	0.74	0.93
Villus height: crypt depth	1.54	1.54	1.11	1.22	0.09	<0.01	0.63	0.58
Goblet cells	7.14	8.78	5.01	8.58	1.33	0.42	0.08	0.50
Mast cells/mm^2	447.99	393.03	407.80	452.13	53.10	0.87	0.93	0.41
Ileum								
Villus height, μm	377.01	381.22	274.47	285.81	24.39	<0.01	0.74	0.88
Crypt depth, μm	220.92	220.59	217.43	218.50	12.92	0.85	0.98	0.96
Villus height: crypt depth	1.75	1.73	1.25	1.35	0.10	<0.01	0.71	0.56
Goblet cells	17.44	19.48	10.61	12.33	2.26	0.01	0.47	0.95
Mast cells/mm^2	1031.32	1135.82	982.86	1121.16	99.56	0.74	0.21	0.86

[1]TN, thermoneutral.

[2]AF, access to feed.

[3]NF, no feed access.

[2]HSC, heat stress followed by rapid cooling.

[5]Recovery treatment.

[6]Feeding treatment.

Differences at $P \leq 0.05$.
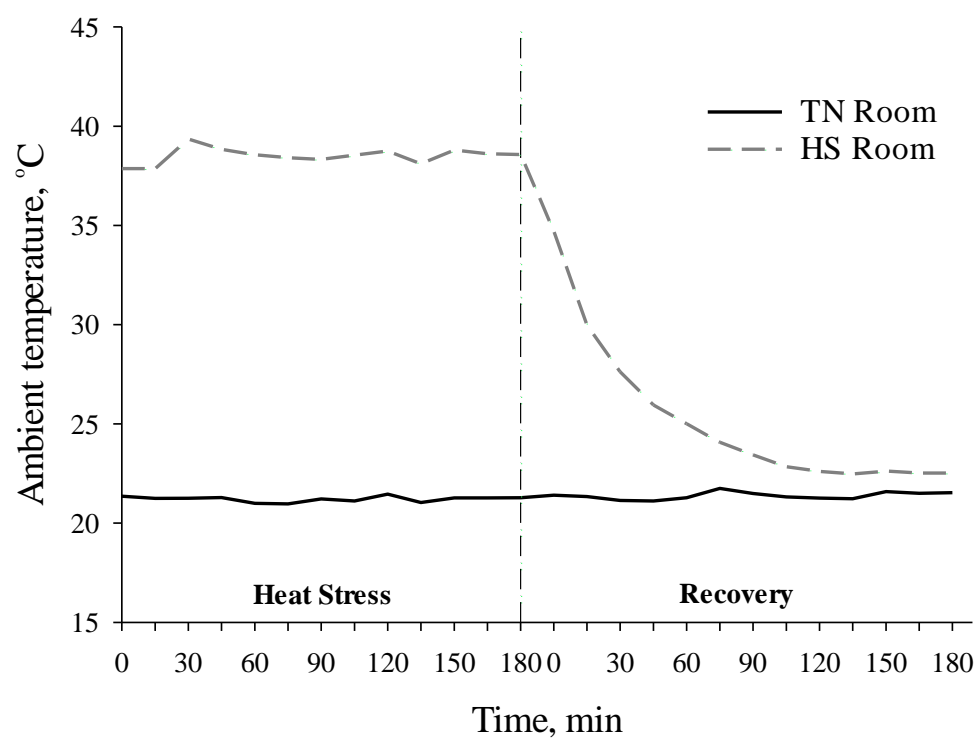
Figure 4.1 Ambient temperature by time during the heat stress and recovery periods.
Abbreviations are thermoneutral (TN) and heat stress (HS).
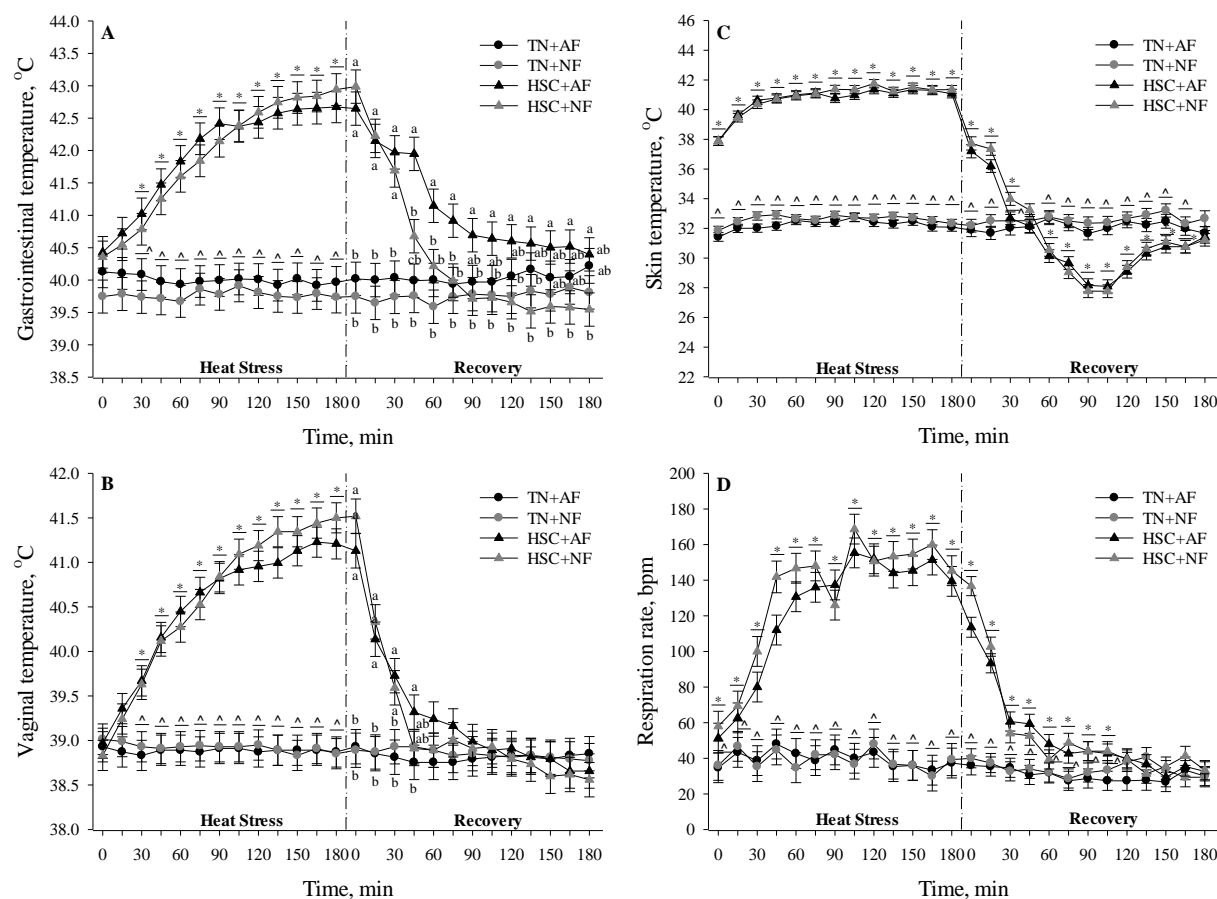
Figure 4.2 Effects of recovery treatment on body temperature indices.

Effects of recovery treatment on body temperature indices recorded every 15 min during the heat stress and recovery periods on (A) gastrointestinal temperature, (B) vaginal temperature, (C) skin temperature, and (D) respiration rate in pigs with or without access to feed. Abbreviations: recovery treatment (R), thermoneutral (TN), heat stress followed by rapid cooling (HSC), feed access (AF), and no feed access (NF). Error bars at each 15-min time point indicate ± 1 SEM. [*],[^]Symbols indicate differences ($P < 0.05$) comparing recovery treatment by time. [a,b,c]Letters indicate differences ($P < 0.05$) comparing recovery treatment by feeding treatment by time at each 15-min time point.
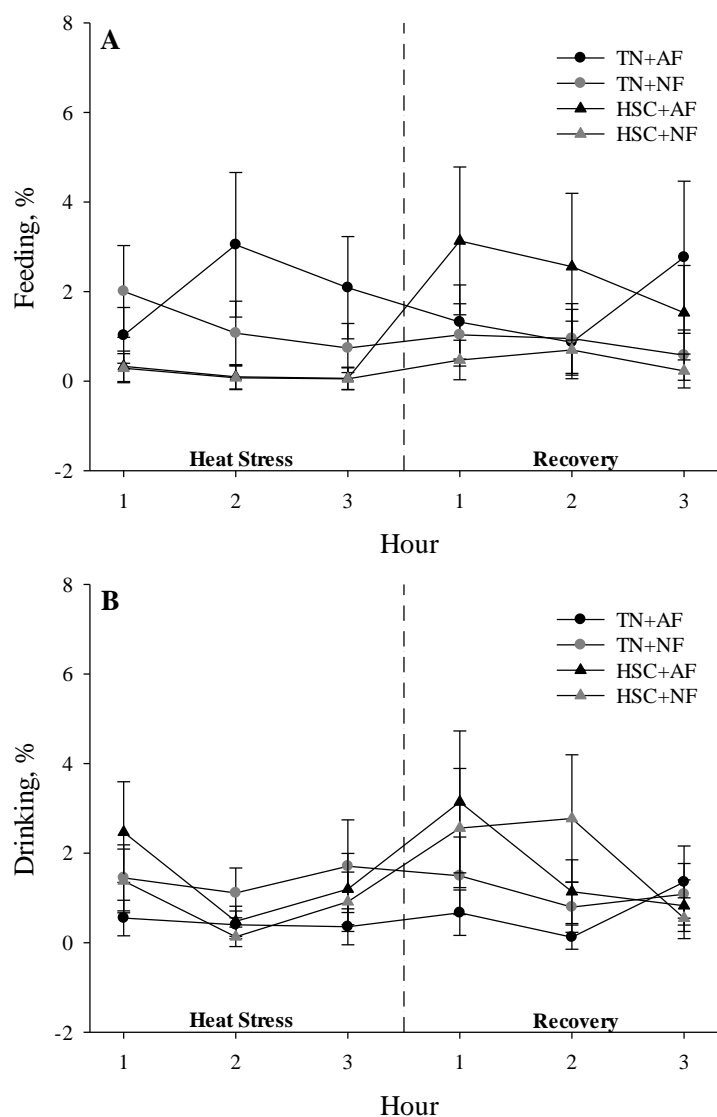
Figure 4.3 Effects of recovery treatment on feeding and drinking attempts.

