INTO THE COMFORT ZONE: UNDERSTANDING SWINE THERMAL PREFERENCE

by

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Dedicated to the pigs and pig farmers

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ABSTRACT

Exposure to thermal stress can negatively impact an animals' overall welfare, resulting in decreased body condition, lower reproductive success, and in severe cases, mortality. Heat stress occurs when temperatures exceed an animal's thermoneutral zone, effecting an animal's production efficiency and overall well-being. The swine industry has prioritized efficient production and as a result has gained rapid improvements in lean growth and increased litter sizes. Unfortunately, modern swine are unable to cope with the negative effects of heat stress as they are unable to sweat, have a small lung capacity for respiration to be effective at heat dissipation, and have increased lean mass with improve growth rates. On the other hand, temperatures that fall below the thermoneutral zone are not usually an issue for swine. This is, in part, due to housing environments being able to maintain warmer temperatures and the animal's reduced surface area to mass ratio making them efficient at heat conservation and cold tolerant. However, piglets are still susceptible to cold temperatures. From a stable temperature of 39°C in the uterus, piglets are born into an environment that is significantly colder and are unable to thermoregulate for the first 72 hours after birth. Because of this, they can suffer from hypothermia quickly. In addition, piglets lack brown adipose tissue, an essential tissue that aids in non-shivering thermogenesis, resulting in lowered resistance to cold environments. Thus, it is crucial to understand the preferred temperatures of swine to create recommendations on when to initiate mitigation strategies to combat the negative effects of thermal stress. However, this is further complicated given that temperature preference can be altered by various factors. Thermal preference differs with age, pregnancy, social context, early life experiences, and behavior, making it exceptionally difficult to classify an animal's thermoneutral zone. Therefore, the central hypothesis of this dissertation was that thermal preference of swine would be impacted by various factors including early life thermal stress, social aggregation, and reproductive stage.

The objective of chapter 2 was to determine if past thermal experiences altered thermal preference in pigs. To test this, piglets were first exposed to one of three early life thermal treatments between 7 to 9 days of age: thermoneutral (25°C with a heating lamp), cold stress (25°C without a heating lamp), and heat stress (cycling 32 to 38°C). At weaning (~20 days of age) pigs were split into groups of 4 same sex and early life thermal treatment and thermal preference was assessed later in life (~15 to 56 days of age) using a thermocline. Pigs differed in thermal

preference across different early life thermal treatments. Early life cold stressed pigs preferred warmer temperatures (+ 2.2°C) compared to their thermoneutral exposed counterparts. However, early life heat stressed pigs did not differ in thermal preference compared to both early life cold and thermoneutral exposed counterparts. These results suggest early life cold stress influences thermal preference later in life. Unrelated to the main objective of this project, early life thermoneutral pigs demonstrated a cooler thermal preference (23.2 to 25.2°C) than recommended guidelines set forth by the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (referred to the *Ag Guide* from hereon) of 26 to 32°C for piglets between 3 to 15 kg. These results suggest that the *Ag Guide* may require an update as the thermoneutral reared pigs did not prefer the *Ag Guide's* temperature recommendations. Additionally, the results of the thermal preference range for thermoneutral exposed pigs did not yield a true thermal preference as only a peak preference and potential upper critical limit was observed. Thus, clarifying this quandary is the objective of the third chapter.

The previous chapter did not elucidate the thermal preference of pigs reared within their thermoneutral zone. Thus, the primary objective of the third chapter was to determine the true thermal preference of 3-15 kg pigs by allowing them to select their preferred temperatures within an adjusted thermal gradient. The previous study looked at piglets tested as a group of 4 and did not examine how group size may influence thermal preference in a social species. As such, previous research has indicated that temperature preference is altered based on the number of individuals tested as this can alter their thermal comfort. That is, a greater number of individuals perceive a cooler temperature as being within their comfort zone whereas an individual does not have access to the thermal benefits of social aggregation. Social aggregation, through huddling, results in greater heat conservation and animals find cooler temperatures more comfortable. Furthermore, the previous study did not block by weight prior to conducting the research which might be why weight category had no influence on thermal preference. By blocking by weight, this study is better able to examine how body weight might influence thermal preference. The secondary objective of this study was to determine if thermal preference was altered in pigs based on group size. We predicted that pigs tested in larger groups (4 pigs) would prefer cooler temperatures compared to those tested as an individual or in a group of 2. Thermal preference was altered by both group size (individually tested pigs, a group of 2 and a group of 4) and body weight (small: 5.20 ± 1.15 kg; medium: 8.79 ± 1.30 kg; and large: 13.95 ± 1.26 kg). Individual pigs preferred a warmer temperature compared to both groups of 2 and 4 pigs (+ 10.0°C). However, groups of 2 and 4 did not differ in thermal preference. Small pigs chose a warmer temperature compared to large pigs (+ 10.2°C) but did not differ when compared to medium sized pigs. In addition, large pigs preferred cooler temperatures compared to medium sized pigs. These results demonstrate that increasing piglet body weight and group size resulted in cooler thermal preferences. Since this study demonstrated a shift in thermal preference of pigs (3 to 15 kg) compared to the recommendation of the *Ag Guide*, it may be reasonable to assume that other production stages need to be re-evaluated for their thermal preference.

One major gap that exists in the preferred temperature recommendations of the *Ag Guide* is the grouping of any pig over 100 kg. The *Ag Guide* states that these pigs prefer temperatures between 10 to 25°C. Since reproductive stage can alter metabolic activity, and heat production, there appears to be a gap in the literature addressing how reproductive stage may alter temperature preference of sows. Thus, the goal of the fourth chapter was to determine if reproductive stage altered the thermal preference of sows. Current recommendations for housing sows within their thermal comfort zone do not reflect how sow reproductive stage might influence temperature preference. In humans, core body temperature increases with fetal development resulting in pregnant women preferring cooler temperatures. This increase in metabolic activity from gestation could similarly result in sows preferring cooler temperatures to increase heat loss as they progress through gestation. In addition, sows can vary in weight based on their reproductive stage and parity. Body weight is well known to alter thermal preference with increasing weight resulting in a smaller surface area to volume ratio and increasing heat conservation which could alter thermal preference. Thus, we hypothesized that the thermal preference of sows would differ based on reproductive stage. Specifically, late-gestation sows would prefer cooler temperatures compared to open and mid-gestation sows. Late-gestation sows did indeed prefer cooler temperatures compared to both mid-gestation and open sows (-0.8°C). However, no differences were observed between open and mid-gestation sows. These results demonstrate that thermal preference is altered later in gestation, and that the thermal preference of sows, while within the *Ag Guide's* recommendations, are perhaps a narrower range, indicating sows are heat stressed at lower temperatures than expected. Further, as these sows were tested individually rather than a group, the thermal preference might be even cooler when considering group housed sows.

These experiments demonstrate that various factors can alter thermal preference of swine and that the preferred temperatures do not align with current recommendations. Updating these guidelines will help producers reduce thermal stress in swine and improve overall well-being of these animals. Further, as researchers will sometimes house pigs individually, this research helps provide temperature recommendations to improve welfare by reducing thermal stress.

CHAPTER 1. THERMOREGULATION AND WELFARE IN SWINE: A LITERATURE REVIEW

1.1 Introduction

Animals can generally be split into one of two groups based on how they thermoregulate, ectotherms and endotherms. The term ectotherm refers to an animal whose body temperature tends to follow that of the environment (Cowles, 1940). That is, the body temperature of ectotherms are adaptable based on the external environment and their body temperature will rise and fall based on the ambient temperature. Ectotherms include reptiles, amphibians, fish, and invertebrates, all of which rely heavily on behavior to help regulate their body temperature in fluctuating environmental climates. For example, iguanas move in and out of the shade to control heat gain; essentially, they find environments that meet their thermoregulatory needs (McGinnis, 1966; Hirth, 1963). On the other hand, endotherms can generate heat internally, beyond simple muscle contraction, which allows them to maintain homeothermy in a wider range of environmental temperatures. When exposed to cold temperatures, metabolic heat production increases to maintain homeothermy. Because of this, endotherms have a relatively constant core body temperature which, to a certain extent, is independent of the ambient temperature. To do this so effectively, endotherms have evolved with various behavioral and physiological adaptations to aid in the regulation of their core temperature (Gordon, 1993).

In environments where temperatures can be extreme and fluctuate seasonally, endotherms are better suited to permit appropriate thermogenic changes. To match the rate of heat loss to the external environment, more heat must be generated and a higher metabolic demand is established. The rate of heat loss is heavily dependent upon the surface area to volume ratio of an animal (Dawson, 1982). Smaller animals, such as piglets, have a higher ratio compared to larger swine, indicating a higher degree of heat loss (Schmidt-Nielsen, 1975). Simply, piglets have a relatively large surface area compared to their small mass and the metabolic demand is much higher compared to grow-finish pigs.

When the environmental temperature is too cold, a pig will adapt physiologically and behaviorally to minimize heat exchange to the external environment (Lin et al., 2017). Thermoreceptors in the skin will react to fluctuations in environmental temperature which will activate the hypothalamus to trigger a change in blood flow in order to alter the rate of heat loss

(Curtis, 1983). Skin blood vessels will constrict, causing warm blood to flow from nonessential body parts (i.e. limbs) to the core (McDowell, 1972). Vasoconstriction decreases heat exchange between the animal and the surrounding environment (Curtis, 1983; Blatteis, 1998). Shivering and food consumption will result in increased heat production. Behaviorally, pigs will huddle to reduce the surface area of the body exposed to the cold environment, which also creates a localized heating source at the center of the huddle. Exposure to cold conditions is further compounded by the fact that piglets are unable to thermoregulate for the first 72 hours after birth, resulting in increased mortality rates for new born piglets (Mount, 1960).

Despite the negative effects of cold exposure, cold stress seldom represents an issue for swine, since modern facilities are heated. Furthermore, genetic selection for efficient production traits (i.e. lean tissue accretion, increased number of piglets born per year, and faster-growing pigs) has led to increased metabolic heat production, and as a result has shaped an animal that is more heat sensitive and cold tolerant (Renaudeau et al., 2012). As ambient temperatures increase, blood vessels in the skin will vasodilate, allowing warm blood to move from the core organs to the extremities. This allows for greater heat dissipation as the thermal gradient between the animal and the external environment will be improved (i.e. heat travels from hot to cold; Curtis, 1983a; Blatteis, 1998). However, when this mode of heat exchange becomes ineffective, pigs will increase their respiration rate to dissipate additional heat. By blood moving from the core to the extremities there is a risk of reducing the amount of blood flow to necessary organs (i.e. less blood circulating in the intestinal system to absorb nutrients from the feed, resulting in reduced supply for cells with nutrients and oxygen), which could negatively impact production and overall well-being of the animal (Martin, 2012).

Pigs, even without genetic selection making them more heat sensitive, are naturally more susceptible to the effects of heat stress. Behaviorally they are unable to dissipate heat in most commercial settings and compared to other livestock species, they physiologically lack the ability to dissipate heat well. When given the choice, pigs will seek out sources of water or mud to roll or lie in, called wallowing, to keep cool. Unfortunately, in commercial settings, pigs do not have the ability to express this thermal behavior. In addition, pigs do not possess functional sweat glands and thus are unable to dissipate heat through this useful mechanism (Richards, 1971). These physiological limitations, along with their genetic selection for improved production, decreases a pigs' ability to dissipate heat and are ultimately more susceptible to heat stress (Johnson, 2018).

Thus, heat stress is a major concern in the swine industry, adversely impacting pig performance through decreased growth, reproduction rates, feed conversion, health, and welfare (Baumgard and Rhoads, 2013). This impact results in swine producers losing between 299 to 315 million dollars per year in the United States alone (St-Pierre et al., 2003). Although not as imposing of a challenge as heat stress, cold stress can also result in compromised production efficiency (Qi et al., 2014).

It is well known that market weight pigs suffer from heat stress (e.g. a decrease of feed intake, growth, and milk production during periods of warm temperatures; Close, 1971; Collin et al., 2001), but piglets suffer negative effects due to cold stress. Harmful effects of cold stress result in slower growth rate, poor feed efficiency, and higher mortality rates. The reason younger pigs are more susceptible to colder temperatures is, in part, due to their larger surface area to mass ratio, resulting in a relatively more heat loss to the environment. Due to this higher rate of heat loss, piglets have a warmer lower critical temperature (LCT) compared to market weight pigs. In addition to this higher rate of heat loss, piglets cannot thermoregulate within the first three days of life (Curtis and Rogler, 1970). Unlike many other neonatal mammals, piglets lack brown fat, which allows for non-shivering thermogenesis (Trayhurn et al., 1989). When temperatures are at or begin to drop below the LCT, the need to conserve heat is crucial. To help maintain a stable core body temperature, piglets must expend additional energy in physiological and behavioral ways, such as increased activity or feed intake.

On the opposite end of the thermoneutral zone is the upper critical temperature (UCT). As temperatures exceed the UCT, negative effects can be observed in market weight pigs, but since piglets have a warmer UCT, this is not seen until extreme hot temperatures are experienced. Pig growth can be reduced, a decrease in reproductive efficiency is observed, altered metabolism and body composition, and in extreme cases, morbidity and mortality can occur (Close, 1971; Collin et al., 2001). The temperatures between the UCT and LCT is considered the thermoneutral zone. Within this range of temperatures, heat exchange between the animal and external environment is minimal and homeostasis is achieved. Thus, additional resources are not needed to maintain normothermia, normal core body temperature.

Within the thermal comfort zone (i.e. the temperature at which an individual or group feels comfortable), pigs are most productive (Ingram et al., 1973). However, research has suggested that the thermoneutral zone is encompassed by the thermal comfort zone (i.e. the ambient temperature range in which an organism feels thermal comfort: van Marken Lichtenbelt and Kingma, 2014).

Within the thermal comfort zone, animals can behaviorally adapt to find or create preferable conditions. Similar to a human jogging on a cold day, pigs can increase activity levels to generate more heat when exposed to cool temperatures or consume less food to avoid increased heat production when warm, thus allowing them to remain within their thermal comfort zone (Young, 1981). The thermal comfort zone can be affected by several factors, such as the surface area to mass ratio, muscle retention, age, previous life experience, behavior, and social aggregation (De Dear and Brager, 1998; Gordon, 1993).

Most research regarding thermoregulatory behavior has used either a thermocline, an apparatus that creates a thermal gradient (Gordon 1993; Balsbaugh et al., 1986), or operant chambers which allow an animal to press a lever to gain access to a heat source (Weiss and Laties, 1961; Baldwin and Ingram, 1967). These methods take advantage of an animal's innate motivation to seek out their preferred temperature, commonly referred to as thermopreferendum.

Here I review the literature on thermal stress and behavioral thermoregulation in swine, regarding welfare. The main principles of welfare and how they relate to thermal stress are addressed in this review to better understand the problem that the swine industry faces. This review focuses on how to better understand thermal comfort in swine by looking at what influences the "real-feel" temperature they experience through thermal preference testing. Overall, this review highlights what questions have been answered with previous research and how that can be applied to help us understand the welfare impacts of thermal stress and how thermal preference can be used as a guideline to evaluate the thermal comfort of commercial swine.

1.2 Thermoneutrality and thermal comfort

The thermoneutral zone can be defined as the range of ambient temperatures where the metabolic heat production is equal to the rate of heat loss (IUPS, 2001). The thermal comfort zone on the other hand is when an individual feels satisfied within a wider range of ambient temperatures but can still experience small amounts heat exchange and metabolic changes (Kingma et al., 2012). The thermal comfort zone includes the thermoneutral zone, since animals can alter their behavior or posture adapt to alter heat loss or gain to the environment (van Marken Lichtenbelt and Kingma, 2014). This zone, like the thermoneutral zone, is variable based on a variety of factors. The following have all been shown to alter an animal's thermal comfort zone: circadian rhythm (mice prefer cooler temperatures when at rest during their dark hours compared to their light hours;

Okamoto-Mizuno and Mizuno, 2012); social interactions such as huddling (as group size increases, the surface to mass ratio decreases; as reviewed by Gilbert et al., 2010); body weight (Wilson and Sinha, 1985); behavior (as activity produces heat via ATP and muscle contraction animals prefer cooler temperatures; Mount, 1960) or postural adjustments (as pigs lay laterally they increase the body surface exposed to a cold floor; Mount, 1960); previous exposure to different thermal conditions and acclimation (humans from warmer climates prefer warmer temperatures compared to those from colder climates; De Dear and Brager, 1998); and energy intake (increased food intake increases heat production; Batavia et al., 2010). The thermal comfort zone of an animal is preferable when making temperature recommendations compared to the thermoneutral zone since an animal has a wider range of temperatures that they can easily be comfortable in without an alteration to metabolic rate (Figure 1.1).

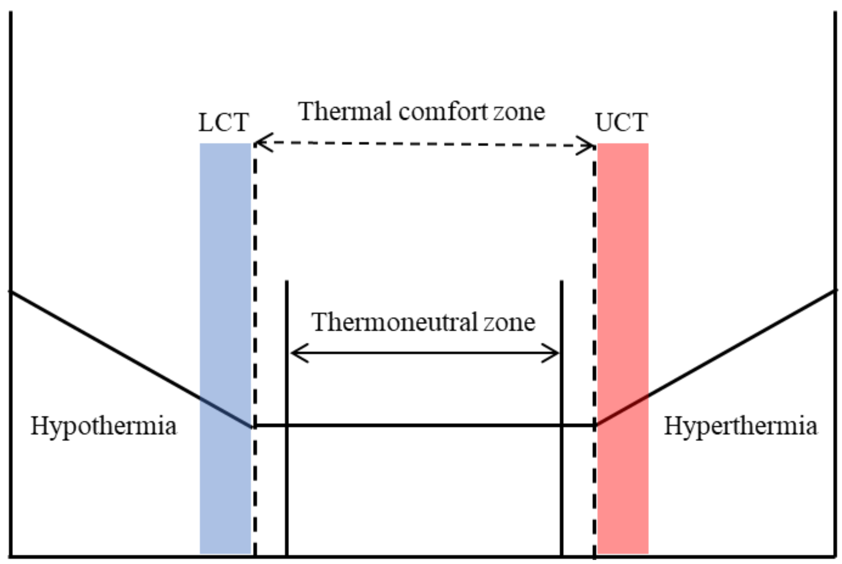

Figure 1.1. Depiction of the thermoneutral zone (i.e. the ambient temperature range in which no changes in metabolic rate are utilized) and the thermal comfort zone (i.e. the temperature in which an animal feels comfortable). Below the lower critical temperature (LCT), cold stress is experienced, and metabolic heat production increases. Above the upper critical temperature (UCT), heat stress occurs and heat dissipation methods like increased respiration rate will occur. Adapted in part Schellen et al., 2014.

1.3 Animal Welfare and Thermal Stress

Keeping animals within their thermal comfort zone is imperative as thermal stress can negatively influence the well-being of animals (Johnson, 2018). The term animal welfare has many definitions as it is found in many disciplines and covers complex relationships. There are three conceptions that help us understand and evaluate animal welfare: 1) biological functioning and health, a homeostasis approach that looks at how well an animal can cope within its environment, 2) affective states, an emotional-based approach that takes into consideration what the animal feels, and 3) natural living, an approach that looks at what is natural for the animal and the freedom it has to express species specific behaviors (Fraser et al., 1997). Animals that have a reduced life expectancy, ability to grow or breed, exhibit disease or injury, and/or do not display the same amount of normal or natural behaviors can be considered to have poor welfare (Broom, 1998). Animals that display normal behaviors, especially highly motivated ones, as well as indicators of pleasure are considered to have good welfare (Broom, 1998).

Thermal stressors negatively impact the overall well-being of swine. Typically, an animal's response to thermal stress includes an alteration to one, if not all, of the conceptions of welfare (Figure 1.2): a decrease in biological functioning (i.e. fewer piglets born per year), affective states (i.e. discomfort), and natural living (i.e. increased time seeking or engaging in wallowing behavior). Despite the abundance of issues associated with heat stress, cold stress is not a major concern for pigs in modern housing facilities. For one, barns are readily designed to conserve heat. The second, is due to the genetic selection for production which favors cold-tolerance (i.e. faster-growing, increased number of piglets born, increased lean tissue: Nienaber and Hahn, 2007). Although cold stress is not an issue for fully grown pigs, it can however pose a significant threat to piglets (Young, 1981).