Effects of recovery treatment on (A) feeding attempts and (B) drinking attempts as % of hour during the heat stress and the recovery periods in pigs with or without access to feed. Abbreviations: recovery treatment (R), thermoneutral (TN), heat stress followed by cooling (HSC), access to feed (AF), and no feed access (NF). Error bars at each hour indicate ± 1 SEM. No differences observed ($P > 0.05$).

CHAPTER 5. EFFECTS OF FEED REMOVAL DURING ACUTE HEAT STRESS ON THE INFLAMMATORY RESPONSE OF GROW-FINISH PIGS

5.1 Abstract

Feeding increases body temperature and can delay the return to euthermia during heat stress (**HS**) in pigs. As a result, the systemic inflammatory response may be increased in HS pigs allowed *ad libitum* feed access. Therefore, the study objective was to evaluate the effects of feed removal during acute HS on the systemic inflammatory response and its short-term effect on growth performance in grow-finish pigs. Thirty-two pigs (93.29 ± 3.14 kg initial BW; 50% barrows and 50% gilts) were subjected to thermoneutral conditions (**TN**; 23.47 ± 0.10°C; n = 16 pigs) for 24 h, or HS for 24 h (cycling 25-36°C; n = 16 pigs). Within each temperature treatment, one-half of the pigs were provided feed (**AF**; n = 8 pigs) and one-half had no feed access (**NF**; n = 8 pigs). At the end of the 24-h temperature treatment period, all pigs were given *ad libitum* access to feed and water and maintained under similar TN conditions for 6 d. During the 24-h temperature and feeding treatments (**TF**) period, gastrointestinal (T_{GI}) and skin (T_{sk}) temperatures were recorded every 30 min during the first 12 h. Serum samples were collected for cytokine analyses at 0, 4, 8, 12, and 24 h of the TF period and then on d 3 and 6 after the TF period. Body weight and feed intake were measured on d 0, 1, 3, and 6 to determine average daily gain (**ADG**) and average daily feed intake (**ADFI**). Pigs were video-recorded throughout the entire trial to assess feeding behavior and posture. During the first 12 h of the TF period, T_{GI} and T_{sk} were greater overall ($P < 0.02$; 1.08 and 4.02°C, respectively) in HS compared to TN pigs, regardless of feeding treatment. During the post-TF period, IL-1α was greater overall ($P < 0.01$; 201.41%) in HS+NF compared to HS+AF and TN+NF pigs. During the TF period, weight loss was greater overall ($P = 0.01$; 53.37%) in HS compared to TN pigs, regardless of feeding treatment. During the TF period weight loss was reduced ($P = 0.02$; 37.92%) in HS+AF compared to HS+NF and TN+NF pigs. During d 1 to 2 post-TF period, ADG was reduced overall ($P < 0.01$; 59.65%) in TN+AF pigs compared to HS+AF, HS+NF, and TN+NF pigs. From d 1 to 2 of the post-TF period, ADFI was reduced ($P = 0.02$; 12.31%) in HS compared to TN pigs, regardless of feeding treatment, whereas G:F was increased overall ($P < 0.01$; 37.27%) in HS compared to TN pigs, regardless of feeding treatment. Following the TF period, overall (0800-0800 h), drinking behavior was greater ($P = 0.03$; 64.86%) in HS

compared to TN pigs, regardless of feeding treatment. After the TF period, daytime (0800-2000 h) sitting behavior was greater ($P = 0.05$; 59.29%) in HS compared to TN pigs, regardless of feeding treatment. In conclusion, feed removal during an acute HS challenge did not reduce the systemic inflammatory response or improve short-term growth performance of grow-finish pigs.

Keywords: acute heat stress, inflammation, cytokine, feed removal, pigs.

5.2   Introduction

Elevated environmental temperatures subject pigs to heat stress (**HS**) and can cause substantial economic losses to the U.S. swine industry (St-Pierre et al., 2003) due to reduced performance and carcass quality as well as increased morbidity and mortality (as reviewed by Johnson, 2018). Economic losses are expected to increase as global temperatures continue to rise (NOAA, 2018), and identifying risk factors and mitigating the effects of acute hyperthermia are of utmost importance to maintain producer profitability and improve animal welfare. Heat stress causes a well-described increase in intestinal permeability resulting in greater bacterial translocation and increased systemic inflammation (Lambert, 2004), which persists even after the HS insult has ceased (Johnson et al., 2016a,b). In addition, HS causes a release of inflammatory cytokines from muscles into circulation that can negatively impact animal health (Bouchama and Knochel, 2002). Extreme heat events during summer months put pigs at a greater risk for acute hyperthermia due to their limited ability to use evaporative heat loss (Renaudeau et al., 2012). Therefore, developing HS recovery strategies is an important step for improving swine health and well-being.

When exposed to elevated environmental temperatures, pigs reduce feed intake to decrease metabolic heat load (Renaudeau et al., 2012). However, feed intake increases immediately after the heat load is removed (Xin and DeShazer, 1992), and this has the potential to add heat to the body and delay the return to euthermia due to the heat of nutrient processing (Cervantes et al., 2018). Previous studies in pigs given feed access compared with those not given feed access during HS recovery have demonstrated that body temperature return to euthermia is delayed (Kpodo et al., 2019), and this may exacerbate the systemic inflammatory response since the negative consequences of HS may be related to the intensity and duration of heat exposure (Eshel et al.,

2001). Therefore, the study objective was to evaluate the effects of feed removal during an acute heat event on the systemic inflammatory response and the short-term effect on growth performance in grow-finish pigs. We hypothesized that feed removal during an acute heat event would reduce the systemic inflammatory response and improve short-term growth performance in grow-finish pigs.

5.3   Materials and methods

5.3.1   Animal and Experimental Design

All procedures involving animals were approved by the Purdue University Animal Care and Use Committee (PACUC no. 1811001826), and animal care and use followed the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (Federation of Animal Science Society, 2010). The study was conducted in February 2019. In two repetitions, 32 crossbred [Duroc x (Landrace x Yorkshire)] pigs [n = 16 barrows/repetition and 16 gilts/repetition; 93.29 ± 3.14 kg initial body weight (**BW**)] were used. One day prior to the experiment, all pigs were moved into individual pens (1.22 m x 2.01 m) within two environmental rooms [thermoneutral (**TN**) and HS] at the Purdue University Animal Sciences Research and Education Center. Within each environmental room, two data loggers (HOBO, data logger temp/RH; accuracy ± 0.2°C; Onset, Bourne, MA) were used to record ambient temperature (**T_A**) and relative humidity (**RH)** in 5-min intervals for the duration of the experiment. At 1500 h on the same day pigs were moved, each pig was orally administered a CorTemp temperature sensor (model HT150002, accuracy ± 0.1°C; HQ, Inc, Palmetto, FL) to monitor gastrointestinal temperature (**T_{GI}**). The temperature sensor was expected to be between the duodenum and the jejunum on the following day when treatments were applied as previously determined (Johnson et al., 2016a).

For the experiment, pigs were either subjected to TN conditions (n = 8 pigs/repetition; 23.47 ± 0.10°C; 62.49 ± 0.61% RH) or a cyclic HS condition (n = 8 pigs/repetition) for 24 h. To achieve the cyclic HS, T_A was gradually increased from 23.78 to 36.39°C, over a 4-h period, and then maintained constant at 36.39 ± 0.09°C for another 4 h. Thereafter, T_A was gradually decreased to 25.26 ± 0.11°C, over a 4-h period where it remained for the next 12 h (Figure 5.1). During this 24-h period within each temperature treatment, one-half of the pigs had *ad libitum* feed access (**AF**;

n = 4 pigs/temperature treatment), whereas the other half did not have feed access (**NF**; n = 4 pigs/temperature treatment); however, all pigs had *ad libitum* access to water. Following the 24-h period of temperature and feeding treatments, which is to be hereafter referred to as the **TF** period, all pigs were kept under TN conditions ($23.30 \pm 0.81°C$; $57.22 \pm 10.48\%$) as defined by the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (Federation of Animal Science Society, 2010) for 6 d and a 12L:12D light cycle starting at 0800 h was maintained. All pigs had *ad libitum* feed and water access for the remainder of the trial, and pigs were fed a standard corn and soybean meal diet formulated to meet or exceed the NRC (2012) requirements for grow-finish pigs.