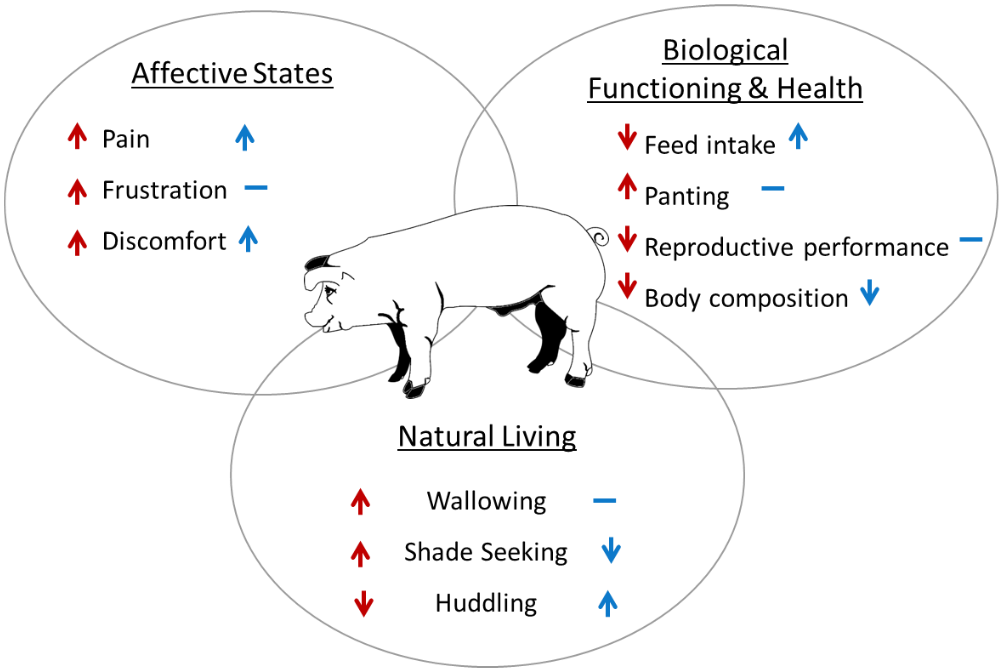

Figure 1.2. Negative influence of thermal stress on the three key constructs of animal welfare: (1) affective states (i.e. emotional states) the animal is experiencing, (2) biological functioning and health of the animal, and (3) natural living (i.e. the expression of natural behaviors). Red arrows indicate heat stress exposure, blue cold stress. Arrows indicate if something increased (up) or decreased (down), and a dashed line indicates no change or research on the subject is limited. Adapted in part from Polsky et al., 2017.

1.3.1 Biological functioning and health

To be considered in good welfare, animals should be free from disease, injury, and malnutrition (Fraser et al., 1997). Producers are committed to promoting the biological functioning of their livestock, as this approach results in higher levels of performance (e.g. increased growth, number of piglets born, decrease the time between estrus), thereby increasing efficiency and profitability of the farm (Randolph et al., 2007). When exposed to temperatures outside the thermal comfort zone, productivity decreases and in extreme cases, morbidity and mortality rates rise.

One of the largest welfare problems in the swine industry that correlates with economic losses from poor biological functioning, is heat stress (St-Pierre et al., 2003). Poor reproductive performance in sows is often observed during times of elevated temperatures, increasing wean-to-estrus interval, decreased farrowing rate, number of piglets born alive, milk yield, and piglet weaning weight (Bertoldo et al., 2012). Furthermore, semen quality is reduced when boars are exposed to heat stress, resulting in low conception rates (Waberski et al., 1994; Rozeboom et al., 2000). In addition to poor reproductive performance, pigs exposed to heat stress grow less efficiently (St-Pierre et al., 2003). On the other hand, piglets are prone to cold stress conditions and due to their differences in size compared to market weight pigs, have a higher LCT and a narrower thermal comfort zone. Furthermore, due to their inability to thermoregulate before three days of age, lack of brown fat, and relatively larger surface area to mass ratio (Schmidt-Nielsen, 1975) they are more prone to cold temperatures. To compensate for the rate of heat loss to the external environment, heat production must be increased to maintain euthermia (Klain and Hannon, 1969).

1.3.2 Affective states

How an animal feels as it experiences and perceives its surrounding environment is one aspect used to determine an animal's welfare. Substantial research has been conducted to investigate negative affective states such as pain and suffering through physiological (e.g. stress hormones) and behavioral (e.g. avoidance, grimace scale) measures (as reviewed by Weary et al., 2006; Von Keyserlingk et al., 2009). However, good welfare implies that animals should have positive experiences, such as comfort and be free from distress (Fraser, 1993). This aspect is difficult to measure and is often the most controversial as one cannot simply ask the animal how they feel. When exposed to temperatures outside of their thermal comfort zone, animals will experience discomfort and will seek means to alleviate it. For instance, animals will move away from temperatures they find aversive, thus alleviating that thermal discomfort (Ramot et al., 2008). In addition to removing discomfort, animals may experience frustration when they are unable to achieve a motivated goal (Fraser, 1993; as reviewed by Weary et al., 2006).

Any object, event, or activity that an animal is motivated to gain access to, is thus rewarding. Neurons in different areas of the brain comprise the reward system and communicate using dopamine, for example when stimulating specific regions of the brain, rats will work to receive the same stimulus in the same way they would work to receive food (Olds and Milner, 1954). Thus, stimulation of the reward system results in the animal feeling good and increased motivation to seek out the reward. Because being thermally comfortable feels good, animals are motivated to find an environment where the strongest positive affective state occurs. Pigs given access to a panel, will learn to press it for access to heat, and will continue pressing the panel until the temperature is within their thermal comfort zone (Baldwin and Ingram, 1967; as reviewed by Curtis, 1983b). If animals are unable to find or create temperatures that are comfortable and rewarding, the situation may lead to frustration. The frustration-aggression hypothesis states that feelings of frustration can manifest into aggressive behavior (Berkowitz, 1989). Pigs housed in high temperature barns demonstrate higher incidences of tail biting, potentially brought on by the higher temperature as a frustration-aggression manifested behavior (Schrøder-Petersen and Simonsen, 2001).

1.3.3 Natural living

The natural living aspect of animal welfare implies that animals should be free to engage in natural behaviors, such as play and exploration (Fraser, 1993). The ability to thermoregulate is an evolutionary adaptation that allows mammals to maintain homeostasis despite environmental temperature fluctuations (Silanikove, 2000). When temperatures increase, a pig's natural motivation would be to seek out shade or place to wallow (Stolba and Wood-Gush, 1989). However, in commercial settings, this behavioral adaptation is not available. Commercial facilities sometimes provide sprinkler or mister systems to help cool pigs during the summer months but may not adequately help dissipate heat. In this instance, pigs will be motivated to spend more time at these systems in order to cool down. However, outdoor facilities provide shelters, which allows pigs to engage in natural shade seeking behaviors, to reduce the radiant heat load. In contrast to these heat dissipation behaviors, thermal conservation behavior, such as huddling, can be easily expressed in commercial settings. Thus, these behaviors provide pigs with some ability to use behavior to control their environment, as was naturally intended.

1.4 Mechanisms of Heat Exchange

Undoubtedly, thermal stress is a major problem with implications to the overall well-being of pigs, but how is body temperature influenced? Swine are homeotherms, that is, they maintain a constant body temperature over a range of varying ambient temperatures through internal heat production (Baldwin and Ingram, 1967). In order to maintain this, pigs must balance the heat gained with the heat lost to the external environment. The control of thermal exchange begins with the hypothalamus and thermoreceptors, which act as a thermostat to regulate the body's core temperature (Curtis, 1983a; Gleeson, 1998). When temperatures increase and are sensed by thermoreceptors, the hypothalamus initiates processes aimed at increasing heat dissipation (i.e. sweating to allow evaporation of water on the skin to cool the exposed body surface, increased panting, and redistribution of blood flow: Curtis, 1983a; Gleeson, 1998). Conversely, when temperatures drop below the LCT, the hypothalamus can initiate shivering, redistribution of blood flow, and increase thyroid hormone to stimulate energy and heat production by cells throughout the body (Curtis, 1983a; Gleeson, 1998). To maintain this constant body temperature, pigs will lose heat in the form of sensible (conduction, convection, and radiation) and latent (evaporative) heat loss (Figure 1.3).

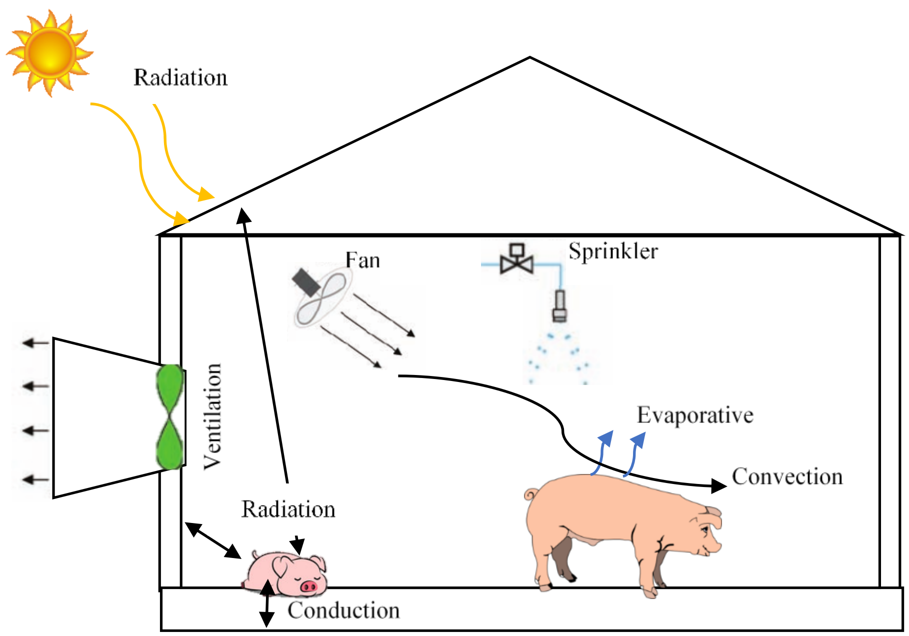

Figure 1.3. The thermal exchange between pigs and their surroundings with different cooling strategies meant to reduce heat stress. The thermal exchange is influenced by parameters that influence heat exchange: radiation, convection, conduction and evaporative heat exchange can be influenced based on the housing design. Adapted in part from Mayorga et al., 2019.

1.4.1 Conduction

Heat exchange by conduction is based on a thermal gradient, where energy transfers from hotter to cooler molecules. For instance, heat is transferred from the body to other, cooler, objects that are in contact with the skin surface (Blatteis, 1998). This heat exchange is dependent upon the thermal gradient (i.e. the temperature difference between the object and animal: Blatteis, 1998) and the conductive properties of the object. For instance, wallowing allows for the rate of heat loss to be greater than laying on a solid floor. When given access to a wet substrate (e.g. mud), pigs will wallow when the ambient temperature is high for longer durations and will seek out a substrate to wallow in more frequently (Olsen et al., 2001). Although an effective mechanism for heat loss, this is not an option for commercially housed pigs. However, pigs generally have access to concrete flooring which will allow for conductive heat loss. This heat transfer is most effective when the floor temperature is cooler than the animal. In fact, pigs spend more time lying on the floor as temperatures get hotter (Aarnink et al., 2006). When lying, pigs typically adopt a lateral posture, which increases the amount of skin in contact with the cool floor (Bruce and Clark, 1979). However, as the ambient temperature decreases, pigs will adopt a sternal laying posture to minimize contact with the floor, to decrease heat loss. They will also reduce their surface area to volume ratio by lying near each other, often on top of one another, increasing heat conservation (Mount and Stephens, 1970).

1.4.2 Convection

Convection is the transfer of energy through the movement of air or water (Bergman et al., 2011). Depending on the temperature differential, convection happens between the pig's skin and the surrounding air (DeShazer et al., 2009). Indoors, pigs are typically provided with fans and ventilation which allows for forced convection. Forced convection is achieved when air is moved over the surface of an animal, allowing for greater heat dissipation by creating a thermal gradient (Mount, 1979; Blatteis, 1998).

1.4.3 Radiation

Unlike the previously mentioned modes of heat loss, radiant heat exchange occurs between objects that are not in contact with one another. Radiant heat exchange involves short-wave (e.g. from the sun) and long-wave radiation (e.g. from the ground or surrounding objects to the animal; DeShazer et al., 2009). The magnitude of radiation that is emitted or absorbed by an animal depends on the emissivity (a measure of an object's ability to emit infrared energy which is species-dependent), surface temperature, and coat color (Morimotot, 1998). In an outdoor system, pigs are exposed to short-wave radiation, because of this, coat color becomes a determinant factor in energy absorption (Mount, 1979). As radiant temperature increases, shade seeking behaviors will increase to reduce exposure to short-wave radiation from the sun. However, in confinement, the amount of radiant heat exchange is determined by the temperature between two surfaces (Mount, 1979).

1.4.4 Latent heat loss: Evaporation

Latent heat loss, unlike the previously mentioned heat exchange methods, does not require a thermal gradient. Instead, this type of heat loss relies on the water vapor pressure gradient between the exchange surfaces and the environment (Guthrie and Lund, 1998). The energy required to vaporize water comes from a combination of the respiratory tract and the skin. This process allows for the cooling of the vascular bed and the surrounding tissues (Blatteis, 1998). For humans evaporative cooling takes place on the skin, mostly from sweat (Guthrie and Lund, 1998). Due to the lack of sweat glands, this is not a mechanism utilized in pigs (Richards, 1971; Ingram, 1974). In pigs however, latent heat loss is mainly through increased respiration rate (i.e. panting). Each increased degree of ambient temperature (above 26°C, age dependent) results in a linear increase in pig skin temperature (Renaudeau et al., 2007). Evaporative heat loss remains the only efficient means for heat loss when the thermal gradient is too narrow or negative (Curtis, 1983a).

1.5 Thermal Preference

Thermal preference tests take advantage of an animal's innate motivation to seek out their preferred temperatures, referred to as thermopreferendum (Gordon, 1993). This motivation typically results in animals selecting temperatures with minimal energy expenditure (Gordon, 1993). That is, animals will select temperatures that feel comfortable and would result in minimal heat exchange between the animal and its external environment, indicating their thermal comfort zone. By presenting an animal with different temperatures, researchers can determine the thermal comfort zone by determining which temperatures the animal either selects most often or spends a greater amount of time in.

A subject of interest in behavior and welfare research is determining what resources or aspects of an animals' environment are of importance. Preference testing allows researchers to identify important aspects of how an animal perceives the world around them. Typically, a preference test can give two or more options to an animal in an enclosed space (as reviewed by

Vorhees and Williams, 2014). In this type of design, animals are often given free access to potential options, and researchers determine how often and how long an animal spends with a certain resource. This then creates a time budget and elucidates what resource the animal prefers by either duration or frequency with the resource options. In theory, animals will spend more time and visit the resource that they prefer more frequently.

Three main ways exist to test thermal preference. In the first method, animals are trained to press or pull a lever to gain access to a reward, referred to as operant conditioning. The second, utilizes thermal compartments which allow researchers to monitor and control specific temperatures that are presented to animals (Fig. 1.4). The third, a thermocline, not to be confused with thermal compartments, allows an animal free access within one enclosed, and constant, thermal gradient (Fig. 1.5). This design allows animals to freely select a wide range of temperatures.

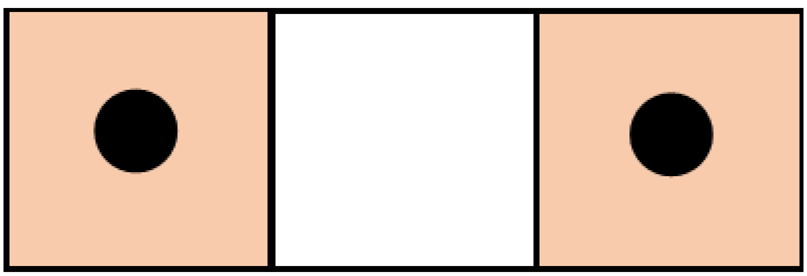

Figure 1.4. A three-choice housing system for preference testing in piglets, as described by Vasdal et al., (2010). The apparatus includes two thermal compartments (left and right) with infrared heating lamps located above (black circles) to control temperature within each compartment. The middle compartment was a neutral compartment that was 2-20°C cooler than either thermal compartment. Temperatures were altered such that piglets experienced a combination of: 26°C vs. 34°C, 26°C vs. 42°C, or 34°C vs. 42°C.

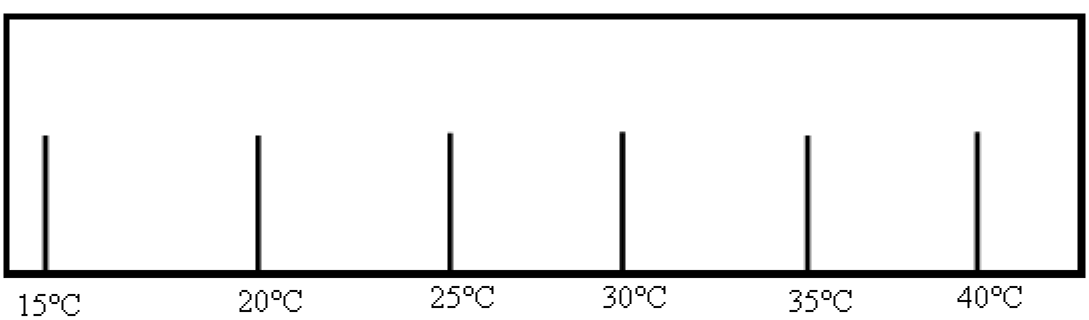

Figure 1.5. Depiction of a thermocline. Typically used in rodents where one end is cooled (left) and the other warmed (right) creating a constant thermal gradient for animals to select their preferred temperature.

1.5.1 Operant conditioning

Operant conditioning is a method of learning that creates an association between a specific behavior and a consequence (Skinner, 1938). Thermal preference can be studied through this technique. In most studies, animals are placed inside an enclosed space starting at a temperature that may be near or below the animals' LCT. Animals are then trained to operate a mechanism to gain access to a reward, in this instance a source of heat (either a heating lamp or a gust of hot air).

Operant conditioning has been used to determine the thermal preference of piglets in several experiments (Baldwin and Ingram, 1967; Baldwin and Lipton, 1973; Ingram, 1965). In these experiments, piglets were exposed to cold temperatures and were trained to press a panel with their snouts to obtain access to radiant or convection heat sources. Research has suggested that piglets in cold environments will learn to operate radiant heaters and when access to the reward slows, that implies a reduction to their metabolic rate to levels within their thermoneutral zone (Baldwin and Ingram, 1967; Ingram, 1965). In a study by Mount (1968), piglets reduced their lever pressing at 25°C. In this case, there is little physiological advantage in continuing to turn on the heaters above 25°C, indicating this as the piglet's preferred temperature. However, these studies were conducted on individual piglets less than 5 weeks of age. At this age, piglets prefer warmer temperatures compared to older piglets as their surface area to mass ratio is smaller. Furthermore, as a social species, piglets are susceptible to social stress and testing in isolation may not accurately reflect thermal preference but rather a desire to leave the enclosed space.

A more recent operant conditioning study assessed piglets thermal preference at 3 to 5 weeks old, allowing them to gain access to an infrared heat lamp for 60 seconds when a level was pressed (Bench and Gonyou, 2007). This study demonstrated that as age (and weight) increased, the average preferred temperature decreased by approximately 0.5 to 0.6°C per week. With the mean temperature preferences being 26.31°C (3 weeks of age), 25.69°C (4 weeks), and 25.27°C (5 weeks). This study demonstrated that keeping the thermal environment constant over time would not be preferred by piglets, as opposed to the previous study which only looked at piglets 8 to 14 weeks old. Thus, understanding that age, and consequently weight, will influence preference is important especially as pigs grow.

Operant methods enable researchers to determine an animal's thermal motivation based on acquisition of the reward, or heat in these examples. Thus, if motivation for heat reward is high, those temperatures are not ideal for the animal. However, if motivation is low, this indicates that the animal is comfortable. While useful, this technique may only indicate the lower end of the preferred temperatures rather than a true thermal comfort zone.

1.5.2 Thermal compartments

Thermal compartments are tightly controlled enclosed spaces that are set to a specific set of conditions. Usually a select number of thermal choices are offered to an animal to select from. By using thermal compartments, researchers can test more than 1 piglet at a time, which is a more realistic approach, since pigs are typically housed in groups in commercial settings. Furthermore, this set-up can be used to not only monitor the thermal preference of an animal but also look at and the animal's motivation for thermal comfort. However, there have been few studies conducted in such a manner.