5.3.2 Body temperature indices

The T_{GI} was measured in 30-min intervals in all pigs for the first 12 h of the TF period using the pre-administered CorTemp temperature sensors. Skin temperature (**T_{Sk}**) was measured in all pigs in 30-min intervals for the first 12 h of the TF period. Skin temperature was measured by taking a broad side photo of each pig using an infrared camera (FLIR Model T440, accuracy $\pm 0.1°C$; emissivity = 0.95; FLIR Systems Inc., USA). Photos were analyzed with the FLIR Tools Software (Version 5.13) by one individual blind to the treatments. The T_{Sk} was determined by drawing a standardized circle on the trunk area (all skin caudal to the neck and dorsal to the elbow and stifle) and recording the mean temperature.

5.3.3 Blood collection and analyses

One blood tube (BD vacutainers; Franklin Lakes, NJ; serum) was collected from all pigs via jugular venipuncture. Blood was collected at 0, 4, 8, 12, and 24 h during the 24-h TF period, and on d 2 and 6 at 0800 h during the post-TF period. Blood samples were centrifuged at 1900 x *g* for 15 min at 4°C to collect serum. Serum samples were stored at -80°C and later submitted to the University of Minnesota Cytokine Reference Laboratory for interleukins (IL-1α, IL-1β, IL-6, IL-12, IL-10, and TNFα) analyses using a multiplex assay. The intra-assay and inter-assay coefficients of variation for cytokines were less than 10 and 20%, respectively. The TNFα concentrations were below the detectable limits and were not considered for further analysis.

5.3.4 Production parameters

Initial BW was measured on d 0 prior to and at the end of the 24-h TF period. Body weight and feed intake were measured on d 2 and 6 after the TF period. Weight gain during the TF period, ADFI, and ADG for d 1 to 2, 2 to 6, and 1 to 6 post-TF period were calculated and used in the growth performance data analysis.

5.3.5 Behavior recording and analyses

Pigs were video-recorded from d 1 to 6 during the post-TF period using ceiling mounted cameras (Panasonic WV-CP254H, Matsushita Electric Industrial Co. Ltd.., Osaka, Japan), and each camera was oriented to capture two adjacent pens (1 AF and 1 NF pig). Video was recorded during the 12 h of light and 12 h of dark from d 1 to 6 post-TF period. Video data were analyzed in Observer XT 11.5 (Noldus; The Netherlands) using the scan-sampling technique in 10-min intervals. Videos were analyzed for consumption behavior and posture by two trained individuals who were blind to the treatments and maintained at least 90% agreement. Consumption behavior included feeding behavior, drinking behavior and other. Posture included sitting, lying, and standing. A definition of each behavior is presented in an ethogram (Table 5.1).

5.3.6 Statistics

Data were analyzed as a 2 x 2 factorial treatment arrangement [temperature treatment (TN and HS) and feeding treatment (AF and NF)] using the PROC MIXED procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). The linear additive model was $Y_{ijk} = \mu + T_i + F_j + K_k + T*F_{ij} + e_{ijk,}$ where Y = dependent variable of interest, μ = mean, T = temperature treatment, F = feeding treatment, K= replication, and e = error term. Cytokine data were analyzed with repeated measures using hour within the TF period (4, 8, 12, and 24 h) or day post-TF period (2 and 6) as the repeated effect when required. For cytokine analyses, initial concentrations of each variable were used as a covariate when significant. Initial BW was included in the growth performance data analyses as a covariate when significant. Growth performance data were analyzed within the TF period and the post-TF period. For the post-TF period, growth performance data were analyzed separately from d 1-2, d 2-6, and d 1-6. Individual pigs were considered the experimental unit and repetition was included as a random factor in all analyses. For the cytokine analyses, plate was included in the model as a random factor. For the behavior data, only 5 d (d 1, 2, 4, 5, and 6) were analyzed

because pig's behavior was disturbed by weighing and blood collection on d 3. A percent of total observations for each pig was calculated and used in the analyses. Consumption behavior (feeding%, drinking%) and posture (standing%, sitting%, and lying%) data were determined for 24 h (0800-0800 h) and further separated by daytime (0800-2000 h) and nighttime (2000-0800 h) for analysis. Other behavior was not included in the final analysis. Behavior and cytokine data were log-transformed to meet normality assumptions when needed, and back-transformed LSmeans are reported. Hour, day, and sex were included as fixed effects in each analysis; however, they are reported only when significant for clarity. Statistical significance was considered at $P \leq$ 0.05, and a tendency was defined as $0.05 < P \leq 0.10$.

5.4 Results

5.4.1 Body temperature

5.4.1.1 Gastrointestinal temperature

During the TF period, T_{GI} was greater overall ($P < 0.01$; 1.08°C) in HS compared to TN pigs, regardless of feeding treatment (Table 5.2). At 180 and 210 min of the TF period, T_{GI} was greater ($P < 0.01$; 0.59°C) in HS+AF compared to TN+AF and TN+NF pigs (Figure 5.2). At 240 min of the TF period, T_{GI} was greater ($P < 0.01$; 0.62°C) in HS+AF compared to TN+AF and TN+NF pigs, whereas T_{GI} was greater ($P < 0.01$; 0.62°C) in HS+NF compared to TN+NF pigs (Figure 5.2). From 270 to 720 min of the TF period, T_{GI} was greater ($P < 0.01$; 1.49°C) in HS compared to TN pigs, regardless of feeding treatment (Figure 5.2). No other T_{GI} differences were detected ($P > 0.10$) during the TF period (Table 5.2; Figure 5.2).

5.4.1.2 Skin temperature

During the TF period, T_{sk} was greater overall ($P < 0.01$; 4.02°C) in HS compared to TN pigs, regardless of feeding treatment (Table 5.2; Figure 5.3). At 180, 420, 450, and 540 min of the TF period, T_{sk} was greater ($P < 0.01$; 0.94, 0.55, 0.80, and 0.76°C, respectively) in TN+AF compared to TN+NF pigs (Figure 5.3). No other T_{sk} differences were detected ($P \geq 0.19$) during the TF period (Table 5.2; Figure 5.3).

5.4.2 Cytokines

5.4.2.1 TF period

During the TF period, serum IL-1α, IL-6, IL-10, and IL-12 were greater overall ($P \leq 0.03$; 47.23, 39.91, 152.60, and 24.78%, respectively) in AF compared to NF pigs, regardless of temperature treatment (Table 5.3). A time difference was observed where IL-6 was greater ($P < 0.01$) at 8 h (19.51 ± 2.33 pg/mL) and 12 h (21.83 ± 4.11 pg/mL) compared to 4 h (13.26 ± 1.10 pg/mL) and 24 h (12.34 ± 2.10 pg/mL) of the TF period, regardless of temperature and feeding treatments (Figure 5.4). No differences were detected between 8 h and 12 h or 4 h and 24 h of the TF period (Figure 5.4). Serum IL-12 was greater ($P < 0.01$) at 4 h (511.59 ± 14.76 pg/mL), 8 h (530.67 ± 16.74 pg/mL), and 12 h (477.03 ± 20.78 pg/mL) when compared to 24 h (432.22 ± 22.44 pg/mL) of the TF period, regardless of temperature and feeding treatments, but no differences were detected between 4 h and 8 h or 4 h and 12 h of the TF period (Figure 5.5). Interleukin-12 was greater overall ($P = 0.05$) in gilts (514.81 ± 18.67 pg/mL) compared to barrows (463.29 ± 18.67 pg/mL), regardless of temperature and feeding treatments (Figure 5.6). No other cytokine differences were observed ($P \geq 0.11$) during the TF period (Table 5.3).

5.4.2.2 Post-TF period

On d 2 and d 6 post-TF period, serum IL-1α tended to be greater overall ($P = 0.09$; 45.91%) in HS compared to TN pigs, regardless of feeding treatment (Table 5.3). Overall, IL-6 and IL-12 were greater ($P < 0.01$; 73.49 and 21.73%, respectively) in AF compared to NF pigs, regardless of temperature treatment (Table 5.3). Interleukin-10 tended to be greater overall ($P = 0.06$; 169.71%) in AF compared to NF pigs, regardless of temperature treatment (Table 5.3). Interleukin-1α was greater overall ($P < 0.01$; 201.41%) in HS+NF compared to HS+AF and TN+NF pigs (Table 5.3). In addition, IL-1α was increased overall ($P < 0.01$; 265.37%) in TN+AF compared to TN+NF pigs (Table 5.3). Interleukin-6 was reduced overall ($P < 0.01$; 75.61%) in TN+NF compared to TN+AF, HS+AF, and HS+NF pigs (Table 5.3). Interleukin-1β tended to be greater overall ($P = 0.08$; 89.06%) in TN+AF compared to HS+AF pigs (Table 5.3). Interleukin-12 tended to be greater ($P = 0.10$; 29.77%) in TN+AF pigs compared to TN+NF, HS+AF, and HS+NF pigs (Table 5.3). No other cytokine differences were detected on d 2 or 6 after the TF period ($P > 0.17$; Table 5.3).