One of the few studies using this technique demonstrated social motivation outweighed thermal comfort of pigs at 3 days old (Hrupka et al., 2000). This study looked at 2 thermal preference elements: 1) determine the average piglet preference when presented with 5 different ambient temperatures randomly assigned to a thermal compartment, and 2) determine if thermal preference was a higher drive than social interactions. This experiment consisted of 4 chambers with one chamber heated to 23, 40, 48, 56, or 64°C and leaving the remaining 3 sections unheated (24°C). When tested alone, piglets selected 40°C most often compared to the other temperature options. Piglets in this study were between 1 to 2 days of age and thus unable to thermoregulate. This temperature selection makes sense as piglets are born into an environment that is significantly colder (18 to 23°C: Brown-Brandl et al., 2001; Yan and Yamamoto, 2000) than the uterine temperature of a sow (39 to 40°C: Mount, 1959; Caldara et al., 2014). Since piglets are unable to generate their own heat, they would need to seek out temperatures closer to their own body temperature to maintain euthermia. However, Hrupka et al. (2000) found that piglets were more attracted to an anesthetized piglet in a cold chamber than an empty warm chamber (i.e. their thermal comfort).These results suggest that social motivation outweighs acute thermal comfort, with piglets preferring the presence of another pig to that of a heated chamber. This study highlights the importance of testing thermal preference in a group when evaluating a gregarious species. It is possible that pigs may select a cooler temperature either because their litter or pen mate is located there or because when pigs huddle, this lowers heat loss and the temperatures they chose.

The second major study using thermal compartments presented two thermal options to piglets aged 12 to 24 hours old (Vasdal et al., 2010). The testing apparatus had three thermal compartments: two test compartments, which held one of two thermal treatments (described below), and one central compartment that was considered thermoneutral (this compartment was cooler than the test compartments by 12 to 20°C, dependent on the temperature being presented). Six litters were first exposed, in a pairwise test, to the following thermal options: 26°C vs. 34°C, 26°C vs. 42°C, or 34°C vs. 42°C. In a follow up experiment, researchers repeated the first study but with different thermal combinations; 30°C vs. 34°C, 30°C vs. 38°C, and 34°C vs. 38°C. In the first experiment, piglets preferred 42°C, compared to 34°C and 26°C, but in the second experiment, piglets' showed no preference between the thermal compartments. Again, this is not a surprising result as at this age range, piglets are unable to thermoregulate and would undoubtedly prefer the warmest temperatures. Although the data found may make sense for piglets tested in groups, but the piglets were only subjected to each treatment for 30 min before testing (60 min) and may not have allowed appropriate time for piglets to acclimate to the new environment.

1.5.3 Thermocline

Thermoclines are different from thermal compartments, as they simultaneously offer more thermal choices to animals than compartments do. Measuring an animal's preferred ambient temperature utilizes their innate motivation to seek out their thermopreferendum with behavior. This choice permits the least amount of heat exchange between the organism and its environment. Behavioral responses are less energetically costly than physiological ones and are typically utilized before physiological alterations (Gordon et al., 1998). Thermoclines have been used to evaluate the thermal preference of rodents, marsupials, bats, and rabbits (as reviewed by Gordon, 1993). Thermal preference is species-dependent due to differences in energetic requirements. For instance, the average preferred ambient temperature is 30.9°C in mice, 30.6°C in guinea pigs, 28.2°C in golden hamsters, and 23.4°C in fischer rats (as reviewed by Gordon, 1993). This method of preference testing has been used in piglets but with conflicting results.

The environmental temperature preferred by a solitary piglet, less than 1 day of age, is approximately 32.3°C, while those aged 1 to 7 days, preferred 29.3°C (Mount, 1963). The thermal preference for a warmer temperature of younger piglets is assumed to reflect compensation for being born metabolically immature, with low body insulation and a lower metabolic rate on the first postnatal day. However, Balsbaugh et al. (1986) found that piglets (12 to 72 hours old) displayed a higher mean thermal preference of 35.7°C. This might be due, in part, to the age differences in the piglets being studied and the amount of time pigs were tested inside the thermocline. Newborn piglets have a high ratio of surface area to body mass, allowing for greater heat loss to their environment, while also having limited insulation from body fat. Thus, piglets' thermal insulation is low and testing young piglets would yield a result of a warmer thermal preference compared to older piglets. Since both the previously mentioned experiments were conducted on individual piglets and previous work has shown that thermal preference can be altered by social context, piglets here might have selected warmer temperatures as they are unable to reap the benefits of huddling.

1.5.4 Thermal indices for swine

Thermal indices for livestock species have been developed using the temperature-humidity index (THI), a combination of temperature and humidity inputs that provides an output of how much discomfort an individual animal might experience in warmer weather (as reviewed by Herbut et al., 2018). Most of these indices have led to recommendations aimed at helping farmers monitor when heat stress conditions might occur. While helpful, these indices may not be accurate. For instance, thermal indices used for dairy cattle extrapolated and applied to pigs (Hahn et al., 2009). While a good starting point, the data from this work is likely to be flawed due to differences in various factors that influence thermoregulation between production species (i.e., surface area to mass ratio, hair cover, and ability to sweat). Further, the metabolic heat production differences between a milking dairy cow and a pig can make adapting thermal indices difficult (Curtis, 1983a). Swine are typically housed indoors where behavioral thermoregulation (i.e. wallowing) is limited; whereas, cattle will often have access to outdoor and indoor space allowing for improved behavioral thermoregulation (i.e. shade seeking), these different management strategies allow for the animals to adapt to the environment and remain within their thermal comfort zone (Hillman, 2009; Renaudeau et al., 2012). Thus, making it nearly impossible to translate an index meant for cattle, to swine.

Table 1.1. Recommended thermal conditions for swine. Adapted from the *Ag Guide* (FASS, 2010)

Production stage	Preferred temperature range[1]	Lower critical temperature	Upper critical temperature
Prenursery (3-15 kg)	26-32°C	15°C	35°C
Nursery (15-35 kg)	18-26°C	5°C	35°C
Growing stage (35-70 kg)	15-25°C	-5°C	35°C
Finishing stage (70-100 kg)	10-25°C	-20°C	35°C
Sows or boars > 100 kg	10-25°C	-20°C	32°C

[1]Based on values from the NRC (1981), DeShazer and Overhults (1982), Curtis (1985), and Hahn (1985)

Other previous work has been utilized to develop recommendations for housing temperature of pigs. The *Ag Guide* provides information on the preferred temperature of swine (FASS, 2010: Table 1.1) but is not without its own flaws. The references used to develop the recommendations in the guide date back to the 1980s (NRC, 1981; DeShazer and Overhults, 1982; Curtis, 1985; Hahn, 1985). The problems here are two-fold. One, those citations actually reference data from the 1970s (Holmes and McLean 1974; Verstegen van Es and Nijkamp, 1971;; Bruce and Clark, 1979) and two, modern pig genetics have increased metabolic heat production, on average, by 16% (Brown-Brandl et al., 2014). Thus, a shift in preferred temperature ranges are expected between modern swine compared to those of ~50 years ago. Further, most of these citations used theoretical formulas to calculate the rate of heat loss for different stages of production pigs, instead of data from real animals. Thus, none of the referenced work did research on the thermal preference of this species to detect thermal comfort.

Finally, animals' age, sex, production stage, and reproductive status can greatly influence an animal's susceptibility to thermal stress (Ross et al., 2015) as well as their thermal comfort zone. Thermal indices should have specific categories to provide the most accurate information for producers and researchers. Thermal preference studies have only been conducted on piglets less than 5 weeks of age, and some studies looked at early weaned piglets which is unlikely to occur in the commercial setting (Vasdal et al., 2010; Blasbaugh et al., 1986). There are no studies that have evaluated the preferred ambient temperature of later stage production swine. Thus, there is currently no scientific evidence available to generate a reliable thermal index for swine, nor provide recommendations for producers on what temperatures are optimal for their pigs.

1.6 Factors That Influence Thermal Preference

The thermal comfort zone, and by extension thermal preference, can shift depending on an animal's age, body weight, sex, production stage, and reproductive status. All of which can greatly influence an animals' susceptibility to thermal stress. Thus, recommendations for keeping animals housed within their thermal comfort zone should have specific targets (e.g. different production stages for pigs) to provide the most accurate information for producers and researchers. To provide this accurate data, more thermal categories may be needed in order to consider the various aspects listed in this section.

1.6.1 Body mass

Body weight is an important factor that is highly correlated with thermal preference (Dauncy and Ingram, 1986). Lighter weight animals have a larger surface area to mass ratio and prefer warmer temperatures due to high rates of heat loss (Dauncy and Ingram, 1986). As the surface area to mass decreases, with increasing body mass, endotherms conserve more heat for a given rise in ambient temperature (Gordon, 1993). Thus, piglets have a relatively large surface area to mass ratio, and as endotherms, rely on changing their metabolic heat production to regulate core body temperature. As pigs grow, their body weight increases, reducing the surface area to mass ratio and shifting preference to a cooler thermal range (Dauncy and Ingram, 1986).

1.6.2 Behavior and posture

Thermoregulatory behavior is an important response in conjunction with autonomic responses (Benzinger, 1969). The thermoregulatory system relies on behavior as the initial means of maintaining homeostasis because it is relatively cheap energetically. Under natural conditions, animals will exhibit thermotaxic behaviors, such as finding shade on a warm day or basking in the sun on a cool day (Gordon, 1993). Consequently, an animal will seek their thermopreferendum to decrease the temperature difference between the environment and themselves. Reducing this thermal gradient allows an animal to minimize energetic costs (as reviewed by Terrien et al., 2011). However, thermal preference may change based on activity. With increased activity, a significant amount of heat is generated from muscle contraction and active individuals will often seek cooler temperatures to increase heat loss (Gordon, 1993).

While animals generally seek out cooler temperatures when active, the opposite behavior is observed during times of inactivity. Mammals typically experience a decline in core body temperature that will correlate with the circadian rhythm of the species. In mice, body temperature can drop by approximately 2°C as they transition from their active phase to sleep (as reviewed by Harding et al., 2019). During sleep, the animal's metabolic rate is typically lower than while inactive and a warmer thermal preference can be observed. Mice have demonstrated thermal preference based on circadian rhythm, that is, they had a different thermal preference during the sleep phase compared to their awake phase (Gaskill et al., 2012). Previous literature has also demonstrated that piglets will select cooler temperatures when sleeping (Bench and Gonyou, 2007) compared to when active.

Posture is another mode of behavioral thermoregulation that alters the rate of heat loss by changing the amount of surface area exposed to the environment. When housed within their thermal comfort zone, piglets prefer to lay near each other with minimal contact and predominately lie in a lateral posture (Huynh et al., 2005). Typically, there is a linear relationship between lateral lying and environmental temperature in pigs (Huynh et al., 2005; Aarnink et al., 2006). Increasing skin contact with the floor allows for greater heat exchange and lessens the heat load if an animal is experiencing heat stress. An increase in ambient temperature reduces contact with conspecifics and increases the amount of lateral lying (Huynh et al., 2005; Aarnink et al., 2006). Reducing physical contact increases the amount of surface area exposed to the air or slatted floor, increasing the opportunity for heat loss through conduction. A decrease in ambient temperature, however, results in more sternal lying to reduce skin exposure to the cool concrete flooring in order to reduce heat loss (Mount, 1960). The decrease in ambient temperature will also result in pigs huddling together, increasing body contact with conspecifics.

1.6.3 Reproductive stage

Reproduction is an energetically demanding process, with the greatest energetic costs occurring in late pregnancy and lactation (Kaczmarski, 1966). During pregnancy, increased heat production is caused by one of two modes: the first, is the increase in metabolic rate of the mother and the second is the energy released by the developing fetus (Kaczmarski, 1966). As a result, pregnant females have a decreased tolerance for heat with an increased basal metabolic rate. Further, an increase of 0.5°C, in both core and skin temperature, during pregnancy results in cooler temperatures being preferred (Hartgill et al., 2011). In humans for instance, preferred ambient temperature went from 26.5°C at week 8 to 23.0°C by week 36 (Hartgill et al., 2011). This increase in metabolic activity could result in cooler temperature preferences the later in gestation a sow is. Furthermore, as sows progress through reproductive stages, body mass increases along with energetic demands, reducing the surface area to mass ratio, adding to that shift in preference to a cooler thermal range (Gordon, 1993).

1.6.4 Social aggregation

Huddling is effective because the ambient temperature surrounding each individual increases due to the combined heat loss of the other animals in the huddle, resulting in a localized heat source. Thus, the thermal gradient between the animal and environment is reduced (i.e. minimal heat exchange between individuals and external environment). The heat gained by other animals in the huddle results in the group being able to tolerate cooler temperatures, thus shifting their thermal comfort zone.

Huddling also reduces the amount of body surface area exposed to the cold environment. Less exposed skin reduces heat loss and metabolic rate. For example, huddling in mice has been shown to decrease energy expenditure by as much as 53% (Moinard et al., 1992). The benefits of huddling however are correlated with the number of individuals in the huddle. Simply put, larger group sizes allow a greater reduction in skin exposed to the cold. However, for every additional animal to huddle, there are diminishing benefits in terms of heat conservation. That is, as more individuals huddle together their heat conservation benefits begin to plateau. For example, research in rodents has demonstrated that mice housed in groups of 2 to 5 will benefit from huddling and this will increase significantly as a function of group size (i.e. mice generate more heat in a group of 5 and can withstand cooler temperatures compared to a group of 2; Martin et al., 1980). However, there will be a maximum group size where any additional individuals will result in negligible heat conservation benefits (Canals et al., 1989). Once group sizes exceed 5 mice, there is no change in thermal preference nor metabolic heat exchange, indicating that any group over the size of 5 begin to reap little to no benefits from huddling. How this affects piglet thermal preference has yet to be studied. Piglets have been tested for thermal preference as either an individual or in a group, but no study looked at how group size alters piglet thermal preference.

1.6.5 Early life experience

Previous research on poultry and rodents have indicated that early exposure to heat stress can boost future thermotolerance later in life (i.e. reduction in heat shock protein, body temperature when thermally challenged later in life: Tetievsky and Horowitz, 2010; Morita et al., 2016). In humans, the adaptive hypothesis predicts that past thermal history modifies humans' thermal preferences. Thus, people who have grown-up in warm climates prefer warmer indoor temperatures compared to those who live in cold climates (de Dear and Brager, 1998). This demonstrates an alteration in thermotolerance, and potentially indicating a shift in thermal preference. For piglets, when exposed to early life heat stress, there is an increase in core body temperature. As core temperature increases, skin temperature (Johnson et al., 2018) also increases. In order to maintain a balance between heat production and heat dissipation, a piglet might select cooler temperatures to improve heat dissipation capacity. Alternatively, cold stressed piglets have a reduction in body temperature (Heldmaier, 1974), which may lead to greater vasoconstriction to conserve body heat. Keeping warm blood near the core results in a preference for warmer temperatures. Although we may speculate at what temperatures these animals exposed to early life thermal stress might choose, this data has yet to be collected.

1.7 Aims of This Project

Previous research attempting to elucidate the thermal comfort of swine has predominately been tested on piglets using thermal preference and theoretical models calculating the rate of heat loss from larger swine to determine the expected thermal comfort of grow-finish pigs and sows (Homes et al., 1977; Mount, 1975; Hahn, 1985 and 1987; Curtis, 1983a; Holmes and McLean, 1974; Verstegen et al., 1971). These theoretical models are the basis for current thermal recommendations for any pig over 100 kg which are used by producers and researchers (FASS, 2010). However, no research has been done to determine the preferred ambient temperature of sows, or to examine if thermal preference of piglets is altered based on group dynamics or early life exposure. Understanding the thermal comfort of swine will help identify the optimal temperatures in which pigs should be housed and identify a potential thermal comfort zone. This information will allow for the development of better guidelines that accurately identify when pigs are thermally stressed.

1.8 References

Aarnink, A. J. A., J. W. Schrama, M. J. W. Heetkamp, J. Stefanowska, and T. T. T. Huynh. 2006. Temperature and body weight affect fouling of pig pens. Journal of animal science. 84:2224–2231.

Baldwin, B. A., and D. L. Ingram. 1967. Behavioural thermoregulation in pigs. Physiology & Behavior. 2:15–16.

Balsbaugh, R. K., S. E. Curtis, R. C. Meyer, and H. W. Norton. 1986. Cold resistance and environmental temperature preference in diarrheic piglets. Journal of animal science. 62:315–326.

Batavia, M., A. Matsushima, O. Eboigboden, and I. Zucker. 2010. Influence of pelage insulation and ambient temperature on energy intake and growth of juvenile Siberian hamsters. Physiology & behavior. 101:376–380.

Baumgard, L. H., and R. P. Rhoads Jr. 2013. Effects of heat stress on postabsorptive metabolism and energetics. Annu. Rev. Anim. Biosci. 1:311–337.

Benzinger, T. H. 1969. Heat regulation: homeostasis of central temperature in man. Physiological reviews. 49:671–759.

Berkowitz, L. 1989. Frustration-aggression hypothesis: examination and reformulation. Psychological bulletin. 106:59.

Bertoldo, M. J., P. K. Holyoake, G. Evans, and C. G. Grupen. 2012. Seasonal variation in the ovarian function of sows. Reproduction, Fertility and Development. 24:822–834.

Blatteis, C. M. 1998. Physiology and pathophysiology of temperature regulation. World Scientific.

Broom, D. M. 1998. Welfare, stress, and the evolution of feelings. Advances in the Study of Behavior: stress and behavior. 27:371.

Brown-Brandl, T. M., M. D. Hayes, H. Xin, J. A. Nienaber, and H. Li. 2014. Heat and moisture production of modern swine. ASHRAE Trans. 120: 469–489. doi:10.13031/ trans.57.1071

Bruce, J. M., and J. J. Clark. 1979. Models of heat production and critical temperature for growing pigs. Animal Science. 28:353–369.

Canals, M., M. Rosenmann, and F. Bozinovic. 1989. Energetics and geometry of huddling in small mammals. Journal of theoretical Biology. 141:181–189.

Close, W. H., L. E. Mount, and I. B. Start. 1971. The influence of environmental temperature and plane of nutrition on heat losses from groups of growing pigs. Animal Science. 13:285–294.

Collin, A., J. van Milgen, S. Dubois, and J. Noblet. 2001. Effect of high temperature on feeding behaviour and heat production in group-housed young pigs. British Journal of Nutrition. 86:63–70.

Cowles, R. B. 1940. Additional implications of reptilian sensitivity to high temperatures. The American Naturalist. 74:542–561.

Curtis, S. E. 1983a. Environmental management in animal agriculture. Iowa State University Press.

Curtis, S. E. 1983b. Perception of thermal comfort by farm animals. Farm Animal Housing and Welfare. 24:59.

Curtis, S. 1985. Physiological response and adaptation of swine in Stress Physiology in Livestock. Vol. II: Ungulates. M. D. Yousef, ed. CRC Press, Boca Raton, FL. p. 59–65 and 129–139.

Curtis, S. E., and J. C. Rogler. 1970. Thermoregulatory ontogeny in piglets: sympathetic and adipokinetic responses to cold. American Journal of Physiology-Legacy Content. 218:149–152.

Dauncey, M. J., and D.L. Ingram. 1986. Acclimatization to warm or cold temperatures and the role of food intake. J. Thermal Bio. 11: 89-93. doi:10.1016/0306-4565(86)90025-2.

Dawson, W. R. 1982. Evaporative losses of water by birds. Comparative Biochemistry and Physiology Part A: Physiology. 71:495–509.

De Dear, R., and G. S. Brager. 1998. Developing an adaptive model of thermal comfort and preference.

DeShazer, J., and D. Overhults. 1982. Energy demand in livestock production. In: Livestock Environment II. Proc. 2nd Int. Livest. Environ. Symp. ASAE, St. Joseph, MI. p 17-27.

Federation of Animal Science Societies. (FASS). 2010. Guide for the care and use of agricultural animals in research and teaching. 3rd ed. Champaign (IL): Fed. Anim. Sci. Soc. Chap. 11.

Fraser, D. 1993. Assessing animal well-being: common sense, uncommon science.

Fraser, D., D. M. Weary, E. A. Pajor, and B. N. Milligan. 1997. A scientific conception of animal welfare that reflects ethical concerns.

Gaskill, B. N., C. J. Gordon, E. A. Pajor, J. R. Lucas, J. K. Davis, and J. P. Garner. 2012. Heat or insulation: behavioral titration of mouse preference for warmth or access to a nest. PloS one. 7:e32799.

Gilbert, C., D. McCafferty, Y. Le Maho, J.-M. Martrette, S. Giroud, S. Blanc, and A. Ancel. 2010. One for all and all for one: the energetic benefits of huddling in endotherms. Biological Reviews. 85:545–569.

Gleeson, M. 1998. Temperature regulation during exercise. International Journal of Sports Medicine. 19:S96–S99.

Gordon, C. J. 1993. Temperature regulation in laboratory rodents. Cambridge University Press.