5.4.3   Growth Performance

5.4.3.1   TF period

During the 24-h TF period, weight loss was greater overall ($P = 0.01$; 53.37%) in HS compared to TN pigs, regardless of feeding treatment (Table 5.4). Weight loss was greater overall during the TF period ($P < 0.01$; 186.39%) in NF compared to AF pigs, regardless of temperature treatment (Table 5.4). Weight loss was reduced ($P = 0.02$; 37.92%) in HS+AF pigs compared to HS+NF and TN+NF pigs (Table 5.4). In addition, weight loss was reduced ($P = 0.02$; 91.12%) in TN+AF pigs compared to HS+AF, HS+NF, and TN+NF pigs (Table 5.4). No other growth performance differences were detected during the TF period ($P > 0.10$; Table 5.4).

5.4.3.2   Post-TF period

5.4.3.2.1   Average daily gain

When data were analyzed from d 1 to 2 post-TF period, ADG was greater overall ($P < 0.01$; 75.93%) in NF compared to AF pigs, regardless of temperature treatment (Table 5.4). From d 1 to 2 post-TF period, ADG was reduced overall ($P < 0.01$; 59.65%) in TN+AF pigs compared to HS+AF, HS+NF, and TN+NF pigs (Table 5.4). In addition, from d 1 to 2 post-TF period, ADG was reduced overall ($P < 0.01$; 29.76%) in HS+AF compared to TN+NF pigs (Table 5.4). When data were analyzed for the entire post-TF period (d 1 to 6), ADG was greater overall ($P < 0.01$; 40.29%) in NF compared to AF pigs, regardless of temperature treatment (Table 5.4). From d 1 to 6 post-TF period, overall, ADG was increased overall ($P < 0.01$; 32.93%) in TN+NF pigs and was reduced in TN+AF pigs (32.93%) compared to HS+AF and HS+NF pigs (Table 5.4). In addition, from d 1 to 6 post-TF period, ADG was greater ($P < 0.01$; 98.18%) in TN+NF pigs compared to TN+AF pigs (Table 5.4). No other ADG differences were detected during the post-TF period ($P > 0.10$; Table 5.4).

5.4.3.2.2   Average daily feed intake

From d 1 to 2 of the post-TF period, ADFI was reduced ($P = 0.02$; 12.31%) in HS compared to TN pigs, regardless of feeding treatment (Table 5.4). From d 1 to 2 of the post-TF period, overall, ADFI was reduced ($P = 0.02$; 12.02%) in AF compared to NF pigs, regardless of temperature treatment (Table 5.4). From d 1 to 2 of the post-TF period, ADFI was greater ($P = 0.01$; 14.42%) in for barrows to gilts, regardless of temperature and feeding treatment (Table 5.5). From d 2 to 6

of the post-TF period, ADFI tended to be greater ($P = 0.07$; 10.36%) in TN+NF compared to TN+AF and HS+NF pigs (Table 5.4). From d 2 to 6 of the post-TF period, overall, ADFI was greater ($P = 0.01$: 11.40%) in barrows compared to gilts, regardless of temperature and feeding treatment (Table 5.5). From d 2 to 6 of the post-TF period, overall, ADFI was reduced in TN+AF-gilts and HS+NF-gilts ($P = 0.01$; 18.97 and 19.59%, respectively) compared to TN+AF-barrows, TN+NF-barrows, TN+NF-gilts, HS+NF-barrows and HS+AF-gilts (Table 5.5). When data were analyzed for the entire post-TF period (d 1 to 6), ADFI tended to be decreased overall ($P = 0.08$; 5.84%) in HS compared to TN pigs, regardless of temperature treatment (Table 5.4). From d 1 to 6 of the post-TF period, ADFI was greater ($P = 0.03$; 13.09%) in TN+NF pigs compared to TN+AF, HS+AF, and HS+NF pigs (Table 5.4). From d 1 to 6 of the post-TF period, ADFI, overall, ADFI was greater ($P < 0.01$; 10. 07%) in barrows compared to gilts (Table 5.5). From d 1 to 6 of the post-TF period, ADFI was reduced ($P = 0.02$) in TN+AF-gilts and HS+NF-gilts (19.76 and 17.94%) compared to TN+AF-barrows, HS+NF-barrows, TN+NF-gilts, and HS+NF-barrows (Table 5.5). No other ADFI differences were detected during the post-TF period ($P > 0.10$; Table 5.4; Table 5.5).

5.4.3.2.3   Feed efficiency

From d 1 to 2, G:F was greater overall ($P < 0.01$; 37.27%) in HS compared to TN pigs, regardless of feeding treatment (Table 5.4). From d 1 to 2, overall, G:F was greater ($P < 0.01$; 49.67%) in NF compared to AF pigs, regardless of temperature treatment (Table 5.4). From d 1 to 2, in G:F was reduced ($P < 0.01$; 57.91%) in TN+AF pigs compared to TN+NF, HS+AF, and HS+NF pigs (Table 5.4). When the data were analyzed for the entire post-TF period (d 1 to 6), G:F was greater overall ($P < 0.01$; 32.61%) for NF compared to AF pigs, regardless of temperature treatment (Table 5.4). From d 1 to 6 of the post-TF period, G:F was reduced overall ($P < 0.01$; 37.29%) in TN+AF pigs compared to TN+NF, HS+AF, and HN+NF pigs (Table 5.4). No other G:F differences were detected ($P \geq 0.12$) during the post-TF period (Table 5.4).

5.4.4   Behavior

From 0800-0800 h, drinking behavior was greater ($P = 0.03$; 64.86%) in HS compared to TN pigs, regardless of feeding treatment (Table 5.6). During the daytime (0800-2000 h), eating behavior tended to be reduced overall ($P = 0.06$; 26.00%) in HS+AF compared to TN+AF pigs (Table 5.6).

During the daytime, drinking behavior tended to be increased overall ($P = 0.07$; 27.94%) in NF compared to AF pigs, regardless of temperature treatment (Table 5.6). No other consumption behavior differences were detected ($P \geq 0.12$; Table 5.6).

From 0800-0800 h, standing behavior tended to be reduced ($P = 0.06$; 19.38%) in HS+AF compared to TN+AF pigs (Table 5.6). From 0800-0800 h sitting behavior tended to be greater ($P = 0.10$; 40.90%) in HS compared to TN pigs, regardless of feeding treatment (Table 5.6). From 0800-0800 h, lying behavior was reduced ($P = 0.02$; 2.85%) in TN+AF compared to TN+NF and HS+AF pigs (Table 5.6).

During the daytime (0800-2000 h), standing behavior was reduced ($P = 0.01$; 29.30%) in HS+AF compared to TN+AF pigs (Table 5.6). During the daytime, standing behavior tended to be reduced ($P = 0.07$; 13.70%) in HS compared to TN pigs, regardless of feeding treatment (Table 5.6). Sitting behavior was greater throughout the daytime ($P = 0.05$; 59.29%) in HS compared to TN pigs, regardless of feeding treatment (Table 5.6). Lying behavior was reduced overall during the daytime ($P = 0.01$; 06.03%) in TN+AF pigs compared to TN+NF and HS+AF pigs (Table 5.6). Standing behavior was greater overall during the nighttime ($P = 0.04$) in barrows (7.83 ± 0.71%) compared to gilts (6.23 ± 0.69%; Figure 5.7). No other posture differences were detected ($P \geq 0.33$; Table 5.6).

5.5   Discussion

Acute hyperthermia is associated with an increased systemic inflammatory response and reduced overall health and well-being of animals (Lambert, 2009). Previous studies reported that cooling in combination with feed removal may be a viable mitigation strategy for reducing the negative impacts of acute hyperthermia in pigs (Kpodo et al., 2018; Kpodo et al., 2019). However, these studies were conducted under constant HS conditions and used a rapid cooling method. In the current study, pigs with or without access to feed were exposed to a cyclic HS to mimic typical summer heat events. Heat stress exposure increased the T_{GI} and T_{sk} compared to TN conditions, regardless of feeding treatment suggesting that the HS protocol was successfully implemented, and that all HS pigs suffered from hyperthermia as previously described (Smith, 2005).

Hyperthermic mammals redistribute blood to the periphery to maximize heat dissipation (Romanovsky and Blatteis, 1996). However, this occurs at the expense of the gastrointestinal tract leading to hypoxia, nutrient depletion, and oxidative stress (Hall et al., 1999). As a result, the intestinal epithelium is damaged leading to an increase in bacteria and endotoxin translocation into systemic circulation (Lambert, 2009). Although HS increases endotoxin translocation in the blood, cytokine concentrations are not always elevated consistently from study-to-study (as reviewed by Lee et al., 2016). Limited data on the effects of HS on circulating IL-1α, L-10, and IL-12 exist in pigs, and most studies measuring these cytokines were conducted in rodent species (Lin et al., 1994; Wang, et al., 2005; Helwig and Leon, 2011). In the current study, no serum cytokine differences were observed between HS and TN pigs during the TF period regardless of feeding treatment. The IL-1β and IL-6 results are in accordance with previous reports (Pearce et al., 2013; Campos et al., 2014; Johnson et al., 2016a) where no differences were detected in pigs exposed to HS conditions. Furthermore, the lack of differences in IL-1α during the TF period are in accordance with those reported in hyperthermic mice (Leon et al., 2006). In contrast to the results in the current study, previous research reported that circulating IL-1β, IL-6, IL-10, and IL-12 were increased in hyperthermic mice (Leon et al., 2006). The discrepancies between these studies and the current study may be due to species and HS protocol differences. In the current study, pigs were exposed to a cyclic HS as opposed to acute hyperthermia and then immediate cooling (Leon et al., 2006). Additionally, IL-12 is increased in HS mice when their maximum core body temperature is reached, while IL-1β, IL-6, and IL-10 are increased during recovery when mice developed hypothermia (Leon et al., 2006). Reasons for the lack of cytokine differences observed in the current study are unknown; however, it is possible that pigs in the present study had a suppressed immune response since HS has been shown to reduce the immune function (Meng et al., 2013). Alternatively, HS increases heat shock protein 70 (HSP70) production (Pearce et al., 2015; Bharati et al., 2017), which can inhibit NF-kB and downregulate cytokine production (Chen et al., 2006). Although HSP70 was not measured in the current study, it is possible that HS may have increased HSP70 which downregulated IL-1α, IL-1β, and IL-6 gene expression (and subsequently production) during the TF period.

In addition to the effects of HS on cytokine production, cytokine concentrations were reduced overall in NF compared to AF pigs, regardless of temperature treatment. Limited data exists on

the effects of feeding on circulating cytokine concentration in pigs; however, a study in humans reported decreased IL-6 during fasting (Aksungar et al., 2007). While reasons why cytokine concentrations were reduced in NF compared to AF pigs are unclear, it is possible that reduced energy availability for NF pigs may have inhibited their immune response since the immune system requires glucose for activation and function (Kvidera et al., 2017). As a result, the production of cytokines by immune cells (monocytes, macrophages, etc.) may have been decreased (as reviewed Adawi et al., 2017) due to reduced energy availability. The reduction in proinflammatory cytokines during the TF period in the current study could have implications towards the ability of NF pigs to mount appropriate innate immune response in the case of infections.

Although no cytokine differences were detected in the TF period, IL-1α was greater overall in HS+NF compared to HS+AF pigs during the post-TF period. Interleukin-1α is a proinflammatory cytokine known to regulate the body response to infection and inflammation (Di Paolo and Shayakhmetov, 2016). It is increased by hyperthermia as evidenced by elevated concentrations in heat stroke victims before and after cooling (Bouchama et al., 1991). Reasons why IL-1α was increased in HS+NF compared to HS+AF after the TF period are unclear. However, it is possible that increased IL-1α in HS+NF pigs was due to re-feeding, as IL-1α was reduced in NF compared to AF pigs during the TF-period. Alternatively, it is possible that re-feeding following a 24-h period of fasting may have caused intestinal damage as previously reported (Lallès and David, 2011), and that IL-1α was increased as a result. It is tempting to speculate that feed removal followed by re-feeding may have been the cause for the increased inflammatory response after the TF period. However, because IL-1α was reduced in TN+NF compared to TN+AF pigs, this may suggest that the increase in IL-1α observed in HS+NF compared to HS+AF pigs was due to a delayed HS effect.

During times of HS, growth performance is reduced, and this reduction may depend on HS intensity (Pearce et al., 2014). In the present study, the 24-h TF period resulted in a marked decrease in BW in HS compared to TN pigs, regardless of feeding treatment. These data agree with previous reports (Kumar et al., 2017; Abuajamieh et al., 2018), and are likely due to gut fill because acute HS reduces feed intake (Pearce et al., 2014; Gabler et al., 2018). In addition, the

weight loss was greater in NF compared to AF within each temperature treatment, and this was expected because NF pigs did not have feed access. In addition to gut fill, NF pigs may have mobilized body energy reserves during the TF period resulting in weight loss (Mayes et al., 1988; Lallès and David, 2011). Furthermore, from d 1-2 of the post-TF period, ADG and G:F were greater in HS+AF, HS+NF, and TN+NF compared to TN+AF pigs. Increased ADG was likely due to compensatory growth caused by greater G:F as previously reported (Lovatto et al., 2006; Heyer and Lebret, 2007). Interestingly, despite this compensatory growth, no differences were detected in final BW, suggesting that feed removal during an extreme heat event did not affect overall growth performance.

In addition to a reduction in feed intake, HS increased water consumption in pigs (Patience et al., 2005). In the present study, a greater drinking frequency was observed in HS compared with TN pigs during the post-TF period, regardless of feeding treatment. Increased drinking frequency for HS pigs was expected because HS reduces body water reserves due to increased evaporative heat loss (Huynh et al., 2007). Alternatively, increased drinking behavior (polydipsia) has been observed in pigs overcoming stress (Byrd et al., 2018) and has been described as a stereotypic behavior (Dantzer, 1991). Hence, increased drinking behavior of HS pigs may be due to a greater stress response during the post-TF period. In addition to polydipsia, HS-exposed pigs had an increase in sitting behavior during the daytime compared to TN-exposed pigs in the current study. Previous reports have associated a greater sitting frequency with increased stress in pigs following transport under HS conditions (Johnson et al., 2018) and in barren environments (Wood-Gush and Beilharz, 1983). Taken together, the increase in sitting and drinking behavior for HS-exposed pigs may indicate that the stress response resulting from hyperthermia persisted even after the temperature insult ceased and that these behaviors could be used as indicators of stress recovery following HS-exposure in pigs.

Although, no recovery treatment and feeding treatment interaction was detected for T_{GI}, some caveats should be noted. It was previously determined that when the CorTemp sensor was orally administered by 1500 h on the day prior to temperature challenge, the sensor would be located between the duodenum and the jejunum during HS and recovery procedure on the following day (Johnson et al., 2016a). However, the location of the sensor was based on a 6-h HS and recovery

procedure contrary to 12 h in the current study. Therefore, it is possible that the CorTemp sensor might have been located at different sections of the intestine than previously determined. Additionally, one-half of the pigs was fasted and the emptiness of the intestine may have affected the CorTemp sensor movement. Therefore, it is important to determine how fasting might affect the movement for future studies. Regardless, these data improve our understanding of fasting effects on the intestinal temperature during HS.

5.6   Conclusions

We hypothesized that feed removal during an acute heat event would reduce the systemic inflammatory responses and improve short-term growth performance in grow-finish pigs. However, contrary to our hypothesis, feed removal during acute heat event did not reduce the systemic inflammatory response in pigs. On the contrary, feed removal appears to have induced a short-term inflammatory response following the TF period and this may have been a result of re-feeding, which has been shown to increase intestinal damage which can lead to systemic inflammation. In addition, HS caused polydipsia and increased sitting frequency during the post-TF period, which are indicative of a greater stress response. Although, this study disproved our hypothesis, it suggests that feed removal alone may not prevent systemic inflammation during HS, and that the negative effects of acute HS on swine welfare and stress response may last several days following the initial insult.

5.7   References

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Table 5.1 Ethogram used for behavior analyses

Category	Behavior	Definition
Consumption	Eating	The pig has its head in the feeder
	Drinking	The pig has its snout in contact with the waterer
	Other	Anything other than head in the feeder and snout in contact with the waterer
Posture	Sitting	The pig is on the thigh and forelegs, active or inactive
	Lying	The pig is lying on the floor sternally or laterally
	Standing	The pig is on its four legs, active or inactive

Table 5.2 Body temperature indices during the first 12-h temperature and feeding treatment period (**TF period**) in grow-finish pigs.

Parameter	Temperature treatment + Feeding treatment					P-value		
	TN[1]+AF[2]	TN+NF[3]	HS[4]+AF	HS+NF	SEM	T[5]	F[6]	T x F
T_{sk}[7], °C	33.51	33.15	37.34	37.36	0.26	<0.01	0.23	0.19
T_{GI}[8], °C	40.13	39.91	41.04	41.16	0.25	<0.01	0.70	0.28

[1]TN, thermoneutral.
[2]AF, access to feed.
[3]NF, no feed access.
[4]HS, heat stress.
[5]Temperature treatment.
[6]Feeding treatment.
[7]T_{sk}, trunk skin temperature.
[8]T_{GI}, gastrointestinal temperature.
Significant ($P \leq 0.05$).

Table 5.3 Serum cytokines measured during the 24-h temperature and feeding treatment period (**TF period**) and the post-TF period (d 2 and 6) in grow-finish pigs.

Parameter	Temperature treatment + Feeding treatment				SEM	*P*-value		
	TN[1]+AF[2]	TN+NF[3]	HS[4]+AF	HS+NF		T[5]	F[6]	T x F
TF period								
IL[7]-1α, pg/mL	5.59	3.04	5.32	4.37	0.83	0.38	0.02	0.25
IL-1β, pg/mL	16.30	14.32	19.01	9.16	4.27	0.59	0.11	0.26
IL-6, pg/mL	21.36	16.47	17.52	11.32	2.96	0.11	0.05	0.63
IL-10, pg/mL	19.77	7.70	17.69	7.13	5.35	0.82	0.03	0.96
IL-12, pg/mL	560.72	430.67	522.61	437.50	26.50	0.54	<0.01	0.39
Post-TF period								
IL-1α, pg/mL	7.49[ab]	2.05[c]	4.32[bc]	9.60[a]	1.59	0.09	0.42	<0.01
IL-1β, pg/mL	24.54[x]	13.84[xy]	12.98[y]	19.79[xy]	6.81	0.62	0.78	0.08
IL-6, pg/mL	24.39[a]	4.46[b]	13.24[a]	17.23[a]	3.86	0.18	<0.01	<0.01
IL-10, pg/mL	33.73	4.72	22.37	16.08	10.78	0.49	0.06	0.17
IL-12, pg/mL	748.78[x]	546.14[y]	612.61[y]	572.21[y]	62.89	0.24	0.01	0.10

[1]TN, thermoneutral.
[2]AF, access to feed.
[3]NF, no feed access.
[4]HS, heat stress.
[5]Temperture treatment.
[6]Feeding treatment.
[7]Interleukin.
[a,b,c]Letters indicate significant differences (*P* ≤ 0.05) within a row.
[x,y]Letters indicate tendency (0.05 < *P* ≤ 0.10).