Hahn, G. 1985. Managing and housing of farm animals in hot environments in Stress Physiology in Livestock. Vol II: Ungulates. M. K. Yousef, ed. CRC Press, Boca Raton, FL. p. 151–174.

Hahn, G. L., J. A. Nienaber, and J. A. DeShazer. 1987. Air temperature influences on swine performance and behavior. Applied Engineering in Agriculture. 3:295–302.

Harding, E. C., N. P. Franks, and W. Wisden. 2019. The temperature dependence of sleep. Frontiers in neuroscience. 13:336.

Hartgill, T. W., T. K. Bergersen, and J. Pirhonen. 2011. Core body temperature and the thermoneutral zone: a longitudinal study of normal human pregnancy. Acta Physiologica. 201:467–474.

Heldmaier, G. 1975. The influence of the social thermoregulation on the cold-adaptive growth of BAT in hairless and furred mice. Pflügers Archiv. 355:261–266.

Holmes, C., W. Close, W. Haresign, H. Swan, and D. Lewis. 1977. Nutrition and the climatic environment. Eds W. Haresign, H. Swan and D. Lewis. London, UK. p. 51-74

Hirth, H. F. 1963. The ecology of two lizards on a tropical beach. Ecological Monographs. 33:83–112.

Holmes, C. W., and N. R. McLean. 1974. The effect of low ambient temperatures on the energy metabolism of sows. Animal Science. 19:1–12.

Huynh, T. T. T., A. J. A. Aarnink, W. J. J. Gerrits, M. J. H. Heetkamp, T. T. Canh, H. A. M. Spoolder, B. Kemp, and M. W. A. Verstegen. 2005. Thermal behaviour of growing pigs in response to high temperature and humidity. Applied animal behaviour science. 91:1–16.

Ingram, D. L., J. L. Monteith, and L. E. Mount. 1973. Heat loss and its control in pigs. Heat loss from animals and man. Assessment and Control. 235–254.

IUPS, T. C. 2001. Glossary of terms for thermal physiology. Revised by the commission for thermal physiology of the international union of physiological sciences (IUPS thermal commission). The Japanese Journal of Physiology. 51:245–280.

Johnson, J. S. 2018. Heat stress: impact on livestock well-being and productivity and mitigation strategies to alleviate the negative effects. Animal Production Science. 58:1404–1413.

Kaczmarski, F. 1966. Bioenergetics oí Pregnancy and Lactation in the Bank Vole1.

Kingma, B., A. Frijns, and W. van Marken Lichtenbelt. 2012. The thermoneutral zone: implications for metabolic studies. Frontiers in bioscience (Elite edition). 4:1975–1985.

Klain, G. J., and J. P. Hannon. 1969. Gluconeogenesis in cold-exposed rats. In: Federation proceedings. Vol. 28. p. 965.

Lin, J., C. Cao, C. Tao, R. Ye, M. Dong, Q. Zheng, C. Wang, X. Jiang, G. Qin, and C. Yan. 2017. Cold adaptation in pigs depends on UCP3 in beige adipocytes. Journal of molecular cell biology. 9:364–375.

van Marken Lichtenbelt, W., B. Kingma, A. Van Der Lans, and L. Schellen. 2014. Cold exposure–an approach to increasing energy expenditure in humans. Trends in Endocrinology & Metabolism. 25:165–167.

Mayorga, E. J., D. Renaudeau, B. C. Ramirez, J. W. Ross, and L. H. Baumgard. 2019. Heat stress adaptations in pigs. Animal Frontiers. 9:54–61.

McDowell, R. E. 1972. Improvement of livestock production in warm climates. Improvement of livestock production in warm climates.

McGinnis, S. M. 1966. Sceloporus occidentalis: preferred body temperature of the western fence lizard. Science. 152:1090–1091.

Moinard, C., C. P. Doncaster, and H. Barré. 1992. Indirect calorimetry measurements of behavioral thermoregulation in a semiaquatic social rodent, Myocastor coypus. Canadian Journal of Zoology. 70:907–911.

Morita, V. S., V. R. Almeida, J. M. Junior, T. I. Vicentini, H. Van Den Brand, and I. C. Boleli. 2016. Incubation temperature alters thermal preference and response to heat stress of broiler chickens along the rearing phase. Poultry Science. 95:1795–1804.

Mount, L. E. 1975. The assessment of thermal environment in relation to pig production. Livestock Production Science. 2:381–392.

Mount, L. E. 1960. The influence of huddling and body size on the metabolic rate of the young pig. The Journal of Agricultural Science. 55:101–105.

Mount, L. E., and D. B. Stephens. 1970. The relation between body size and maximum and minimum metabolic rates in the new-born pig. The Journal of physiology. 207:417–427.

Nienaber, J. A., and G. L. Hahn. 2007. Livestock production system management responses to thermal challenges. International Journal of Biometeorology. 52:149–157.

NRC. 1981. Effects of Environment on Nutrient Requirements of Domestic Animals. Natl. Acad. Press, Washington, DC.

Okamoto-Mizuno, K., and K. Mizuno. 2012. Effects of thermal environment on sleep and circadian rhythm. Journal of physiological anthropology. 31:14.

Olds, J., and P. Milner. 1954. Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. Journal of comparative and physiological psychology. 47:419.

Olsen, A. W., L. Dybkjær, and H. B. Simonsen. 2001. Behaviour of growing pigs kept in pens with outdoor runs: II. Temperature regulatory behaviour, comfort behaviour and dunging preferences. Livestock production science. 69:265–278.

Polsky, L., and M. A. von Keyserlingk. 2017. Invited review: Effects of heat stress on dairy cattle welfare. Journal of dairy science. 100:8645–8657.

Ramot, D., B. L. MacInnis, and M. B. Goodman. 2008. Bidirectional temperature-sensing by a single thermosensory neuron in C. elegans. Nature neuroscience. 11:908.

Randolph, T. F., E. Schelling, D. Grace, C. F. Nicholson, J. L. Leroy, D. C. Cole, M. W. Demment, A. Omore, J. Zinsstag, and M. Ruel. 2007. Invited review: Role of livestock in human nutrition and health for poverty reduction in developing countries. Journal of animal science. 85:2788–2800.

Renaudeau, D., A. Collin, S. Yahav, V. De Basilio, J.-L. Gourdine, and R. J. Collier. 2012. Adaptation to hot climate and strategies to alleviate heat stress in livestock production. Animal: an international journal of animal bioscience. 6:707.

Richards, S. A. 1971. The significance of changes in the temperature of the skin and body core of the chicken in the regulation of heat loss. The Journal of physiology. 216:1–10.

Rozeboom, K. J., D. L. Reicks, and M. E. Wilson. 2004. The reproductive performance and factors affecting on-farm application of low-dose intrauterine deposit of semen in sows. Journal of animal science. 82:2164–2168.

Schmidt-Nielsen, K. 1975. Scaling in biology: the consequences of size. Journal of Experimental Zoology. 194:287–307.

Schmidt-Nielsen, K. 1997. Animal physiology: adaptation and environment. Cambridge University Press.

Schrøder-Petersen, D. L., and H. B. Simonsen. 2001. Tail biting in pigs. The Veterinary Journal. 162:196–210.

Silanikove, N. 2000. Effects of heat stress on the welfare of extensively managed domestic ruminants. Livestock production science. 67:1–18.

Stolba, A., and D. G. M. Wood-Gush. 1989. The behaviour of pigs in a semi-natural environment. Animal Science. 48:419–425.

St-Pierre, N. R., B. Cobanov, and G. Schnitkey. 2003. Economic losses from heat stress by US livestock industries. Journal of dairy science. 86:E52–E77.

Terrien, J., M. Perret, and F. Aujard. 2011. Behavioral thermoregulation in mammals: a review. Front Biosci. 16:1428–1444.

Tetievsky, A., and M. Horowitz. 2010. Posttranslational modifications in histones underlie heat acclimation-mediated cytoprotective memory. Journal of Applied Physiology. 109:1552–1561.

Trayhurn, P., N. J. Temple, and J. V. Aerde. 1989. Evidence from immunoblotting studies on uncoupling protein that brown adipose tissue is not present in the domestic pig. Canadian journal of physiology and pharmacology. 67:1480–1485.

Verstegen, M. W. A., A. J. H. Van Es, and H. J. Nijkamp. 1971. Some aspects of energy metabolism of the sow during pregnancy. Animal Science. 13:677–683.

Von Keyserlingk, M. A. G., J. Rushen, A. M. de Passillé, and D. M. Weary. 2009. Invited review: The welfare of dairy cattle—Key concepts and the role of science. Journal of dairy science. 92:4101–4111.

Waberski, D., S. Meding, G. Dirksen, K. F. Weitze, C. Leiding, and R. Hahn. 1994. Fertility of long-term-stored boar semen: Influence of extender (Androhep and Kiev), storage time and plasma droplets in the semen. Animal Reproduction Science. 36:145–151.

Weary, D. M., L. Niel, F. C. Flower, and D. Fraser. 2006. Identifying and preventing pain in animals. Applied animal behaviour science. 100:64–76.

Weiss, B., and V. G. Laties. 1961. Behavioral thermoregulation. Science. 133:1588–1588.

Wilson, L. M., and H. L. Sinha. 1985. Thermal preference behavior of genetically obese (ob/ob) and genetically lean (+/?) mice. Physiology & behavior. 35:545–548.

Young, B. A. 1981. Cold stress as it affects animal production. Journal of Animal Science. 52:154–163.

CHAPTER 2. EARLY LIFE THERMAL STRESS: IMPACTS ON FUTURE THERMAL PREFERENCE IN WEANED PIGS (3 TO 15 KG)

Thermal stress can result in productivity losses, morbidity, and mortality if proper management practices are not employed. A basic understanding of the relationship between animals and the thermal environment is crucial to assess the environment's impact on livestock performance. Therefore, the study objective was to evaluate whether different early life thermal stressors (**ELTS**) altered the temperature preference of pigs later in life. Twelve sows and their litters were randomly exposed to one of three ELTS treatments from 7 to 9 d of age: early life heat stress (**ELHS**; cycling 32 to 38ºC; n=4), early life cold stress (**ELCS**; 25.4±1.1ºC without heating lamp; n=4), or early life thermoneutral (**ELTN**; 25.4±1.1ºC with a heating lamp; n=4) conditions. From 10 to 20 d (weaning) all piglets were exposed to ELTN conditions. At weaning, pigs were randomly assigned to groups of 4 of the same sex and ELTS treatment. Temperature preference, where pigs freely choose a temperature, was assessed in 21 groups (n=7 groups per ELTS treatment) using one of three thermal gradient apparatuses (22 to 40°C). Testing began at 26±1.3 d of age to give pigs time to acclimate to solid food after weaning and one group per ELTS treatment were tested simultaneously in each apparatus. Pigs were given 24 h to acclimate followed by a 24 h testing period. Behavior (active and inactive), posture (upright, sternal and lateral lying), and location were documented every 20 min using instantaneous scan samples. Preferred feeding temperature was determined by the latency to empty a feeder in each location. Data were analyzed using PROC MIXED in SAS 9.4. A cubic regression model was used to calculate the peak temperature preference of pigs based on the temperature pigs spent most of their time. The preference range was calculated using peak temperature preference ±SE for each ELTS treatment group. Early life thermal stress altered where pigs spent most of their time within the thermal gradient (P =0.03) with ELTN pigs preferring cooler temperatures (peak preference of 23.8°C) compared to their ELCS exposed counterparts (peak preference of 26.0°C; P <0.01). However, ELHS exposed pigs (peak preference of 25.6°C) did not differ in their temperature preference compared to ELTN or ELCS exposed counterparts (P >0.05). In summary, ELCS exposure altered pig temperature preference later in life indicating ELTS can alter temperature preference in pigs.

2.1 Introduction

Prior exposure to temperature extremes may have a long-term impact on animal thermoregulation and thermopreferendum. Studies in early life heat stressed (**ELHS**) rodents have described improved thermotolerance to heat stress (**HS**) exposure later in life (Tetievsky and Horowitz, 2010). In addition, piglets exposed to early life cold stress (**ELCS**) may have a permanent reduction in body temperature (Heldmaier, 1974). Furthermore, a recent study determined that ELHS exposed pigs have reduced thermotolerance when exposed to an HS challenge immediately following weaning (Johnson et al., 2018). Taken together, these data indicate that early life temperature extremes can influence thermoregulation, which may have implications for future temperature preference.

Thermotolerance and body temperature are influenced by heat exchange between the animal and its environment (Johnston and Bennett, 2008), and a permanent shift in an animals' thermotolerance may result in an altered thermopreferendum. This is because the surface temperature (**T_S**) to ambient temperature (**T_A**) differential is the driving force for sensible heat exchange (Kingma et al., 2014). For instance, as body temperature increases, T_S increases to maintain a balance between heat production and heat dissipation (Kingma, et al., 2014). Since body temperature is increased more rapidly in ELHS pigs exposed to a HS challenge without a similar absolute T_S increase (Johnson et al., 2018), this suggests that a cooler T_A would be preferred to increase the thermal gradient and improve heat dissipation capacity. Alternatively, because ELCS pigs have a reduction in body temperature (Heldmaier, 1974), this may lead to greater vasoconstriction to conserve body heat (Campbell, 2008) resulting in a preference for warmer temperatures to reduce the thermal gradient. Therefore, the study's objective was to investigate whether early life thermal stress (**ELTS**) alters the temperature preference of pigs later in life. We hypothesized that pigs exposed to ELHS would prefer a cooler T_A and that ELCS pigs would prefer warmer temperatures relative to early life thermoneutral (**ELTN**) exposed pigs.

2.2 Methods and Materials

All procedures involving animal use were approved by the Institutional Animal Care and Use Committee at Purdue University (protocol #1701001525), and 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).

2.2.1 Early life thermal stress exposure

As described in Johnson et al. (2018), 12 first-parity sows with similar-sized litters [n = 11.8 piglets/litter; Duroc x (Landrace x Yorkshire)] were exposed to one of three ELTS treatments: ELTN (25.4 ± 1.1ºC with heating lamp; n = 4), ELHS (cycling 32 to 38ºC; n = 4), or ELCS (25.4 ± 1.1ºC without heating lamp; n = 4) from 7 to 9 d post-farrowing. All temperature treatments were based on the *Guide for the Care and Use of Agricultural Animals in Research and Teaching*'s recommended thermal conditions for swine (Federation of Animal Science Societies, 2010). Thermal stress was verified through rectal temperature (**T_R**), T_S, average daily gain, thermal imaging, and respiration rate (**RR**) and these data are described by Johnson et al. (2018). After thermal treatment exposure (day 10 post-farrowing), piglets were housed under normal production conditions (25.4 ± 1.1ºC with 56.1 ± 8.1% relative humidity (**RH**) and supplemented with a heating lamp) until weaning (20.0 ± 1.3 d of age).

2.2.2 Experimental design: thermal preference

Mead's resource equation was used *a priori* to determine the number of groups needed for the 2 x 3 factorial design (2 sex x 3 ELTS; Mead, 1990). Eighty-four pigs were randomly assigned (via random integer generator, random.org) to one of 21 testing groups that consisted of 4 same-sex pigs of the same early life thermal treatment (table 2.1). Two pigs from each group were from the same litter.

At weaning, all pigs were re-located to nursery room 1 at the Purdue Animal Science Research Education Center (West Lafayette, IN USA). A nursery pen (0.95 m x 1.43 m) held up to 8 pigs; therefore, if two testing groups were co-housed, they were treatment and sex matched. The nursery room received natural lighting via windows (15:9 light:dark), the average ambient temperature inside the nursery was 25 ± 5.8°C with 58.1 ± 10.5% RH, and all pigs were given *ad libitum* access to food and water. Temperature preference testing began on June 7, 2017 (26 d ± 1.3; 6.45 ± 1.26 kg BW) and ran until June 26, 2017 (39 d ± 6.5; 9.71 ± 1.62 kg BW). Body weight was documented before each testing phase and added as a categorical variable by doing a mean split for each treatment (above or below the treatment average).

For temperature preference testing, pigs were transported in a cart approximately 91 m from the nursery to the environmental room. For each temperature preference testing session, 1 group of pigs from each ELTS treatment was randomly selected and simultaneously tested in 1 of 3 thermal apparatuses located within the environmental room. The pigs were allowed 24 h to acclimate to the new enclosure, thermal gradient, and environmental room. During acclimation and temperature preference testing, pigs were able to explore the entirety of the thermal apparatus. Each apparatus was cleaned in between acclimation, temperature preference testing, and between testing groups. Between acclimation and temperature preference testing, pigs were removed from the thermal apparatus and penned in an adjacent environmental room for approximately 2 h to clean and reestablish the thermal gradient. Waste was removed with a pressure washer, and the flooring and surrounding walls within the thermal apparatus were disinfected (LYSOL disinfectant all-purpose cleaner, Reckitt Benckiser LLC, NJ, US). After cleaning, the thermal gradient was considered stable when 3 readings, measured every 15 min, did not vary by more than 0.2°C. Pigs were then returned to their assigned thermal apparatus for an additional testing period of 24 h. Precautions were taken to control for room position and side bias by balancing thermal treatments across the three thermal apparatuses as well as where, within the apparatus, pigs were initially placed. In addition, at least 1 group of barrows and 1 group of gilts were tested in each testing run. During experimental set-up and preference testing, researchers were not blinded to the pigs' ELTS treatment, but animal care staff were. During video coding, observers (LR and CF) were blinded to the ELTS treatment of the pigs and only the testing period was coded.

2.2.3 Thermal apparatus

The methods and materials were adapted in part from Robbins et al., (2018). Briefly, three thermal gradient apparatuses were built (3.05 m x 0.61 m x 0.61 m; L x W x H) to provide the required space per piglet and create the desired temperature gradient (Fig. 2.1). To create the needed thermal gradient (20˚C to 40˚C), ceramic heating lamps (herein referred to as heating elements (Floureon 200W Multi Basking IR Heat Bulb) were placed at strategic locations 0.46 m above the floor to create a constant thermal gradient and were covered with wire mesh (Acorn international, Memphis, TN, USA). The wire mesh was used to prevent the pigs from being accidentally burned should they be able to reach the heating elements. Plexiglas (MFG-Acrylic 1.52 m x 1.91 cm x 0.61 m: Meyer Plastics, Inc., Lafayette, IN, USA) was used to create a lid above each of the two halves of the thermal apparatuses. The cool end of all three thermal apparatuses had two computer fans (Coolermaster silent fan 120 S/2, Cool Master Technology Inc., Taiwan) to push cool ambient air into the apparatus and down the gradient to the hotter end where 6 exhaust holes were cut. Finally, eight containers were used to supply the pigs with feed and water within the apparatus (Fortiflex MF-2 Mineral Feeder, 2 x 1.75 qt. capacity, 32.39 cm x 14.61 cm x 15.88 cm). Total water supplied between the three waterers was 11.36 L, and 2.27 kg total food was evenly allocated across the 5 feeding containers per day. Containers with feed were placed every 0.31 m along the right side of the thermal apparatus wall and containers of water were placed every 0.61 m along the left side of the thermal apparatus wall (Fig. 2.2).

The three thermal apparatuses were installed at Purdue University Animal Science Research and Education Center (West Lafayette, IN, USA) in an environmentally controlled room. The environmental room RH and temperature were documented every 5 min (07:00 to 22:06) for 3 days before pigs being placed inside, with the thermal apparatus air velocity and temperature measurements being taken every 15 minutes (table 2.2). Within the thermal apparatus, lines were drawn every 0.61 m to indicate thermal zones (5 thermal zones total; A to E) and ensure temperature was measured consistently (Fig. 2.2). The lines were used by observers to record the location of the pigs during video analyses.

2.2.4 Behavior and posture observations

The pigs were videotaped continuously over the 24 h testing period for behavior, location, and posture using infrared cameras (Sony Corporation, Tokyo, Japan) and video surveillance software (GeoVision, Taiwan). The scan interval used for recording data from video was determined by comparing different sampling intervals (5, 10, 15, 20, 25, 30 min) based on the frequency of time spent at each location. Data from each subsample were compared pairwise and considered to accurately estimate the behavior or posture if the intervals were not significantly different from each other compared to the 5 min interval (Ledgerwood et al., 2010). Based on this data, a 20-minute sampling interval was selected for this study. Thus, the location, behavior, and posture were recorded for each piglet using instantaneous scan samples every 20 min. The ethogram contained 3 simple behavior categories: active, inactive, and other (table 2.3).

If pigs were observed in more than 1 thermal zone (location), the proportion of a piglet in each zone was documented in 0.25 increments (head, front quarter, mid-section and rump; Fig. 2.3). Postures can indicate thermal comfort (Mount, 1960); therefore, posture was also documented at each scan sample (table 2.4). The frequency of behaviors was calculated for each group of pigs by counting the total number of times each behavior category was observed in each location per day and totaled per group. This calculation was repeated for posture. Any observations of pigs documented in the "other" category for behavior and posture were dropped from the dataset.