Table 5.4 Growth performance monitored during the 24-h temperature and feeding treatment period (**TF period**; d 0) and d 1-2, 2-6, and 1-6 post-TF period in grow-finish pigs.

Parameter	Temperature treatment + Feeding treatment				SEM	P-value		
	TN[1]+AF[2]	TN+NF[3]	HS[4]+AF	HS+NF		T[5]	F[6]	T x F
Initial BW, kg	93.19	93.07	93.10	93.97	1.50	0.73	0.74	0.72
Days 0								
ΔBW, kg	-0.40^a	-5.09^c	-3.20^b	-5.22^c	0.53	0.01	<0.01	0.02
Days 1 to 2								
ADG, kg	1.40^c	4.10^a	2.88^b	3.43^{ab}	0.28	0.17	<0.01	<0.01
ADFI, kg	2.98	3.60	2.80	2.97	0.23	0.02	0.02	0.18
G:F, kg/kg	0.47^b	1.14^a	1.06^a	1.15^a	0.08	<0.01	<0.01	<0.01
Days 2 to 6								
ADG, kg	0.88	0.96	0.91	0.78	0.09	0.38	0.79	0.27
ADFI, kg	2.96^y	3.25^x	3.10^{xy}	2.93^y	0.12	0.48	0.63	0.07
G:F, kg/kg	0.30	0.29	0.29	0.27	0.03	0.55	0.63	0.69
Days 1 to 6								
ADG, kg	1.10^c	2.18^a	1.63^b	1.65^b	0.15	0.99	<0.01	<0.01
ADFI, kg	2.97^b	3.37^a	3.02^b	2.95^b	0.10	0.08	0.12	0.03
G:F, kg/kg	0.37^b	0.65^a	0.55^a	0.57^a	0.04	0.24	<0.01	<0.01
Final BW, kg	99.53	100.12	99.84	98.17	0.67	0.33	0.57	0.16

[1]TN, thermoneutral.
[2]AF, access to feed.
[3]NF, no feed access.
[4]HS, heat stress.
[5]Temperture treatment.
[6]Feeding treatment.
[a,b,c]Letters indicate significant differences ($P \leq 0.05$) within a row.
[x,y]Letters indicate tendency ($0.05 < P \leq 0.10$).

Table 5.5 Effects of temperature and feeding treatments (**TF**) and sex on average daily feed intake monitored on d 1-2, 2-6, and 1-6 post TF period in grow-finish pigs.

Parameter	Temperature treatment + Feeding treatment								SEM	P-value			
	TN^1+AF^2		$TN+NF^3$		HS^4+AF		$HS+NF$			T^5	F^6	S^7	T x F x S
	Barrows	Gilts	Barrows	Gilts	Barrows	Gilts	Barrows	Gilts					
Days 1 to 2													
ADFI, kg	3.29	2.67	3.74	3.45	3.13	2.47	3.01	2.92	0.28	0.02	0.02	0.02	0.72
Days 2 to 6													
ADFI, kg	3.29^a	2.63^b	3.35^a	3.15^a	3.01^{ab}	3.19^a	3.25^a	2.61^b	0.17	0.39	0.71	0.01	0.01
Days 1 to 6													
ADFI, kg	3.29^a	2.65^b	3.38^a	3.36^a	3.05^{ab}	3.00^{ab}	3.18^a	2.71^b	0.15	0.08	0.12	<0.01	0.02

[1]TN, thermoneutral.

[2]AF, access to feed.

[3]NF, no feed access.

[4]HS, heat stress.

[5]Temperture treatment.

[6]Feeding treatment.

[7]Sex.

[a,b]Letters indicate significant differences ($P \leq 0.05$) within a row.

Table 5.6 Consumption behavior and posture measured on d 1, 2, 4, 5 and 6 post temperature and feeding treatment period in grow-finish pigs.

Parameter	TN[1]+AF[2]	TN+NF[3]	HS[4]+AF	HS+NF	SEM	T[5]	F[6]	T x F
Consumption								
0800-0800 h								
Eating, %	7.51	6.25	6.18	7.25	1.13	0.82	0.90	0.12
Drinking, %	0.50	0.61	0.91	0.92	0.17	0.03	0.70	0.73
0800-2000 h								
Eating, %	10.50^x	8.11^{xy}	7.77^y	9.59^{xy}	1.13	0.60	0.85	0.06
Drinking, %	0.72	0.75	0.75	1.29	0.25	0.78	0.07	0.65
2000-0800								
Eating, %	2.95	3.29	3.31	3.45	1.03	0.59	0.61	0.83
Drinking, %	0.30	0.47	0.13	0.05	0.08	<0.01	0.81	0.12
Posture								
0800-0800 h								
Standing, %	13.31^x	11.57^{xy}	10.73^y	12.19^{xy}	0.85	0.25	0.87	0.06
Sitting, %	1.02	0.74	1.19	1.29	0.25	0.10	0.47	0.64
Lying, %	84.83^b	87.29^a	87.34^a	85.98^{ab}	1.23	0.47	0.51	0.02
0800-2000 h								
Standing, %	18.77^a	15.60^{ab}	13.27^b	16.39^{ab}	1.28	0.07	0.87	0.01
Sitting, %	1.32	0.94	1.86	1.74	0.35	0.05	0.42	0.62
Lying, %	77.27^b	81.73^a	82.72^a	79.54^{ab}	1.38	0.23	0.62	0.01
2000-0800 h								
Standing, %	6.98	6.70	7.03	7.41	0.89	0.62	0.95	0.67
Sitting, %	0.35	0.27	0.46	0.28	0.14	0.68	0.33	0.74
Lying, %	92.26	92.75	91.87	91.82	0.90	0.46	0.81	0.76

[1]TN, thermoneutral.

[2]AF, access to feed.

[3]NF, no feed access.

[4]HS, heat stress.

[5]Temperture treatment.

[6]Feeding treatment.

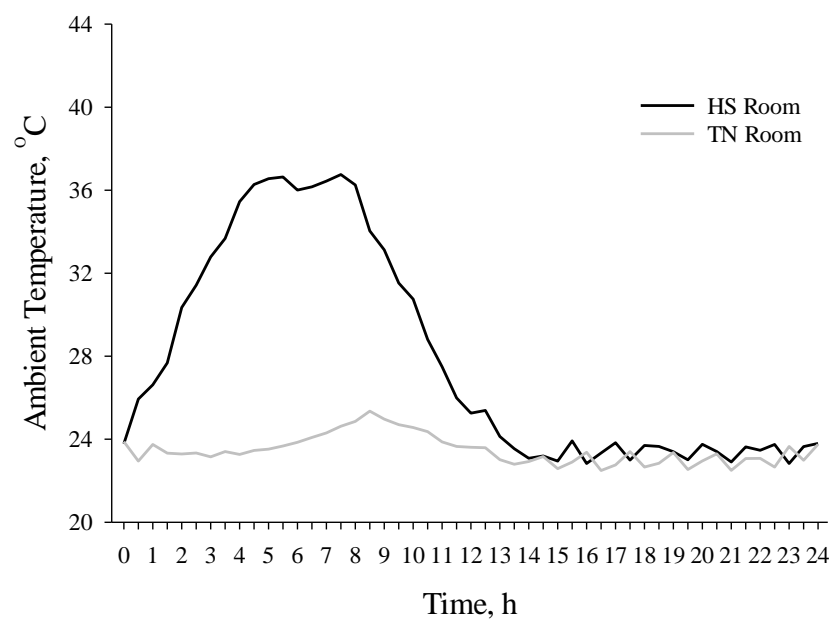[a,b,c]Letters indicate significant differences ($P \leq 0.05$) within a row.

Figure 5.1 Ambient temperature by time point during the 24 h of cyclic heat stress.
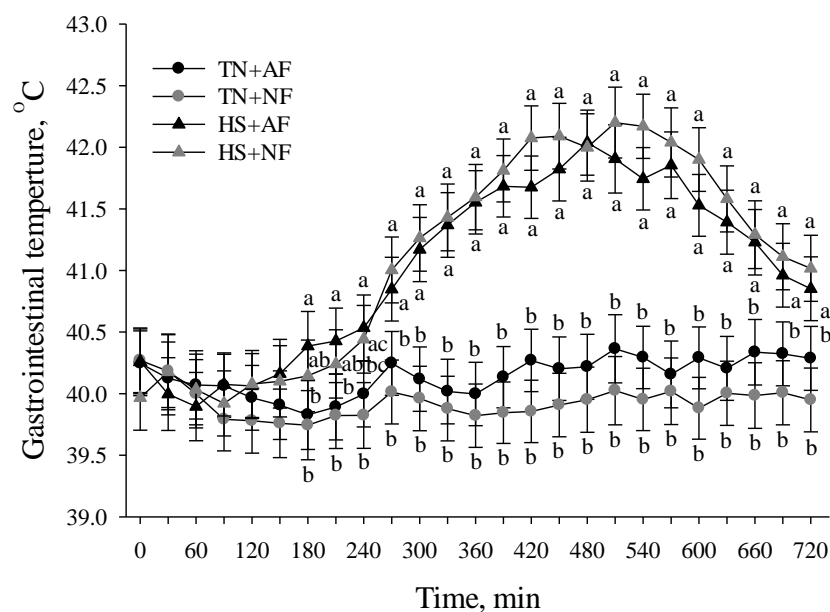Thermoneutral (TN); Heat stress (HS).