2.2.5 Latency to empty feeders

Video was used to determine the latency until each food container was emptied. If the food was not completely consumed, the maximum time pigs were in the apparatus (1440 min) was assigned to that location-specific container. Food latency, rather than consumption, was used in the final analysis because once the pigs consumed all the food in that location, they would be forced to consume food in a location that may not reflect their true temperature preference.

2.2.6 Analyses

All analyses were performed using the PROC MIXED (GLM) procedure in SAS 9.4 (SAS Institute INC., Cary, NC). The assumptions of the GLM (normality of error, homogeneity of variance, and linearity) were confirmed post-hoc, and data were transformed when necessary to meet these assumptions (Grafen and Hails, 2002). The threshold for significance $P < 0.05$ was used and Bonferroni corrected where applicable.

2.3.6.1 Early life thermal stress and body weight

The peak temperature preference was isolated by determining the temperature pigs were observed most often. The average end body weight per group was analyzed with this single temperature preference to determine if body weight varied by group and if body weight predicted the temperature pigs spent most of their time in. Sex and ELTS were also included in this model as fixed effects.

2.3.6.2 Behavior and posture by location

A cubic regression model was performed for both behavior and posture data and both were log10+0.001 transformed to meet the assumption of a GLM. To avoid pseudoreplication and accommodate repeated measures, analyses were blocked by Group of pigs, nested within sex and ELTS. Group of pigs cannot be treated as a random effect, there is not a meaningful wider population of groups of four pigs representing the unique ELTS conditions they were reared in between 7 to 9 d of age to which the results could pertain (Newman et al., 1997), and was therefore treated as fixed effects. Main effects plus three-way interactions of sex, ELTS treatment, behavior or posture, and location were originally tested with a cubic variable of location. However, due to non-orthogonal data, higher order interactions that were non-significant were dropped from the model. Weight category and any interactions were removed from the analysis because they were not significant, and AIC was reduced when this variable was removed. Further, as data were not orthogonal, non-significant higher-order interactions were dropped from the final analysis.

The cubic curve from the final model above was generated in 0.2°C increments starting with the coldest thermal zone temperature (23.2°C) and increasing to the warmest temperature (38.2°C). Peak temperature for ELTS, behavior, and posture was calculated by identifying the temperature with the greatest frequency. The temperature preference range was then calculated from the peak temperature ± SE. Tukey tests for differences in LSM between ELTS, behavior, and posture were run in each thermal zone. Since Tukey tests were run 5 times (for each thermal zone), the alpha was Bonferroni corrected for the multiple tests ($\alpha = 0.05/5 = 0.01$).

2.3.6.3 Latency to empty feeders

Analyses were blocked by group of pigs, nested within sex, ELTS, and weight category. Main effects plus second-order interactions of sex, ELTS treatment, weight category, and temperature corresponding with the feeder location were tested. Data did not require transformation to meet the assumptions of a GLM (normality of error, homogeneity of variance, and linearity). The weight category was included with this model due to a higher R^2 value. Post hoc Tukey tests were used to evaluate significant terms in the model.

2.3 Results

2.3.1 Early life thermal stress and body weight

No differences in body weight were detected between ELTS treatments (table 2.5). Furthermore, BW did not affect peak temperature preference (table 2.6).

2.3.2 Thermal preference and behavior

Early life thermal stress altered where pigs spent their time in the thermal gradient (GLM: $F_{2,176} = 3.47$; $P = 0.03$: Fig. 2.4). Early life thermoneutral pigs had a peak temperature preference of 23.8°C (7.71%) with a preferred range between 23.2 to 25.2°C (table 2.7). This preference was cooler by 2.2°C compared to ELCS pigs ($_{\alpha/3}$: $F_{1,176} = 7.93$; $P < 0.01$: table 2.7). Early life cold stressed pigs spent the most amount of time at 26.0°C (8.68%) but their temperature preference ranged between 24.8 to 27.6°C (table 2.7). Peak temperature preference did not differ when comparing ELHS and ELCS pigs ($_{\alpha/3:}$ $F_{1,176} = 2.79$; $P = 0.10$: table 2.7) Early life heat stressed pigs had a peak temperature of 25.6°C (6.59%) with a preferred range between 24.4 to 27.2°C (table 6). Finally, ELCS pigs spent more time at 34.2°C compared to ELTN pigs ($_{\alpha/5}$: $P < 0.02$: Fig. 2.4).

Behavior altered where pigs were most frequently observed within the thermal gradient ($F_{1,176} = 25.89$; $P < 0.01$: Fig. 2.5). Inactive behavior was observed most often at 24.6°C (24.90%) and observed most frequently between 23.6 to 25.8°C (table 2.7). Active behavior was observed most often at a temperature of 25.8°C (2.97%, range: 24.6 to 27.2°C: table 2.7). Further, Tukey tests ($_{\alpha/5}$) showed that pigs spent more time inactive than active at 23.3°C (19.63%), 26.2°C (18.54%), and 30.1°C (5.07%: $P < 0.01$; Fig. 2.5) and peak temperature differed by 1.2°C when comparing active and inactive behaviors ($_{\alpha/2:}$ $F_{1,176} = 34.87$; $P < 0.01$: table 2.7).

2.3.3 Thermal preference and posture

The frequency of various postures differed across the thermal gradient (GLM: $F_{2,276}$ = 14.99; P < 0.01; Fig. 5). Upright posture was observed most at 25.6°C (23.11%) and frequently observed between 24.4 to 27.0°C (table 2.7). Sternal laying posture was observed equally at 24.6°C and 24.8°C (23.98%) and observed most frequently between 23.6 to 26.0°C (table 2.7). Finally, in the lateral laying posture pigs were observed most at 24.4°C (44.59%; range: 23.2 to 25.6°C: table 6). The peak temperature for upright posture was warmer by an average of 0.9°C compared to sternal ($_{\alpha/3}$: $F_{1,276}$ = 13.31; P < 0.01: table 2.7) and by 1.2°C compared to lateral lying postures ($_{\alpha/3}$: $F_{1,276}$ = 28.73; P < 0.01: table 2.7). No difference was observed between peak temperature preferences when pigs were sternal or lateral laying ($_{\alpha/3}$: $F_{1,276}$ = 2.93; P = 0.09: table 2.7). Further, Tukey tests ($_{\alpha/5}$) showed that pigs were observed more in the lateral laying posture compared to both upright and sternal laying at 23.3°C (24.35% and 20.35%, respectively) and 26.2°C (14.59% and 15.86%, respectively: P < 0.01: Fig. 2.6). Compared to sternal laying, pigs were observed upright most often at 30.1°C (4.08%), 34.2°C (2.79%) and 38.2°C (3.67%: P < 0.01: Fig. 2.6). However, compared to lateral lying, they were more often observed upright at 34.2°C (2.17%) and 38.2°C (3.56%: P < 0.01: Fig. 2.6). Lastly, pigs were observed most often in the lateral compared to the sternal lying posture at 30.1°C (4.13%: P < 0.01: Fig. 2.6).

2.3.4 Latency to empty feeders

The latency to empty the various feeders depended on its thermal location (GLM: $F_{4,56}$ = 4.11; P < 0.01: Fig. 2.7). Pigs consumed food the fastest from the feeder at 26.2°C compared to 34.2°C (175.67 min) and 38.2°C (190.62 min: P < 0.05: Fig. 2.7). An interaction of sex and weight category (GLM: $F_{1,80}$ = 5.00; P = 0.03: table 2.8) and ELTS and sex (GLM: $F_{2,80}$ = 3.57; P = 0.03: table 2.8) altered the latency times; however, post hoc Tukey tests did not identify any significant comparisons (P > 0.05).

2.4 Discussion

This study experimentally looked at the effects of ELTS on the future temperature preference of pigs. Body temperature is influenced by heat exchange between the animal and its environment (Johnston and Bennett, 2008), and previous research has demonstrated a permanent shift in an animals' thermotolerance when exposed to HS during the early stages of development (as reviewed by Horowitz, 2007). This shift in thermotolerance may result in an altered temperature preference due to the T_S to T_A differential as the driving force for sensible heat exchange (Kingma et al., 2014). Thus, this study hypothesized that exposure to ELTS would result in an altered temperature preference in pigs later in life. Specifically, ELHS pigs would prefer cooler temperatures since body temperature has been shown to increase more rapidly in ELHS pigs exposed to an HS challenge, without a similar absolute T_S increase (Johnson et al., 2018). In addition, cooler temperature preference would create a more efficient heat sink (i.e. heat flows warmer to cooler). Consequently, these pigs would seek out cooler temperatures compared to ELCS and ELTN exposed counterparts to optimize this heat exchange. In contrast, ELCS pigs were predicted to prefer warmer temperatures compared to the other thermal treatments. Cold stressed pigs often have a reduction in body temperature that results in greater vasoconstriction and improved heat conservation (Campbell, 2008) that may reflect in a warmer temperature preference compared to other ELTS pigs.

Results in the present study demonstrated that ELTS influenced temperature preference between ELCS and ELTN pigs. Early life cold stressed pigs spent most of their time between 24.8 to 27.6°C, and as predicted, ELCS pigs had a peak temperature preference that was 2.2°C warmer when compared to ELTN pigs. Early life cold stressed pigs may have greater vasoconstriction resulting in improved heat conservation (Heldmaier, 1974), which could explain the shift in temperature preference when compared to ELTN pigs. Unfortunately, due to the nature of this study, body temperature data (e.g. skin surface and body temperature) could not be documented while the animals were inside the thermal apparatus and it cannot be confirmed if vasoconstriction caused the shift in temperature preference for ELCS pigs. Although preferences differed between ELCS and ELTN pigs, the actual treatments only differed by the presence/absence of heat from a heating lamp. Both the ELCS and ELTN pigs were exposed to the same barn level T_A, but the heating lamp for ELCS pigs was turned off. These results support that the radiant heat given off by the lamp is influential on pigs' thermoregulatory development. Past research has indicated that younger piglets (less than 10 d) prefer the heat from the lamp over a mat to rest on, but as they get older and gain more BW, the preference shifts to the mat (Zhang and Xin, 2001; Xin and Zhang, 1999). This might explain why ELCS pigs had a shift in temperature preference compared to ELTN, as they were most affected by the treatment given (i.e. the absence of a heating lamp at 7 to 9 d old).

Temperature preferences were similar between ELHS pigs and the other treatments, but this may be related to the specific ELHS protocol. Smaller pigs have a relatively large surface area to mass ratio making them more susceptible to the effects of CS than HS. Early life heat stressed pigs preferred temperature between 24.4 to 27.2°C and were exposed to an HS treatment (cycling 32 to 38 ºC) that mimicked a diurnal pattern. These temperatures were selected to ensure the safety of the sows but still provide adequate heat stress to the piglets. Previously, ELHS pigs had increased T_R and T_S, indicating that during the treatment (applied at 7 to 9 d of age) they exhibited signs of HS (Johnson et al., 2018). Despite this, the ELHS pigs' preference did not differ from any of the other thermal treatments and this may be related to the ELHS treatment protocol.

Acquiring thermotolerance is a transient process and depends primarily on the severity and duration of the initial HS. In general, the greater the initial heat dose, the greater the duration of thermotolerance. For example, following a sublethal heat exposure, thermotolerance (based on the presence of HSP70) can be observed within several hours and lasts 3 to 5 d (as reviewed by Kregel, 2002). Perhaps, if we had tested temperature preference within 5 d of ELTS, rather than beginning at weaning (d 20), we may have observed a more drastic difference in temperature preference. It is also possible that if the piglets had been exposed to HS temperatures for a longer duration it may have had a stronger effect on their thermal preference. This has been previously shown in rodents and pigs (2 or 30 d in rodents: Tetievsky and Horowitz, 2010; 20 d in pigs Renaudeau et al., 2010). Despite the lack of differences between ELHS and the other treatments, this study provides further knowledge about factors that influence the TCZ of pigs.

Understanding the TCZ of an animal is critical to creating temperature recommendations and providing an optimal environment both for production and welfare. Interestingly, these results demonstrated a shift in the preferred temperature range of group-housed pigs under these conditions. The ELTN pigs appeared to prefer cooler temperatures than what is stated in the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (Federation of Animal Science Societies, 2010), with ELTN pigs spending more time between 23 to 25°C than expected. While our data overlaps with temperatures thought to be preferred by 3 to 15 kg piglets (25 to 32°C; Federation of Animal Science Societies, 2010), pigs in this study spent ≤ 15% of their time in temperatures above 29°C. The shift in temperature preference may be because the *Guide for the*

Care and Use of Agricultural Animals in Research and Teaching (Federation of Animal Science Societies, 2010) recommendations are based on swine genetics from nearly 40 years ago (NRC, 1981; DeShazer and Overhults, 1982; Curtis, 1985; Hahn, 1985). Since the publication of the original research forming the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (Federation of Animal Science Societies, 2010), total heat and moisture production in current genetic lines has increased by an average of 16%, ranging 10 to 32% across production stages (Brown-Brandl et al., 2014). This increase in heat production may have contributed to the decrease in preferred ambient temperatures observed in this study.

An additional explanation of the temperature discrepancy between this study and the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (Federation of Animal Science Societies, 2010) could be due to the social environment. The recommendations in the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (Federation of Animal Science Societies, 2010) are based on data from individually tested piglets, rather than a group. This is a very important distinction since social behavior and the physical contact that generally results, can influence temperature preference. In a preference test, pigs preferred to lie near an anesthetized littermate rather than lie within comfortable temperature alone (Hrupka et al. 2000). Group-housed pigs are often observed huddling together or touching each other when resting. The heat conserved from huddling would make normally cool temperatures for an individual much more comfortable when in a group (Mount, 1963; Hrupka et al., 2000).

Although group preference has not been systematically evaluated, previous research has determined the temperature preference of individual piglets of various ages (< 14 d) and sizes (Vasdal et al., 2010; Balsbaugh et al., 1986; Bench and Gonyou, 2007). When provided with thermal options, 24 h old piglets preferred temperature of 42°C (Vasdal et al., 2010). However, piglets between 12 to 72 h have demonstrated a preference for temperatures between 33 to 37°C (Balsbaugh et al., 1986). These studies demonstrate that individually tested, pre-nursery piglets (3 to 15 kg), prefer warmer temperatures than the recommended temperatures outlined in the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (25 to 32°C; Federation of Animal Science Societies, 2010). Although piglets in this previous research preferred warmer temperatures than the piglets in this study, the former piglets were tested individually, were much younger, and weighed less.

As mentioned above, previous thermal preference work focused on very young, lightweight piglets (Vasdal et al., 2010; Balsbaugh et al., 1986; Bench and Gonyou, 2007). Body weight is an important factor that is highly correlated to temperature preference (Dauncy and Ingram, 1986). Animals with a larger surface area to mass ratio prefer warmer temperatures, due to high rates of heat loss (Dauncy and Ingram, 1986). However, as pigs age, body weight and metabolic heat production increases, reducing this ratio and shifting preference to a cooler thermal range (Dauncy and Ingram, 1986). Older (12 to 14 d), group housed piglets (in groups of 8) selected cooler temperatures (25.77°C) (Bench and Gonyou 2007) which are more similar to results from the current study, indicating piglets tested as a group will prefer cooler temperatures compared to those tested individually. Individually tested and younger piglets may have preferred a warmer temperature, but a large group allows the piglets to maximize heat conservation from huddling. The combination of factors such as age, weight, and the number of group members play a large part in thermal preference. However, it is unknown what the optimal group size would be for various barn temperatures or the degree to which group size influences the temperature preference of piglets within our tested weight range.

The thermoregulatory system relies on behavior as the initial means of maintaining homeostasis because it is relatively cheap energetically. Under natural conditions, animals will exhibit thermotaxic behaviors, such as finding shade on a warm day or basking in the sun on a cool day (Gordon, et al., 1993). Consequently, an animal will alter its behavior to change heat loss or gain. Animals have an innate motivation to seek out a preferred T_A, referred to as thermopreferendum. Thermopreferendum is where animals seek to decrease the temperature difference between the environment and the animal and allowing an animal to minimize energetic costs (Terrien et al., 2011). However, thermal preference may change based on activity. With increased activity, a significant amount of heat is generated from muscle contraction and active individuals will often seek cooler temperatures to increase heat loss (Smith et al., 2005). In this study, pigs spent very little time active (approximately 15%) and the peak temperature preference was not different during inactive behaviors. The minimal amount of time active could have limited our ability to observe temperature preferences based on activity. It is also possible that with so little activity, pigs did not build up enough metabolic heat to alter their preference.

While animals generally seek out cooler temperatures when active, however the opposite behavior is often observed during times of inactivity. Mammals typically experience a decline in core body temperature during sleep that will correlate with the circadian rhythm of the species (as reviewed by Harding et al., 2019). In mice, body temperature can drop by approximately 2°C as they transition from their active phase to sleep (as reviewed by Harding et al., 2019). During sleep, the animal's metabolic rate is typically lower than while inactive (still but alert) and a warmer temperature preference can be observed (as reviewed by Harding et al., 2019). Mice have demonstrated a clear thermal preference during the sleep phase (lights on), choosing warmer environments approaching thermoneutrality (27 to 30°C) and minimizing energy expenditure (Gordon et al., 1994; Gaskill et al., 2012). Previous literature has demonstrated that pigs will select cooler temperatures when sleeping (Bench and Gonyou, 2007) compared to when active. In this study, the ethogram did not distinguish between sleep and animals that may have been still but alert. Had we done so, perhaps we would have observed a difference in thermal preference based on behavior.

Posture is another mode of behavioral thermoregulation that alters the rate of heat loss by changing the amount of surface area exposed to the environment. When housed within their TCZ, pigs prefer to lay near each other with minimal contact and predominately lie in a lateral posture (Huynh et al., 2005). A warmer temperature however reduces conspecific contact and increases the amount of lateral lying (Huynh et al., 2005; Aarnink et al., 2006). The lateral lying posture, increases skin contact with the floor allowing for greater heat exchange and lessens the heat load if an animal is experiencing HS.A decrease in $T_{A,}$ however, results in more sternal lying to reduce the exposed skin surface area to reduce heat loss (Mount, 1960). The decrease in T_A will also result in pigs huddling together, becoming condensed and increasing body contact with conspecifics.

Typically, there is a linear relationship between lateral lying and environmental temperature in pigs (Huynh et al., 2005; Aarnink et al., 2006; Pedersen et al., 2003). Increasing skin contact with the floor allows for greater heat exchange and lessens the heat load if an animal is experiencing HS. However, pigs typically stay in the lateral posture (~70%) within their TCZ, although near littermates (Huynh et al., 2005). In this study, pigs in the three coolest thermal zones (23.3°C, 26.2°C, and 30.1°C) spent more time in a lateral posture (~45%), compared to sternal lying (~24%). Although they spent different amounts of time in these postures, there was no significant difference between the peak temperatures in sternal (24.6 and 24.8°C) or lateral lying (24.4°C). The lack of differences between sternal and lateral laying could be due to space limitations within the thermal apparatus or the piglet's desire to lay near a littermate. Overall, pigs spent most of their time in the lateral posture at a temperature below the *Guide for the Care and*

Use of Agricultural Animals in Research and Teaching (Federation of Animal Science Societies, 2010) recommendations. The fact that the pigs chose cooler temperatures than expected and were lying in a posture that indicates comfort, supports that the preferred temperatures are within their TCZ.

Examining the percentage of time pigs spent within various temperatures is not the only way thermal preference can be assessed. An alternative could look at temperatures that were either avoided or where specific behaviors were performed. Studies have shown that pigs' latrine (Signoret et al., 1969), meaning they establish an elimination location away from rest areas (Petherick 1983; Stolba and Wood-Gush, 1989). Thus, defecation behavior could be a means to observe the least preferred temperatures; however, this behavior was observed so infrequently that it was omitted from analyses. In addition, upright posture was observed most frequently in the two warmest thermal zones (34.2°C and 38.2°C) and had a significantly warmer peak temperature (25.6°C) than either of the lying postures. The increase in an upright posture in the hottest temperatures could be due to pigs walking to the end of the apparatus for elimination. It may indicate the temperature at which there was an aversion. This observation illustrates the importance of not only looking at the amount of time spent within thermal locations but also the actions within those areas.

Animals maintained at high or low T_A for long periods modify their physiological responses to adapt to that T_A. An animal's (bird and mammals) resting metabolic rate declines after exposure to HS and increases after exposure to CS (Chaffee and Roberts, 1971; Herpin et al., 2002). To fuel the increased energetic needs and maintain a positive energy balance during cold exposure, animals need to adapt their food intake to compensate. An increase in caloric intake has been described in numerous species (including pigs), to counteract cold-induced costs of thermoregulation (Dauncey and Ingram, 1986). In response to CS, catabolic mechanisms are enhanced, promoting energy intake and energy expenditure. The increase in feeding during CS conditions allows pigs to meet their thermal heat requirements at lower T_A (Verstegen et al., 1987; Young, 1981). As the ambient temperature rises, swine will consume less food to reduce heat production (Collin et al., 2001). For grow-finish stage pigs, estimates indicate a range of 10 to 30% reduced feed intake as temperatures rise from 19 to 31°C (Hutu and Onan, 2019). Although food consumption was measured in this study, pigs could move freely between temperatures and thus food consumption was not directly altered by temperature. However, the amount of time it took pigs to empty the food bins reveals the temperatures in which they preferred to eat. Regardless of their ELTS, pigs consumed food at a faster rate in the two coolest temperature zones (23.3°C and 26.2°C). This data indicates that those studying nutrition and production, may want to house their pigs between these temperatures to keep pigs within their TCZ and still promote feed intake. This unsurprisingly correlated with the thermal zones they spent the most amount of time in. Unfortunately, this measure also comes with limitations. When the preferred feeders were emptied, the pigs had to move into other thermal zones to consume feed, thus adding variability to both our behavioral and location data. However, this may not have significantly influenced our data since pigs spent only ≈ 15% of their time active, which included feeding behavior.

This study was able to successfully identify the effects of ELTS on thermal choice in group-housed pigs, regardless of the limitations. The inability to achieve the target temperature range (20 to 40°C) was unfortunate since we were unable to locate the lower critical temperature of ELTN pigs. Ideally, the data would have taken on more of a traditional bell curve shape to help us identify temperatures that indicate upper and lower critical temperature limits. However, for ELTN pigs the lower critical temperature was not observed due to the limitations with establishing cooler temperatures within our gradient. Unfortunately, the inability to achieve these desired temperatures was due to the limitation of our facilities during the summer months. The environmental room, where the thermal apparatuses were located, was unable to reduce the T_A enough to achieve the required lower thermal gradient. Additional limitations in our design did not provide the ideal amount of floor space for the larger pigs. A larger floor space within each thermal zone would have allowed for better spacing of both feeders and waterers, and for all animals to fit within each zone easily. Unfortunately, due to size constraints the design used was best situated for the environmental room it was placed in and allowed for all ELTS to be run simultaneously. Overall, a shift in preferred temperatures was detected even though we were unable to demonstrate a preference bell curve for all treatments. Therefore, providing pigs with much lower temperature choices than previously expected will be necessary to fully evaluate the temperature preference of 3 to 15 kg pigs.

2.5 References

Aarnink, A. J. A., J.W. Schrama, M.J.W. Heetkamp, J. Stefanowska, and T.T.T Huynh. 2006. Temperature and body weight affect fouling of pig pens. J. Anim. Sci. 84: 2224-2231. doi:10.2527/jas.2005-521.

Balsbaugh, R. K., S. E. Curtis, R. C. Meyer, and H. W. Norton. 1986. Cold resistance and environmental temperature preference in diarrheic piglets. J. Anim. Sci. 62: 315-326. doi:10.2527/jas1986.622315x.

Bench, C. J., and H. W. Gonyou. 2007. Temperature preference in piglets weaned at 12–14 days of age. Can. J. Anim. Sci. 87: 299-302. doi:10.4141/cjas06040.

Brown-Brandl, T. M., M. D. Hayes, H. Xin, J. A. Nienaber, and H. Li. 2014. Heat and moisture production of modern swine. ASHRAE Trans. 120: 469–489. doi:10.13031/ trans.57.1071.

Campbell, I. 2008. Body temperature and its regulation. Anaest. Intens. Care Med. 9: 259-263. doi:10.1016/j.mpaic.2008.04.009.

Chaffee, R. R. J., and J.C. Roberts. 1971. Temperature acclimation in birds and mammals. Ann. Review of Physiol. 33: 155-202. doi:10.1146/annurev.ph.33.030171.001103.

Collin, A., van Milgent, J., and Le Dividich, J. 2001. Modelling the effect of high, constant temperature on food intake in young growing pigs. Animal Science. 72: 519-527. doi:10.1017/S1357729800052048.

Curtis, S. 1985. Physiological response and adaptation of swine in Stress Physiology in Livestock. Vol. II: Ungulates. M. D. Yousef, ed. CRC Press, Boca Raton, FL. p. 59–65 and 129–139.

Dauncey, M. J., and D.L. Ingram. 1986. Acclimatization to warm or cold temperatures and the role of food intake. J. Thermal Bio. 11: 89-93. doi:10.1016/0306-4565(86)90025-2.

DeShazer, J., and D. Overhults. 1982. Energy demand in livestock production. In: Livestock Environment II. Proc. 2nd Int. Livest. Environ. Symp. ASAE, St. Joseph, MI. p 17-27.

Federation of Animal Science Societies. 2010. Guide for the care and use of agricultural animals in research and teaching. 3rd ed. Champaign (IL): Fed. Anim. Sci. Soc. Chap. 11.

Gaskill, B. N., C.J. Gordon, E.A. Pajor, J.R. Lucas, J.K. Davis, and J.P. Garner. 2012. Heat or insulation: behavioral titration of mouse preference for warmth or access to a nest. PloS one, 7: e32799. doi:10.1371/journal.pone.0032799.

Grafen, A., and R. Hails. 2002. Modern statistics for the life sciences. Oxford University Press, Oxford, NY. p. 155-163.

Gordon, C.J., 1993. Temperature Regulation in Laboratory Rodents. Cambridge University EPress, New York, pp. xii, 276.

Hahn, G. 1985. Managing and housing of farm animals in hot environments in Stress Physiology in Livestock. Vol II: Ungulates. M. K. Yousef, ed. CRC Press, Boca Raton, FL. p. 151–174.

Harding, E. C., N.P. Franks, and W. Wisden. 2019. The temperature dependence of sleep. Front. in Neuro. 13: 336. doi:10.3389/fnins.2019.00336.

Heldmaier, G. 1974. Cold adaptation by short daily cold exposures in the young pig. J. Appl. Physiol. 36: 163-168. doi:10.1152/jappl.1974.36.2.163.

Herpin, P., Damon, M., and Le Dividich, J. (2002). Development of thermoregulation and neonatal survival in pigs. Livestock production science. 78: 25-45. doi:10.1016/S0301-6226(02)00183-5.

Horowitz, M. 2007. Heat acclimation and cross-tolerance against novel stressors: genomic–physiological linkage. Progress in Brain Research. 162: 373-392. doi:10.1016/S0079-6123(06)62018-9.

Hrupka, B. J., V. D. Leibbrandt, T. D. Crenshaw, and N. J. Benevenga. 2000. Effect of sensory stimuli on huddling behavior of pigs. J. Anim. Sci. 78: 592-596. doi:10.2527/2000.783592x.

Hutu, I., & Onan, G. 2019. Alternative Swine Management Systems. Academic Press. p. 62. doi:10.1016/B978-0-12-818967-2.00004-6.

Huynh, T. T. T. et al. 2005. Thermal behaviour of growing pigs in response to high temperature and humidity. Appl. Anim. Behaviour Sci. 91: 1-16. doi:10.1016/j.applanim.2004.10.020.

Johnson, J. S., M. A. Aardsma, A. W. Duttlinger, and K. R. Kpodo. 2018. Early life thermal stress: Impact on future thermotolerance, stress response, behavior, and intestinal morphology in piglets exposed to a heat stress challenge during simulated transport. J. Anim. Sci. 96: 1640-1653. doi:10.1093/jas/sky107.

Johnston, I. A., and A. F. Bennett. 2008. Animals and temperature: phenotypic and evolutionary adaptation. Cambridge University Press, Cambridge, NY. p. 265-286.

Ledgerwood D. N., Winckler C., Tucker C. B. 2010. Evaluation of data loggers, sampling intervals, and editing techniques for measuring the lying behavior of dairy cattle. J. Dairy Sci. 93*:5129–5139. doi:10.3168/jds.2009-2945.*

Kregel, K. C. 2002. Invited review: heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J. App. Physiol. 92: 2177-2186. doi:10.1152/japplphysiol.01267.2001.

Kingma, B. R., A. J. Frijns, L. Schellen, and W. D. van Marken Lichtenbelt. 2014. Beyond the classic thermoneutral zone: Including thermal comfort. Temperature (Austin) 1: 142-149. doi:10.4161/temp.29702.

Mead, R. 1990. The design of experiments: statistical principles for practical applications. Cambridge University Press, Cambridge, NY. p. 620.

Mount, L. E. 1963. The thermal insulation of the new-born pig. J. Physiol. 168: 698. doi:10.1113/jphysiol.1963.sp007216.

Mount, L. 1960. The influence of huddling and body size on the metabolic rate of the young pig. J. Ag. Sci. 55: 101-105. doi:10.1017/S0021859600021651.

Newman, J. A., J. Bergelson, and A. Grafen. 1997. Blocking factors and hypothesis tests in ecology: is your statistics text wrong? Ecology 78: 1312-1320. doi:10.1890/0012-9658(1997)078[1312:BFAHTI]2.0.CO;2.

NRC. 1981. Effects of Environment on Nutrient Requirements of Domestic Animals. Natl. Acad. Press, Washington, DC.

Pedersen, S., P. Sousa, L. Andersen, and K.H. Jensen. 2003. Thermoregulatory Behaviour of Growing–Finishing Pigs in Pens with Access to Outdoor Areas. Ag. Eng. International: CIGR Journal. 5.

Petherick, J. C. 1983. A biological basis for the design of space in livestock housing. In: Baxter, S.H., Baxter, M.R., MacCormack, J.A.D. (Eds.), Farm Animal Housing and Welfare. Martinus Nijhoff, The Hauge. p. 103-120.

Renaudeau, D., C. Anais, L. Tel, and J. L. Gourdine. 2010. Effect of temperature on thermal acclimation in growing pigs estimated using a nonlinear function. J. Anim. Sci. 88: 3715-3724. doi:10.2527/jas.2009-2169.

Robbins, L., A. R. Green-Miller, J. S. Johnson, C. Gonzales, and B. N. Gaskill. 2018. Thermocline Design for Thermal Preference Testing in Piglets. In: 10th International Livestock Environment Symposium (ILES X). p 1. American Society of Agricultural and Biological Engineers.

Signoret, J. P., B. A. Baldwin, D. Fraser, and E. S. E. Hafez. 1975. The behavior of swine. In Behaviour of Domestic Animals. ESE Hafez ed. Baillière Tindall, London, UK. p. 295-329.

Smith, N. P., C.J. Barclay, and D.S. Loiselle. 2005. The efficiency of muscle contraction. Progress in Biophys. and Mol. Bio. 88: 1-58. doi:10.1016/j.pbiomolbio.2003.11.014.

Stolba, A., and D. G. M. Woodgush. 1989. The Behavior of Pigs in a Semi-Natural Environment. Anim. Prod. 48: 419-425. doi:10.1017/S0003356100040411.

Tetievsky, A., and M. Horowitz. 2010. Posttranslational modifications in histones underlie heat acclimation-mediated cytoprotective memory. J. Appl. Physiol. 109:1552–1561. doi:10.1152/japplphysiol.00469.2010.

Terrien, J., M. Perret, and F. Aujard. 2011. Behavioral thermoregulation in mammals: a review. Front Biosci. 16: 1428-1444. doi:10.2741/3797.

Young, B. A. 1981. Cold Stress as It Affects Animal Production. J. Anim. Sci. 52: 154-163. doi:10.2527/jas1983.5761601x.

Vasdal, G., I. Mogedal, K. E. Boe, R. Kirkden, and I. L. Andersen. 2010. Piglet preference for infrared temperature and flooring. Appl. Anim. Behaviour Sci. 122: 92-97. doi:10.1016/j.applanim.2009.12.008.

Verstegen, M., A. Henken, and W. Van der Hel. 1987. Influence of some environmental, animal and feeding factors on energy metabolism in growing pigs. Energy metabolism in farm animals. Dordrecht: Springer. doi:10.1007/978-94-009-3363-7_5.

Xin, H., and Q. Zhang. 1999. Preference for lamp or mat heat by piglets at cool and warm ambient temperatures with low to high drafts. Appl. Eng. in Ag. 15: 547. doi:10.13031/2013.5816.

Zhang, Q., and H. Xin. 2001. Responses of piglets to creep heat type and location in farrowing crate. Appl. Eng. in Ag. 17: 515. doi:10.13031/2013.6467.

2.6 Tables and Figures

Table 2.1. Number of pigs and average weight exposed to early life thermal stress by sex.

Parameter	Number of groups	Average weight, kg (LSM ± SD)
ELTN		
Gilts	4	9.56 ± 2.15
Barrows	3	9.18 ± 1.59
ELCS		
Gilts	3	8.35 ± 1.35
Barrows	4	8.82 ± 1.55
ELHS		
Gilts	3	7.59 ± 1.34
Barrows	4	8.89 ± 2.60

Table 2.2. Temperatures, for the room and thermal apparatus, averaged over the course of three days.

Average room temperature, °C (Mean ± SD: RH ± SD)	**Location within the thermal apparatus**	**Average Temperature, °C (LSM ± SD)**
17 ± 1.88°C: 66.7 ± 8.08%	A	22.65 ± 0.20
	B	25.15 ± 0.12
	C	29.10 ± 0.30
	D	33.13 ± 0.45
	E	38.15 ± 0.23

Table 2.3. Ethogram used for behavioral observations.

Category	Behavior	Description
Active	Active	Pig is walking about, can be seen actively engaged with the environment or with another pig. This includes fighting or head tossing with another pig or interacting with troughs located on the long ends of the thermal apparatuses such as biting or chewing.
	Eating	Pigs head is in the feeding trough, located under heating elements (5 total), can only see back of head and ears while within in the feeding trough. All food troughs are located under the heating lamps.
	Drinking	Pigs head is in the watering trough, located opposite wall to heating elements (3 total), can only see back of head and ears while within in the watering trough. Water troughs are located on the opposite side of the thermal apparatus from the heating elements.
Inactive	Inactive	Pig is motionless and assumed to be sleeping. The animal may be inactive if sitting, standing or lying still and alert. Animal is stationary, slow and small head movements may be seen but their body is motionless.
Other	Other	Pigs' behavior cannot be determined, camera angles or glare do not allow for accurate assessment
	Defecation	Pig is stationary or in a dog-sit position, can see fecal matter being excreted

Table 2.4. Ethogram used for posture observations.

Posture	**Description**
Upright	Pigs' body is erect and top line (back) is to the camera, includes pig standing on all four hoofs on ground and dog-sitting where pig has rump on floor
Sternal Laying	Pig lies up-right with stomach and chest touching the ground, top line is facing the camera. This includes when a pig is sternal on her anterior body and lateral on her posterior body. Sternal includes the medial plane of the head and body being perpendicular to a 45-degree angle to the ceiling.
Lateral Laying	Pig lies on side with shoulder and rump touching the ground, top line is facing a wall. Medial plane of head and body are greater than 45 degrees and approximately 90 degrees to the ceiling.
Other	Any other postures or those that cannot be determined, camera angles or glare do not allow for accurate assessment. When sow is in position transition and down on front knees but stays with hind end up for a while and may still be moving about.

Table 2.5. Average end body weight by ELTS and sex (LSM ± SE) did not influence temperature preference

Parameter	BW, kg (LSM ± SE)	F	*P*-value
ELTS		0.81	0.46
ELTN	9.37 ± 0.64		
ELCS	8.58 ± 0.64		
ELHS	8.24 ± 0.64		
Sex		0.39	0.54
Gilts	8.96 ± 0.51		
Barrows	8.50 ± 0.54		

Table 2.6. Influence of parameters on temperature preference (LSM ± SE)

Parameter	**F**	***P*-value**
ELTS	1.25	0.32
Sex	1.22	0.29
End BW	2.59	0.14

Table 2.7. Peak temperature preference (°C) by ELTS, behavior, and posture (LSM ± SE)

Parameter*	**Peak temperature preference, °C**	**Average time spent at peak temperature, %**
ELTS		
ELTN*	23.8	7.71
ELCS+	26.0	8.68
ELHS*+	25.6	6.59
Behavior		
Inactive	24.6	24.9
Active	25.8	2.97
Posture		
Upright+	25.6	23.11
Sternal*	24.6 and 24.8	23.98
Lateral*	24.4	44.59

* Different symbols denote a significant difference in peak temperature preference between parameters Tukey tests ($P < 0.01$).

Table 2.8. Latency to empty feeders (min) based on interaction effects (LSM ± SE)

Parameter	Interaction parameter	Time to empty feeders, min
Weight*	Sex	
Above	Barrow	1378 ± 31
	Gilt	1315 ± 40
Below	Barrow	1279 ± 50
	Gilt	1397 ± 40
ELTS		
ELTN	Barrow	1272 ± 53
	Gilt	1354 ± 43
ELCS	Barrow	1291 ± 50
	Gilt	1418 ± 53
ELHS	Barrow	1422 ± 50
	Gilt	1297 ± 53

*Weight category was calculated by the average weight of piglets' post testing and separated by ELTS

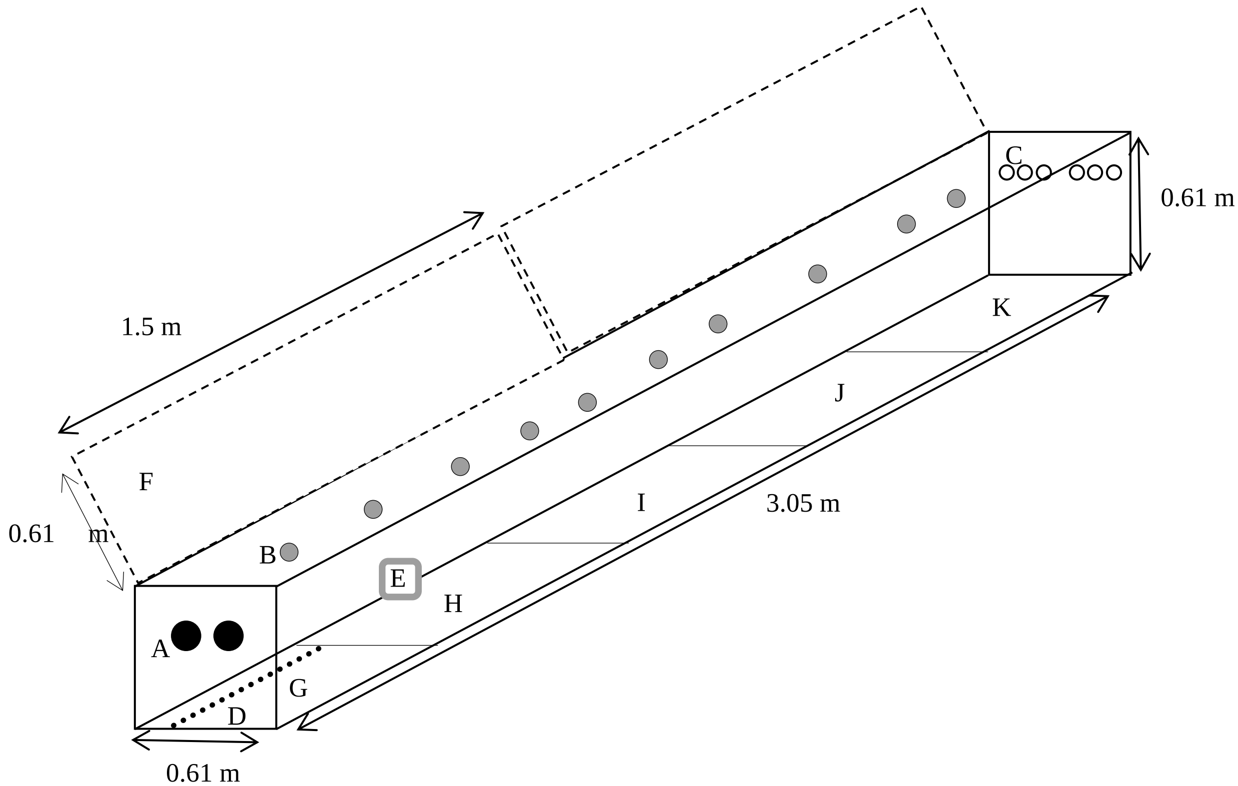

Figure 2.1. Right: photograph of a single thermal apparatus. Legend: A) 2 computer fans were used to push cool air into the apparatus; B) heating elements placed at different intervals to warm the air as it moved down the apparatus ; C) 6 exhaust holes; D) the dotted line indicates a 0.64 m spacing between solid lines used to create thermal zones where piglet location was documented; E) example of height of container used for water and feed (see Figure 2 for more detail about container spacing); F) two plexiglass lids covered the entire apparatus and opened upwards; G to K indicate the five thermal zones with average temperatures at: G) 23.3°C, H) 26.2°C, I) 30.1°C, J) 34.2°C, and K) 38.2°C.