Figure 5.2 Effects of feed removal on gastrointestinal temperature recorded every 30 min in grow-finish pigs subjected to cyclic heat stress.

Effects of feed removal on gastrointestinal temperature recorded every 30 min in grow-finish pigs subjected to cyclic heat stress. Abbreviations are: thermoneutral (TN), heat stress (HS), access to feed (AF), no access to feed (NF), temperature (T), and feeding (F). Error bars indicate ± 1 SEM. [a,b,c]Letters indicate differences at each 30 min time point ($P < 0.01$).
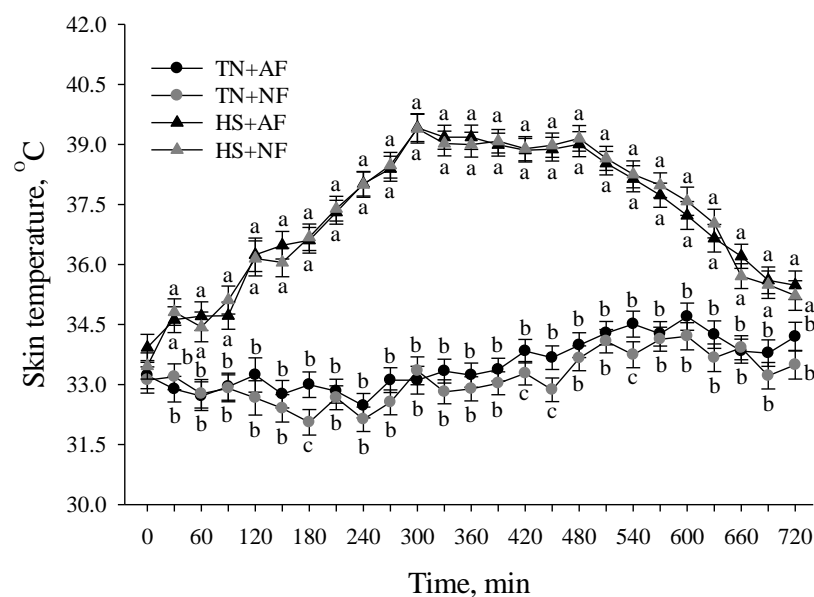
Figure 5.3 Effects of feed removal on skin temperature recorded every 30 min in grow-finish pigs subjected to cyclic heat stress.

Effects of feed removal on skin temperature recorded every 30 min in grow-finish pigs subjected to cyclic heat stress. Abbreviations are: thermoneutral (TN), heat stress (HS), access to feed (AF), no access to feed (NF), temperature (T), and feeding (F). Error bars indicate ± 1 SEM. [a,b,c]Letters indicate differences at each 30 min time point ($P < 0.01$).
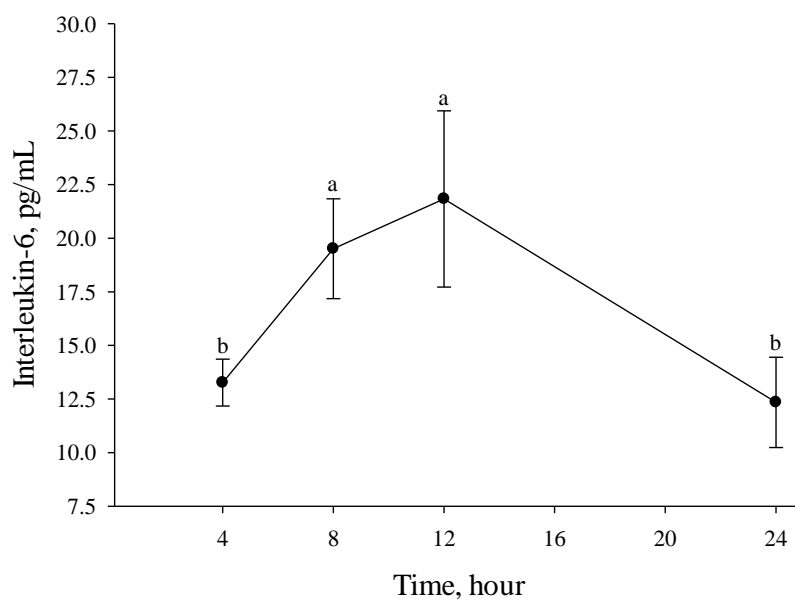
Figure 5.4 Serum interleukin-6 measured every 4 h during 24-h temperature and feeding treatment period in grow-finish pigs.

Serum interleukin-6 measured every 4 h during 24-h temperature and feeding treatment period in grow-finish pigs. Error bars indicate ± 1 SEM. [a,b]Letters indicate hour differences ($P < 0.01$).
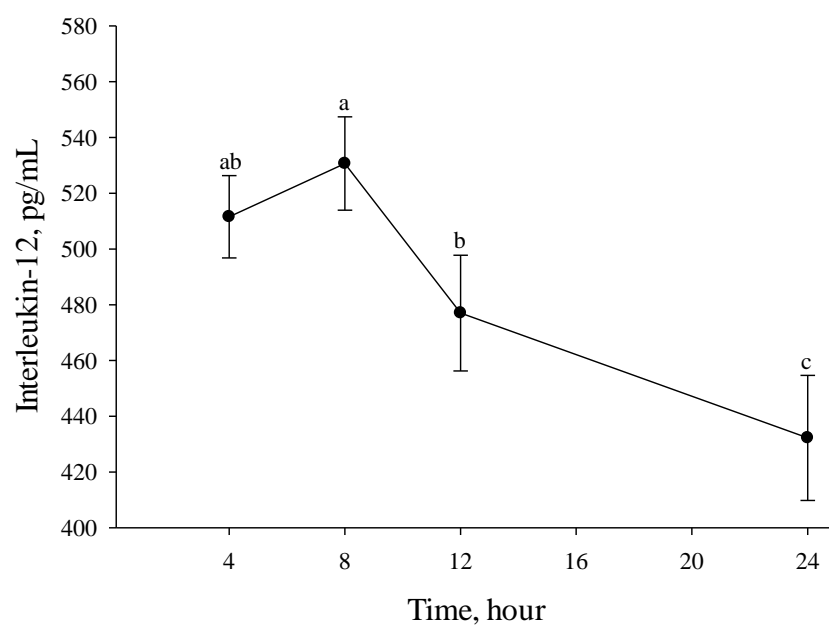
Figure 5.5 Serum interleukin-12 measured every 4 h during 24-h temperature and feeding treatment period in grow-finish pigs.

Serum interleukin-12 measured every 4 h during 24-h temperature and feeding treatment period in grow-finish pigs. Error bars indicate ± 1 SEM. [a,b,c]Letters indicate hour differences ($P < 0.01$).
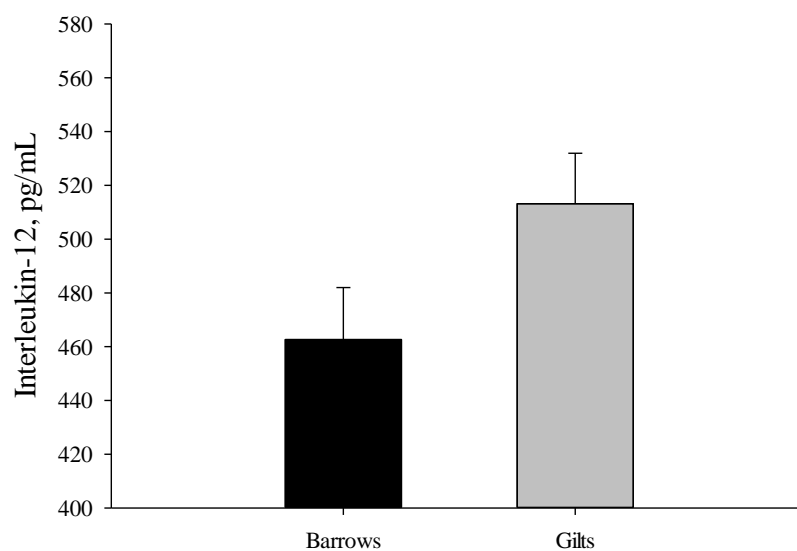
Figure 5.6 Effects of sex on overall serum interleukin-12 measured every 4 h during 24-h temperature and feeding treatment period in grow-finish pigs.