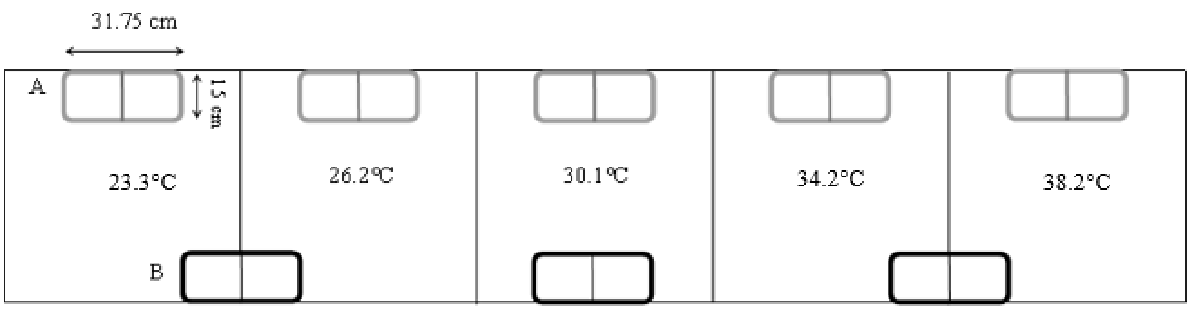

Figure 2.2. Top view of a single thermal apparatus showing average temperature per thermal zone, location of a) containers used for food (in grey) and b) those providing water (in black).

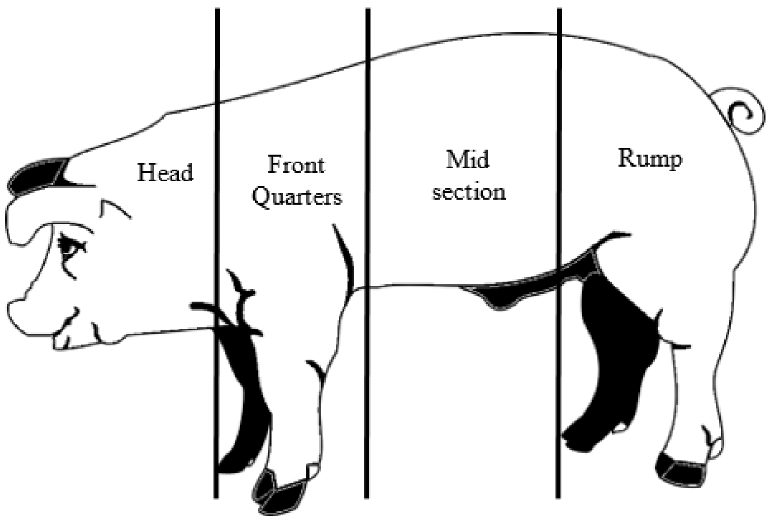

Figure 2.3. Diagram depicting sections of a pig used to assess percentage of body part located within a thermal section, each body section was equivalent to 25%. Head was considered from back of the ears to the snout, front quarters were considered back of the ears to behind the forelimbs, mid-section was from behind the forelimbs to front of the back limbs, and the rump was considered the front of the back limbs to base of the tail.

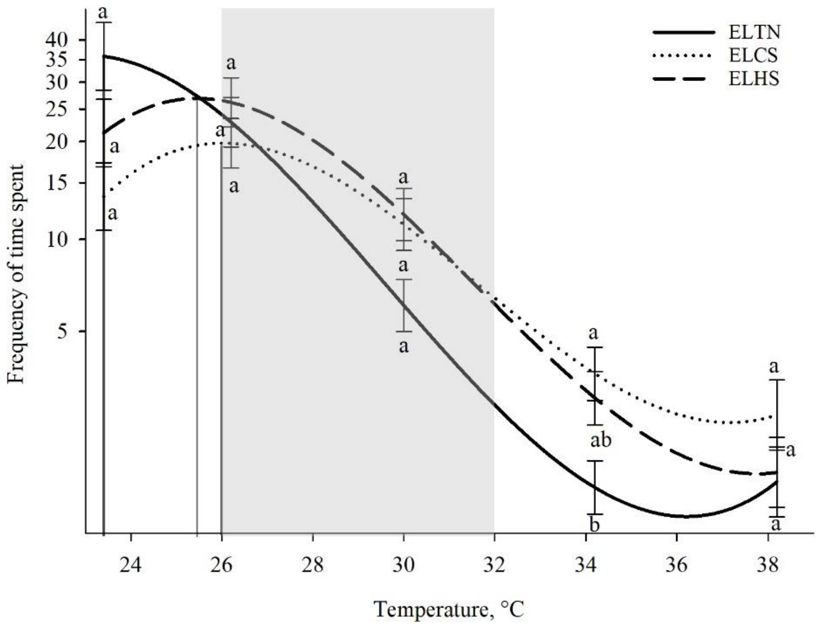

Figure 2.4. Total frequencies of time spent in different temperatures within the thermal gradient based on early life thermal stress conditions (**ELTS**). The effects of early life thermoneutral (**ELTN**), early life cold stress (**ELCS**), and early life heat stress (**ELHS**) on temperature preference. Temperature within the thermal apparatus is plotted on the x-axis and total frequencies are plotted on the y-axis as a log10 scale. Cubic peaks are indicated by vertical lines corresponding to ELTS. Standard error bars are located at the temperatures of the five thermal zones (23.3°C, 26.2°C, 30.1°C, 34.2°C, and 38.2°C) and different letters denote significant Tukey pairwise comparisons ($P < 0.01$). The gray box indicates the recommended temperatures (26 to 32°C) for piglets between 3 to 15 kg (Federation of Animal Science Societies, 2010).

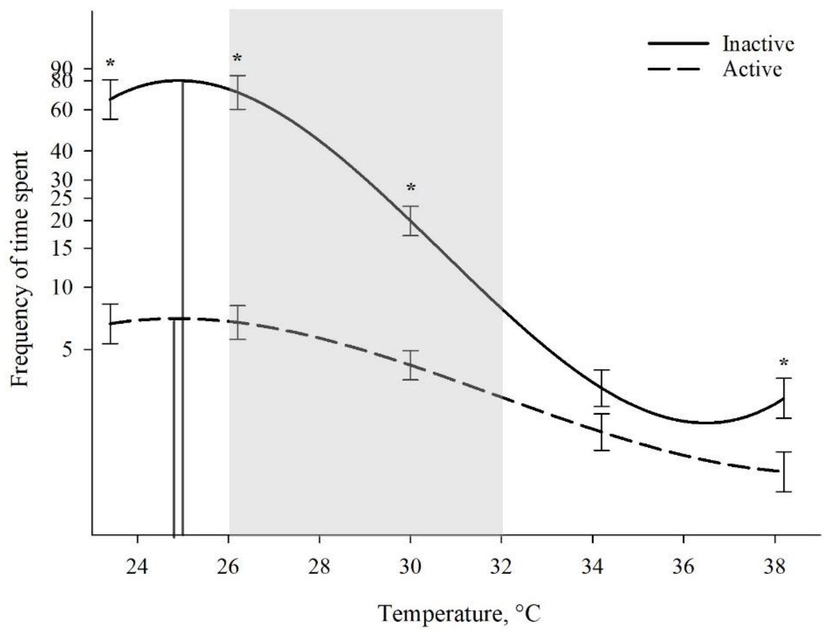

Figure 2.5. Total frequencies of time spent in different temperatures within the thermal gradient based on behavior. Data are plotted by behavior: Active and Inactive. Temperature within the thermal apparatus is plotted on the x-axis and total frequencies are plotted on the y-axis as a log10 scale. Cubic peaks are indicated by solid vertical lines. Standard error bars are located at the temperatures of the five thermal zones (23.3°C, 26.2°C, 30.1°C, 34.2°C, and 38.2°C). An asterisk indicates significant Tukey tests ($P < 0.01$). The gray box indicates the recommended temperatures (26 to 32°C) for piglets between 3 to 15 kg (Federation of Animal Science Societies, 2010).

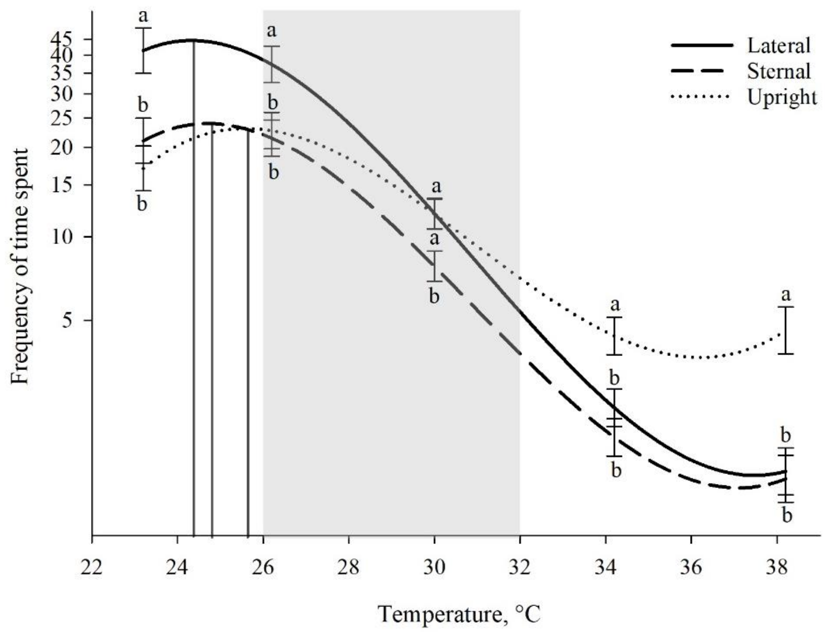

Figure 2.6. Total frequencies time spent in different temperatures within the thermal gradient based on posture. Data are plotted by postures: lateral and sternal lying, and upright. Temperature within the thermal apparatus is plotted on the x-axis and total frequencies are plotted on the y-axis as a log10 scale. Cubic peaks are indicated by solid vertical lines. Standard error bars are located at the temperatures of the five thermal zones (23.3°C, 26.2°C, 30.1°C, 34.2°C, and 38.2°C). Different letters denote significant Tukey pairwise comparisons (*P* < 0.01). The gray box indicates the recommended temperatures (26 to 32°C) for piglets between 3 to 15 kg (Federation of Animal Science Societies, 2010).

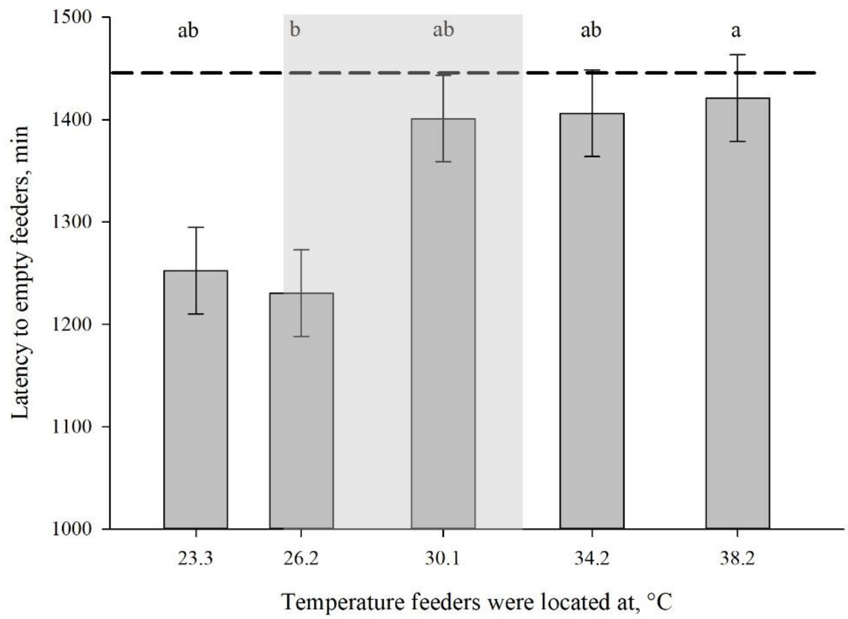

Figure 2.7. Latency to empty feeders located within the 5 thermal zones: 23.3°C, 26.2°C, 30.1°C, 34.2°C, and 38.2°C. Data presented as LSM ± SE (n=7 per ELTS treatment). Different letters denote significant Tukey pairwise comparisons (P < 0.05). Dotted line indicates the maximum amount of time pigs had to consume food (1440 minutes). The gray box indicates the recommended temperatures (26 to 32°C) for piglets between 3 to 15 kg (Federation of Animal Science Societies, 2010).

CHAPTER 3. ONE IS THE COLDEST NUMBER: DETERMINING HOW GROUP SIZE AND BODY WEIGHT AFFECTS THERMAL PREFERENCE IN WEANED PIGS (3 TO 15 KG)

Housing pigs within their thermal comfort zone maximizes productivity and performance. However, fundamental information on behavioral thermoregulatory responses of individual and group housed pigs is meager. As a gregarious species, pigs prefer to be near one another huddling. As pigs huddle together, they decrease their heat loss to the environment by decreasing surface area and increasing mass. Additionally, pigs gain weight rapidly as they age and as an individual's grows their ability to withstand lower temperatures increases. Thus, based on previous knowledge on heat loss and how surface area and mass affect it, we hypothesized that group size would alter pig thermal preference and that this would vary by body weight (**BW**). Thirty-six groups of pigs (n = 2) were tested in a factorial design based on group size (1, 2, or 4) and weight category (small: 5.20 ± 1.15 kg; medium: 8.79 ± 1.30 kg; and large: 13.95 ± 1.26 kg) in both sexes. Treatment groups were placed inside a thermal gradient (4.6 m x 0.9 m x 0.9 m; L x W x H) that ranged in temperature from 18 to 30°C. Pigs could habituate to the gradient for 24 h. The following 24h testing period was continuously video recorded and each pigs' location during inactivity (~70% daily budget) within the thermal apparatus was recorded every 10-min via instantaneous scan sampling. Data were analyzed using a GLM and Log10+0.001 transformed for normality. Tukey tests and Bonferroni corrected custom tests were used for post-hoc comparisons. Peak temperature preference was determined by the maximum amount of time spent at a specific temperature. Both group size ($P = 0.001$) and weight category ($P < 0.001$) influenced the thermal location pigs were observed in. Individual pigs preferred 30.31°C which differed from a group of 2 (20.0°C: $P = 0.003$) and 4 pigs (20.0°C: $P < 0.001$). The peak temperature preference of the small pigs (30.2°C) differed from the large pigs (20.0°C: $P < 0.001$) but did not differ from the medium sized pigs (28.4°C: $P > 0.05$). Overall, a heavier BW and larger group size resulted in cooler thermal preferences. With more individuals, pigs selected a cooler temperature indicating their thermal comfort; however, an individual is unable to conserve nearly as much heat and selects ambient temperature to reduce heat loss

3.1 Introduction

Pigs achieve thermal balance through a combination of physiological and behavioral processes; including panting, huddling and thermotaxis (Glancy et al., 2015; Gordon, 1993). Pigs will seek out their preferred ambient temperature (**T_A**) where they will not have to utilize any physiological mechanisms for thermoregulation and there is minimal heat exchange between the animal and its environment (Gordon, 1993). Heat loss is affected by the thermal gradient between the animal, the T_A, and its thermal conductance (Heldmair et al., 2004). Overall, thermal conductance is dependent on both the total surface area of the animal exposed to its environment and its insulation properties (Gilbert et al., 2010). The surface area to mass ratio will vary among animals depending on their body weight (**BW**). However, as the mass of a body increases, the surface area does not increase proportionately (White and Seymour, 2003). Thus, the surface to mass ratio is higher for smaller pigs compared to heavier pigs (White and Seymour, 2003).

Not only does BW affect the surface area to mass ratio, but behavior, such as huddling, can alter the other side of the ratio. When exposed to cold stress (**CS**), pigs will huddle in a close-packed group, reducing the amount of an individual's surface area exposed to the environment. Reducing the surface area exposure consequently decreases the amount of heat loss (Mount, 1960). Unfortunately, the benefits of huddling do not linearly increase with more individuals. There is a critical number at which the addition of more individuals to the huddle will provide negligible benefits (Canals et al., 1989).

The surface area to mass ratio of an individual, as described above, directly affects heat loss and conservation. Thus, these factors will affect an animal's *thermopreferrendum*, or temperature where heat loss equals heat production (Gordon, 1993). Thus, an animal will choose a temperature where it does not need to use any major physiological mechanisms to heat or cool itself. The thermal preferences of piglets have been previously studied, however these studies have focused on the choices of either individual piglets (Baldwin and Ingram, 1967 and 1968; Mount, 1960) or extremely young and lightweight piglets (20 to 72 h old: Balsbaugh et al., 1986; Vasdal et al., 2010). While this information is important for understanding basic thermoregulatory processes and choice, we do not know the thermal needs of post-weaned pigs and ignores that they are extremely gregarious and in a production setting, are typically housed in groups.

An understanding of how group size (surface area exposure) and BW (mass) affect the temperature preference of young pigs would aid in the development of temperature recommendations for both producers and researchers. Thus, the purpose of the current study was to determine the thermal preference of young pigs in various group sizes and weight categories. We hypothesized that group size would alter thermal preference and that this would vary by BW. Specifically, surface area to mass ratio would be reduced in bigger groups and with heavier pigs, thus reducing overall heat loss and resulting in cooler temperature preferences.

3.2 Methods and Materials

All procedures involving animal use were approved by the Institutional Animal Care and Use Committee at Purdue University (protocol # 1901001844), and animal care and use standards were based upon the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (referred to as the *Ag Guide* from hereon; Federation of Animal Science Societies, 2010.

3.2.1 Animals and housing

Pigs [Duroc x (Landrace x Yorkshire)] were weaned at 20.0 ± 1.3 d of age and housed in nursery room 1 at the Purdue University Animal Science Research Education Center (West Lafayette, IN US). A nursery pen (0.95 m x 1.43 m) held up to 8 pigs; therefore, pigs were housed single-sex and within their randomly assigned treatment group. The nursery room received natural lighting via windows (15:9 light:dark) and the temperature was held at 25 ± 5.8°C with 58.1 ± 10.5% RH. Pigs were given access to food and water ad libitum. Temperature preference testing began on March 15, 2019 (26 d ± 1.3 of age; 6.84 ± 0.19 kg BW) and ran until April 23, 2019 (39 d ± 6.5 of age; 9.71 ± 1.62 kg BW). Body weight was documented before weaning and pigs were randomly assigned into one of three group size categories (1, 2 or 4 pigs per group) and were blocked based on their BW (small: 5.20 ± 1.15 kg; medium: 8.79 ± 1.30 kg; and large: 13.95 ± 1.26 kg). Pigs were re-weighed before testing to ensure pigs were appropriately assigned to each weight category.

3.2.2 Thermal apparatus

The thermal apparatus temperatures were designed based on the *Ag Guide* recommendations for thermal conditions of pigs weighing between 3 to 15 kg (FASS, 2010). The thermal apparatus contained a gradient between 18.2 ± 0.3°C to 30.3 ± 0.5°C (Fig. 3.1). The thermal apparatus was designed to allow pigs to freely walk between and choose between a range of temperatures which encompassed temperatures around the recommendations in the *Ag Guide* (26 to 32°C) to determine if a shift in thermal preference can be observed. The thermal apparatus measured 4.57 m x 0.91 m x 0.91m (L x W x H; Fig. 3.1). Pigs had access to water from waterers (AquaChief™, Hog Slat, Inc. NC, US) and food provided in goat feeders (14.2 L: 15.88 cm x 8.20 cm x 7.32 cm) ad libitum. When goat feeders emptied, it was resupplied by gravity from a clear PVC pipe above the feeder (5.08 cm diameter, 30.48 cm length). These feeders and waterers were placed every 0.76 m, such that each thermal zone contained one feeder and one waterer (Fig. 3.2).

The thermal apparatus had a Plexiglas lid (MFG-Acrylic 1.52 m x 1.91 cm x 0.61 m: Meyer Plastics, Inc., Lafayette, IN, USA) to allow the pigs to be video recorded. The environmental room lights were set on a timer with a 12:12 light:dark cycle (light on at 0800 and off at 2000 h). The temperature gradient was created by heating elements (100 W and 150 W reptile ceramic infrared brooder bulbs, Aiicioo, Shenzhen, China) that added incremental amounts of heat as the air moved down the gradient, pulled by exhaust fans. The conditioning box (0.61 m x 0.61 m x 0.46 m, LxWxH; Fig. 3.3) was located above the two thermal apparatuses and moved air into the gradient at the cool end through ducting.