Sex effects on overall serum interleukin-12 measured every 4 h during 24-h temperature and feeding treatment period in grow-finish pigs. Error bars indicate ± 1 SEM. Significance ($P \leq$ 0.05).
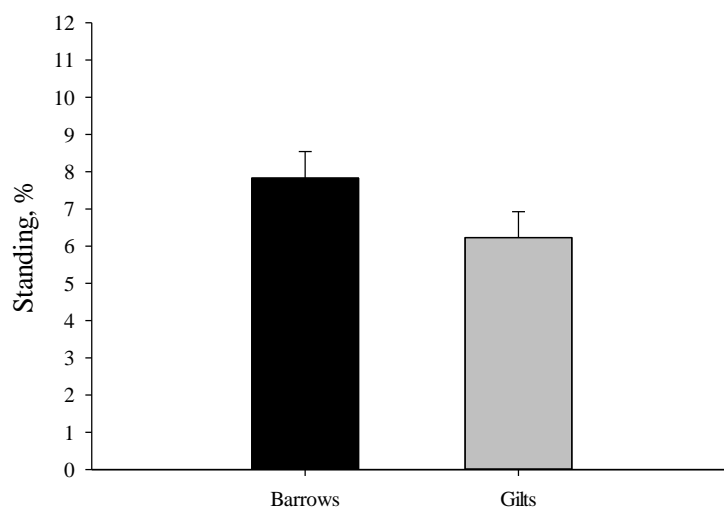
Figure 5.7 Effects of sex on overall standing frequency during nighttime (2000 to 0800 h) on d 1, 2, 4, 5 and 6 post 24-h temperature and feeding treatment period in grow-finish pigs.

Effects of sex on overall standing frequency during nighttime (2000 to 0800 h) d 1, 2, 4, 5 and 6 post 24-h temperature and feeding treatment period in grow-finish pigs. Error bars indicate $\pm$ 1 SEM. Significance ($P \leq 0.05$).

CHAPTER 6.   SUMMARY

Heat stress (**HS**) negatively affects animal health and productivity and is costly for the swine industry (St-Pierre et al., 2003). The economic losses are due to deleterious effects such as reduced growth performance, reproductive efficiency, and carcass quality (Baumgard and Rhoads, 2013). Although, several mitigation strategies are used to alleviate the negative impacts of HS in swine, production is still suboptimal, and morbidity and mortality are increased in extreme cases during warm summer months (Baumgard and Rhoads, 2013). Specifically, extreme heat events put grow-finish pigs at greater risk for acute hyperthermia that can lead to death unless the body temperature is rapidly returned to euthermia to restore thermoregulatory function. Therefore, evaluating mitigation strategies to promote recovery from acute hyperthermia is the first step toward improving pigs' health and well-being and ensuring profitability. Rapid cooling (i.e., cold water immersion or dousing) is effective in returning body temperature to euthermia (based on rectal temperature) when used to treat heat stroke in humans, exercise-induced hyperthermia in horses, and capture-induced hyperthermia in antelopes. However, studies in pigs reported that rapid cooling resulted in a sustained increase in rectal and intestinal temperatures, and exacerbated intestinal damage and inflammatory responses in pigs. Because these studies were conducted for a short period of time and pigs had access to feed during acute hyperthermia and rapid cooling, it was unclear how long intestinal damage and inflammatory responses would last and if feed access had played an important role in the sustained body temperature increase, intestinal damage, and inflammatory responses. In addition to the negative impacts of acute hyperthermia, variation in either T_A, RH, or airspeed creates microenvironments in farrowing barns resulting in reduced productivity in sows, but the existence of microclimate in grow-finish barns and its impact on pigs' thermoregulation and production performance were unknown. Therefore, the studies described in chapters 2 to 5 in this dissertation were conducted to fill the knowledge gaps and advance our understanding of microclimate in pigs' barns and the use of rapid cooling to promote recovery from acute hyperthermia in pigs.

In chapter 2, the study objective was to ascertain the existence of microclimates in grow-finish barns and characterize their impacts on swine productivity and thermoregulation during late summer. Therefore, environmental conditions (airspeed, temperature, and relative humidity) were

measured in two distinct pen locations based on the orientation of the pens to ventilation fans and air inlets. In addition, thermal indices and growth performance of pigs were determined over 6 weeks during late summer. The results indicated that microclimates exist in grow-finish barns and that pigs raised in pens not located directly below air inlets and ventilation fans had greater body temperature and reduced feed efficiency despite similarities in in-barn ambient temperature and relative humidity. These data clearly demonstrate that pen-to-pen environmental variation within grow-finish barns affects pigs' thermoregulation and may have negative impacts on pigs' health due to increased body temperature. Furthermore, this effect on thermoregulation reduced feed efficiency, which has negative implications towards producer profitability. While this study has improved our understanding of the impact of microclimates within grow-finish facilities, future work should be conducted to evaluate these effects over a longer period and with differing ventilation systems.

In chapter 3, the use of rapid and gradual cooling to alleviate the negative impacts of acute hyperthermia were evaluated in grow-finish pigs. The study objective was to determine the temporal effects of different cooling methods on body temperature, intestinal morphology and integrity, and systemic inflammatory responses after acute hyperthermia in pigs. Therefore, grow-finish pigs were exposed to acute HS and then rapidly or gradually cooled. Following acute HS and recovery phase, all pigs were maintained under thermoneutral conditions and then sub-sets of pigs were euthanized over three days to determine the temporal effects of the cooling methods on intestinal physiology and integrity. As opposed to previous research in pigs that were allowed feed access during the hyperthermia recovery period (Johnson et al., 2016), the results showed that rapid cooling following acute hyperthermia was effective in returning body temperature to euthermia when compared to gradual cooling and that rapid cooling prevented further intestinal damage. These data suggest that rapid cooling may be a viable mitigation strategy to combat acute hyperthermia in pigs if feed access is limited during the rapid cooling period. However, because pigs did not have access to feed in this study, it was unclear whether the absence of feeding contributed to the effectiveness of the rapid cooling method.

The study described in chapter 4 was conducted to determine if feed removal plays a role in the effectiveness of rapid cooling in reducing body temperature to euthermia and preventing intestinal

damage following acute hyperthermia. In this study, grow-finish pigs with or without access to feed were exposed to an acute HS challenge and then rapidly cooled. This study demonstrated that feed access was a determinant factor in the cooling outcome, as the gastrointestinal temperature returned to euthermia more rapidly during the rapid cooling period when feed was removed suggesting that feed removal alone may be used as a management tool to combat acute hyperthermia in pigs. However, despite the rapid return of body temperature to euthermia in rapidly cooled pigs without feed access, this did not have an impact on intestinal morphology. It was hypothesized that the lack of intestinal morphological damage in the rapidly cooled pigs given feed was due to the reduced re-feeding behavior [i.e., a 5-fold reduction compared to Johnson et al., (2016)] during the rapid cooling phase. However, this theory could not be confirmed due to study design. Future studies should determine the effects of re-feeding during rapid cooling on body temperature, intestinal integrity, and inflammatory response during rapid cooling following acute hyperthermia in pigs.

The study described in chapter 5 was conducted to evaluate the effects of feed removal in the absence of rapid cooling on the systemic inflammatory response and short-term growth performance of grow-finish pigs. In this study, grow-finish pigs with or without access to feed were exposed to a cyclic HS for 24 h. Following the cyclic HS period, pigs were maintained under thermoneutral conditions for 6 d and inflammatory biomarkers were assessed. It was determined that feed withdrawal independently caused a reduction in pro-inflammatory cytokines during the HS challenge when compared to pigs with feed access. In addition, although HS exposure did not cause an immediate increase in cytokine response, pro-inflammatory cytokine concentrations were increased in the days following the HS-challenge. These results suggest that feed removal alone may not be used for promoting recovery from acute hyperthermia in pigs. It is well-known that pigs drastically reduce feed intake during acute HS, and producers should be aware that pigs may increase feed intake when heat event has ceased and that they may have a delayed inflammatory response to the temperature insult.

Hyperthermia-induced mortality also occurs during transport. Although, mortality during transport has multiple causes including transport length, mixing with unfamiliar mates, trailer stocking density, and environmental conditions (Vitali et al., 2014), the risk for mortality are greater due to

HS during summer months when the ambient temperature is above the upper critical limit of finishing pigs (temperature > 25°C). Failure to regulate and maintain euthermic body temperature results in hyperthermia and death may occur due to systemic inflammation, disseminated intravascular coagulation and cardiovascular failure (Mustafa et al., 1985; Sunstrum et al., 2007). During HS, pigs resort to evaporative heat loss through increased respiration rate to dissipate heat. Maximum respiration rate is generally observed at loading and unloading (Alvarez et al., 2009) suggesting that loading and unloading are the critical periods where rapid cooling should be performed to help pigs regulate body temperature and cope efficiently with HS during transport. Although, studies have investigated the use of cooling methods during transport, the temperature of water were generally greater than 15°C or not reported (Fox et al., 2013; Knowles et al., 1998; Nannoni et al., 2013; Ritter et al., 2006) and ambient temperature ranged from (19.5 to 32.7°C). Because we have determined that rapid cooling with cold water immersion in combination with feed removal was effective in returning body temperature to euthermia, this method may be used during transport to promote recovery from hyperthermia under extreme environmental conditions. In addition, HS damages the intestine and reduces intestinal integrity (Pearce et al., 2013), and this may increase the risk of carcass contamination during processing. Therefore, future studies should investigate rapid cooling with cold water (4°C) during transport under extreme heat conditions to promote recovery from acute hyperthermia and determine its impacts on intestinal integrity and tensile strength during processing.

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