A portable air conditioning unit (24,500 BTU, LG Electronics, Seoul, South Korea) was wired to a CoolBot (Store It Cold LLC, Florida, US) which allowed for the air conditioning unit to circulate 15 ± 0.81°C air into thermal apparatuses below. Two eight-inch duct fans (99.1 L/s airflow, Suncourt Inc. Iowa, US) per thermal apparatus were used to pull the air from the conditioning box into the thermal apparatuses to create the temperature gradient. To avoid any aversion of the pigs to airflow speed, both chambers maintained a low velocity airflow (Alnor air velocity meter, AVM410 FLW Inc., CA; range of 0 to 20 m/s with accuracy of ±5% of reading or ± 0.025 m/s with 0.01 m/s resolution). The temperature was monitored using data loggers (HOBO Data Logger; U12-013, Onset Computer Corporation, MA USA; temperature range of -20°C to 70°C with accuracy of ± 0.35°C and RH range 5% to 95% with accuracy of ± 2.5% to max 3.5%) with temperature probes to assure that the 2 apparatuses were as identical as possible.

3.2.3 Experimental design

Mead's resource equation was used *a priori* to determine the number of groups needed for the 3-weight category x 3 group size x 2 sex factorial design (Mead, 1990). Eighty-four nursery pigs [Duroc x (York x Landrace)] were randomly selected (via random integer generator, random.org) to be tested as either an individual, in a group of 2, or 4 pigs (table 3.1). In addition to this, pigs were blocked by three different weight categories: small (5.20 ± 1.15 kg); medium (8.79 ± 1.30 kg); or large (13.95 ± 1.26 kg).

For temperature preference testing, pigs were transported in a cart approximately 91 meters from the nursery to the environmental room. For each temperature preference testing session, only 2 groups could be run at a given time, thus one pig of each sex was randomly selected and simultaneously tested in 1 of 2 thermal apparatuses located within the environmental room. Group size and weight category were balanced across testing runs and apparatuses. The pigs had 24 h to acclimate to the thermal gradient and environmental room. During both acclimation and temperature preference testing, pigs were able to explore the entirety of the thermal apparatus. Each apparatus was cleaned in between acclimation, temperature preference testing, and subsequent testing groups. Between acclimation and testing, pigs were removed from the thermal apparatus and placed in stalls within the same environmental room for approximately 2 h to clean and re-establish the thermal gradient. Waste was removed with a pressure washer and the apparatus floors and walls were disinfected (LYSOL disinfectant all-purpose cleaner, Reckitt Benckiser LLC, NJ, US). After cleaning, the thermal gradient was stable when 3 readings, measured every 15 min, did not vary by more than 0.2°C. Pigs were then returned to their assigned thermal apparatus. During experimental set-up and testing, researchers were not blinded to the pigs' treatments. Due to the nature of this study, only weight category and sex were blinded to video coders.

3.3.3.1 Behavior and posture by location

Pigs were continuously video recorded during the 24 h testing period for behavior, location, and posture data collection. Infrared cameras (Sony Corporation, Tokyo, Japan) provided both day and night video which was recoded with video surveillance software (GeoVision, Taiwan). The scan interval used for recording data from video was determined by comparing different sampling intervals (5, 10, 15, 20, 25, 30 min) based on the frequency of time spent at each location. Data from each subsample were compared pairwise and considered to accurately estimate the behavior or posture if the intervals were not significantly different from each other compared to the 5 min interval (Ledgerwood et al., 2010). Based on this data, a 10-minute sampling interval was selected for this study. Pig location in a thermal zone, behavior (table 3.2), and posture (table 3.3) were recorded using instantaneous scan samples every 10 min. If pigs were observed in more than one thermal zone (location), the percentage of the pig in each zone was documented in 25% increments (head, front quarter, mid-section and rump; Fig. 3.4).

The proportion of behaviors was calculated for each group of pigs by counting the total number of times each behavior category was observed in each location per day and totaled per group and divided by the total number of observations. This calculation was repeated for posture. Although other behaviors were coded, only inactive behavior was analyzed since pigs spent approximately 70% of their time performing this behavior.

3.2.4 Analyses

All analyses were performed using the PROC MIXED (GLM) procedure in SAS 9.4 (SAS Institute INC., Cary, NC). The assumptions of the GLM (normality of error, homogeneity of variance, and linearity) were confirmed post-hoc, and data were transformed when necessary to meet these assumptions (Grafen and Hails, 2002). The threshold for significance $P < 0.05$ was used and Bonferroni corrected where applicable.

A cubic regression model was performed on the proportion of time for both posture and behavior data. This was then transformed where necessary to meet the assumptions of a GLM. To avoid pseudoreplication and accommodate repeated measures, analyses were blocked by the group of pigs nested within sex, group size and weight category. Pig group was treated as a fixed effect, as there is no meaningful wider population of pigs representing the group size and weight category combinations, to which the results could pertain (Newman et al., 1997).

3.2.5 Behavior by location

The behavior analysis only included data while pigs were inactive. Main effects and second order interactions of sex, group size, weight category, and location were tested with a cubic variable of location. The three-way interaction between group size, weight categories and temperature were also included in this model. The cubic curve from the final model above was generated in 0.2°C increments starting with the coldest thermal zone temperature (18.2°C) and increasing to the warmest temperature (30.4°C). The peak temperature preference was identified by determining the location (temperature) where pigs spent most of their time based on the regression model. The temperature preference range was then calculated from the peak temperature ± SE. In each thermal zone, Tukey tests were used to determine LSM differences within group size and weight category. Since Tukey tests were run 5 times (for each thermal zone), the alpha was Bonferroni corrected for the multiple tests ($\alpha = 0.05/5 = 0.01$).

3.2.6 Posture by location

Posture data were split into two separate models. The first model excluded individually tested pigs and evaluated huddling behavior only. This was done since individual pigs are incapable of performing huddling. This analysis was originally run as a cubic regression model but was reduced to a linear model, since quadratic and cubic terms were not significant. Main effects and second order interactions of sex, group size, weight category, and location were tested. In addition, the three-way interaction of group size, weight category, and temperature was also evaluated. Data were square root transformed to meet the assumptions of a GLM. The second posture model included all group size treatments and analyzed all postures except for huddling. A cubic regression model was used with data log10+0.001 transformed to meet the assumptions of a GLM.

3.3 Results

3.3.1 Behavior by location

During times of inactivity, group size ($P = 0.001$) influenced where pigs spent their time (table 3.4). Individual pigs preferred a warmer peak temperature (30.2°C) compared to pigs tested in a group of 2 ($F_{1,127} = 10.95$; $P = 0.001$; 20.2°C) and 4 ($F_{1,127} = 9.73$; $P = 0.002$; 20.0°C, table 3.5). No differences in peak temperature preference was observed between pigs tested in a group of 2 compared to a group of 4 ($F_{1,127} = 0.04$; $P = 0.852$, Fig. 3.5).

Pigs tested in a group of 2 had a temperature preference range of 18.8°C to 21.0°C and groups of 4 preferred 18.8°C to 21.2°C (table 3.5). No preferred temperature range could be calculated for pigs tested individually because individual piglets do not demonstrate a peak temperature nor a preferred temperature range (Fig. 3.5). Individual pigs spent less time in cooler thermal zones (18.2°C and 21.5°C) compared to pigs tested in a group of 2 (P = 0.002 and < 0.001, respectively) and 4 (P = 0.002 and 0.004, respectively; Fig. 3.5). Individual pigs also spent more time at 30.31°C compared to those tested as a group of 2 and 4 (P's $\leq$ 0.001; Fig. 3.5). No difference between group size was observed at 24.8°C, nor was there any difference observed between pigs tested as a group of 2 and 4 at any thermal zone (Fig. 3.5).

In addition to group size, during times of inactivity weight category (P < 0.001) influenced where pigs spent their time (table 3.4). Small weight category pigs preferred a warmer temperature (30.2°C) than those in the large weight category (20.0°C: $F_{1,127}$ = 20.62; P < 0.001) but did not differ from the medium category pigs (28.4°C; table 3.5). Large weight category pigs' peak thermal preference did differ from the medium category pigs ($F_{1,127}$ = 24.26; P < 0.001; table 3.5). Large weight category pigs spent more time in the 18.2°C and 21.5°C zones compared to both small (P = 0.015 and < 0.001, respectively) and medium weight category pigs (P = 0.008 and < 0.001, respectively; Fig. 3.6). Medium and small weight category pigs spent more time in the 27.3 and 30.3°C zones compared to pigs in the large weight category ($P \leq$ 0.001 for all comparisons; Fig. 3.6). No difference between any weight category at 24.8°C or between small and medium weight category pigs at any thermal zone (Fig. 3.6).

3.3.2 Posture by location

A linear relationship was found between temperature and the percent of observations where pigs were observed huddling; where huddling decreased as temperatures increased (P = 0.009; table 3.6).

When looking at all postures, save for huddling, there was a three-way interaction observed between weight category, posture and the time spent in thermal zones (P < 0.001; table 3.7). While in the lateral posture, small and medium pigs were observed most at a warmer temperature (30.2°C and 29.4°C, respectively) compared to large pigs (20.6°C; $F_{1,459}$ = 6.70 and 14.80; *P's* $\leq$ 0.001, respectively, table 3.5). In comparison to small and medium pigs, large pigs spent more time lying laterally in the 18.2°C and 21.5°C thermal zones (*P's* < 0.001; Fig. 3.7A). Large pigs also spent less time at the warmest thermal zones (27.3°C and 30.3°C) compared to both medium ($P < 0.001$) and small pigs ($P = 0.031$ and $P < 0.001$, respectively) while in lateral position (Fig. 3.7A). No differences between small and medium pigs were observed at any thermal zone and no difference between any weight category while in lateral posture was observed at 24.8°C (Fig. 3.7A).

While in the sternal posture, small and medium pigs were observed at the same peak temperature preference (30.2°C) compared to large pigs (19.6°C; $F_{1,459} = 8.93$ and 4.37; $P = 0.037$ and 0.003, respectively, table 5). No differences in peak thermal preference was observed between medium and small weight category pigs while in the sternal position (Fig 3.7B). While in the sternal posture, large pigs spent more time at the cooler thermal zones (18.2°C and 21.5°C) compared to both small and medium pigs ($P < 0.001$; Fig. 3.7B). Large pigs also spent less time at the warmest thermal zones (27.3°C and 30.3°C) compared to both medium and small pigs ($P < 0.001$) in the sternal position (Fig. 3.7B). No differences between small and medium pigs were observed at any thermal zone and no difference between any weight category while in lateral posture was observed at 24.8°C (Fig. 3.7A).

Finally, while in the upright posture, small and medium weight category pigs were observed at the same peak temperature of 30.2°C, both were different compared to large pigs' peak preference of 19.8°C ($F_{1,459} = 35.25$ and 31.10; $P < 0.001$, respectively, table 3.5). While in the upright posture large pigs spent the most time at 21.5°C compared to both small ($P = 0.038$) and medium pigs ($P = 0.033$; Fig. 3.7C). No differences were observed between small and medium pigs at any thermal zone while in the upright posture and no differences were observed at 18.2°C, 24.8°C, 27.3°C, and 30.3°C between any weight category (Fig. 3.7C). Additionally, when looking at posture there was an interaction between group size, weight category, and time spent in various temperatures, regardless of posture ($P < 0.001$; table 3.7).

3.4 Discussion

The objective of this study was to understand how the thermal preference of pigs was altered based on group size and BW. Group size allows pigs to gather in larger huddles, which reduces the surface area to mass ratio and can result in reduced heat loss, energy conservation, and the generation of a localized heat source (Vickery and Miller, 1984). In addition, animals' exchange heat with their environment across their body surfaces, thus smaller animals tend to lose heat to a cooler environment faster than heavier animals. Because of this, smaller animals would prefer warmer temperatures to maintain euthermia. Thus, this study hypothesized that group size would alter thermal preference and that this would vary by BW.

As predicted, pigs tested individually preferred warmer T_A than pigs tested in a group of 2 or 4. The peak temperature preference of individually tested pigs was 10.0°C warmer than those group housed. A group of pigs have the added benefit of huddling which reduces surface area exposure to the ambient temperature. Several studies in rodents indicate the energetic benefits of huddling as a function of group size (Martin et al., 1980; Fedyk, 1971). When exposed to 5°C, huddling reduces mean energy expenditure by 31% for rodents housed in groups of 2 and 5 compared to an individual, respectively. Like mice, our data supports this basic phenomenon where group housed pigs (2 or 4/group) preferred cooler temperatures than a solitary pig. While clear results appear between an individual animal and those house in a group, the results indicate there is no statistical difference in preference between a group of 2 or 4 pigs. However, this could be due to the thermal zones not being refined enough, over the large range of temperatures tested, to identify these subtle differences, if they exist.

In addition to group size altering thermal preference, weight category influenced where pigs spent their time. As predicted, smaller pigs preferred warmer temperatures compared to large weight category pigs. The peak temperature preference of smaller pigs was 1.8°C and 10.2°C warmer than medium and large weight category pigs, respectively. Lighter weight animals have a larger surface area to mass ratio, which enables them to lose heat more efficiently but also requires a higher energy requirements per unit of body mass compared to heavier individuals (Roverud and Chappell, 1991). Thus, a smaller animal would prefer a warmer temperature, to minimize heat loss, compared to a larger individual (Roverud and Chappell, 1991); which is what was observed in this study. This data indicates that as pigs age and gain weight, the nursery room temperature could be reduced periodically to stay within the growing pig's TCZ. Previous research has suggested lowering T_A for nursery pigs as they age with a 0.5 to 0.6°C decrease per week (Bench and Gonyou, 2007). Even though smaller pigs displayed a clear preference for warmer temperatures, this is likely negated by being housed in groups (there was no significant interaction between our treatment variables), which is common practice on farm. Perhaps a better set of data that could inform nursery room recommendations would be to evaluate the thermal preference of common group sizes used on farm and evaluate their preferences periodically while within this age range.

Though initially included in our statistical model, there was no interaction between weight category, group size, and temperature as we had predicted when looking at pigs during times of inactivity. Anecdotally, individually tested pigs in the small weight category appeared to display escape behaviors (i.e. jumping at walls) during the acclimation phase whereas large weight pigs did not demonstrate this behavior as much. This could be indicative of social stress, with smaller pigs relying on social context more compared to larger weight category pigs and highlights the importance of testing thermal preference in both individual and group settings. Previous research on thermal preference in pigs has focused on the regulatory capacities of an individual (Balsbaugh et al., 1986), which is likely an oversight for such a gregarious species that are usually housed in a group setting.

In addition to looking at behavioral thermoregulation, this study examined how posture altered thermal choice. Pigs typically take a sternal posture when in cooler temperatures to better conserve heat and a lateral posture is often observed when animals are within their TCZ (Mount, 1960). However, our posture data, was only significant when included in a three-way interaction between posture, weight category, and thermal preference. While our data indicates small and medium weight category piglets chose the warmest temperature possible in the thermal gradient, regardless of posture, we don't know if they would have chosen a warmer temperature if it were an option. Though we cannot determined a true peak temperature preference nor preference range for these weight category pigs, this data indicates they preferred warmer temperature. Smaller wight category pigs appeared to go to both extremes resulting in a soft curve rather than a bell curve we would expect to see. These pigs would go to the cold end and switch to the warm end constantly.

The present study expands our knowledge of pig thermal preference and how this is altered by weight and group size. It should be noted that the gradient did not allow for observing the thermal preference of individual and small weight category pigs. To account for this the apparatus would need to be elongated but due to facility constraints this was not possible. Further, food was not measured due to pigs knocking food out of the bowls and in some occasions, though rare, removing the feeder from the wall. It would be of interest for future studies to look at average daily gain and food intake. Though not part of the original purpose of this study, it is worth noting that the temperature preference range of pigs in this study are lower than the recommendations found in the *Ag Guide* (26 to 32°C; FASS, 2020). The thermal recommendations in the *Ag Guide* were based on research conducted over 30 years ago (NRC, 1981; DeShazer and Overhults, 1982; Curtis, 1985; Hahn, 1985). Since the development of these recommendations, genetic selection has focused on lean-growth, which creates an animal with a higher metabolic heat production. This increased heat production could be the reason for the lower preferred temperatures found in this study, especially for heavier weights. As selection for specific traits is likely to change in the future, thermal preference should be evaluated regularly to help avoid thermal stress and improve pig welfare.

3.5 References

Baldwin, B., and D. Ingram. 1967. Behavioural thermoregulation in pigs. Physiology & Behavior 2: 15-16. doi:10.1016/0031-9384(67)90005-4.

Balsbaugh, R. K., S. E. Curtis, R. C. Meyer, and H. W. Norton. 1986. Cold resistance and environmental temperature preference in diarrheic piglets. J Anim Sci 62: 315-326. doi:10.2527/jas1986.622315x.

Bench, C. J., and H. W. Gonyou. 2007. Temperature preference in piglets weaned at 12–14 days of age. Canadian journal of animal science. 87:299–302.

Canals, M., M. Rosenmann, and F. Bozinovic. 1989. Energetics and geometry of huddling in small mammals. Journal of theoretical Biology 141: 181-189. doi:10.1016/S0022-5193(89)80016-5.

Curtis, S. 1985. Physiological response and adaptation of swine in Stress Physiology in Livestock. Vol. II: Ungulates. M. D. Yousef, ed. CRC Press, Boca Raton, FL. p. 59–65 and 129–139.

DeShazer, J., and D. Overhults. 1982. Energy demand in livestock production. In: Livestock Environment II. Proc. 2nd Int. Livest. Environ. Symp. ASAE, St. Joseph, MI. p 17-27.

Federation of Animal Science Societies. 2010. Guide for the care and use of agricultural animals in research and teaching. 3rd ed. Champaign (IL): Fed. Anim. Sci. Soc. Chap. 11.

Gilbert, C., D. McCafferty, Y. Le Maho, J.-M. Martrette, S. Giroud, S. Blanc, and A. Ancel. 2010. One for all and all for one: the energetic benefits of huddling in endotherms. Biological Reviews. 85:545–569.

Glancy, J., R. Groß, J. V. Stone, and S. P. Wilson. 2015. A self-organising model of thermoregulatory huddling. PLoS Comput Biol. 11:e1004283.

Gordon, C. J. 1993. Temperature regulation in laboratory rodents. Cambridge University Press.

Grafen, A., and R. Hails. 2002. Modern statistics for the life sciences. Oxford University Press, Oxford, NY. p. 155-163.

Hahn, G. L. 1981. Housing and management to reduce climatic impacts on livestock. J Anim Sci 52: 175-186. doi:10.2527/jas1981.521175x.

Heldmaier, G., S. Ortmann, and R. Elvert. 2004. Natural hypometabolism during hibernation and daily torpor in mammals. Respiratory physiology & neurobiology 141: 317-329. doi:10.1016/j.resp.2004.03.014.

Ledgerwood, D. N., C. Winckler, and C. B. Tucker. 2010. Evaluation of data loggers, sampling intervals, and editing techniques for measuring the lying behavior of dairy cattle. Journal of dairy science. 93:5129–5139.

Martin, R. A., M. Fiorentini, and F. Connors. 1980. Social facilitation of reduced oxygen consumption in Mus musculus and Meriones unguiculatus. Comparative Biochemistry and Physiology Part A: Physiology 65: 519-522. doi:10.1016/0300-9629(80)90072-9.

Mead, R. 1990. The design of experiments: statistical principles for practical applications. Cambridge University Press, Cambridge, NY. p. 620.

Moinard, C., C. P. Doncaster, and H. Barré. 1992. Indirect calorimetry measurements of behavioral thermoregulation in a semiaquatic social rodent, Myocastor coypus. Canadian Journal of Zoology 70: 907-911. doi:10.1139/z92-129.

Mount, L. 1960. The influence of huddling and body size on the metabolic rate of the young pig. The Journal of Agricultural Science 55: 101-105. doi:10.1017/S0021859600021651.

Mount, L. E. 1975. The assessment of thermal environment in relation to pig production. Livestock Production Science. 2:381–392.

Mount, L. E., and D. B. Stephens. 1970. The relation between body size and maximum and minimum metabolic rates in the new-born pig. The Journal of physiology. 207:417–427.

Newman, J. A., J. Bergelson, and A. Grafen. 1997. Blocking factors and hypothesis tests in ecology: is your statistics text wrong? Ecology 78: 1312-1320. doi:10.1890/0012-9658(1997)078[1312:BFAHTI]2.0.CO;2.

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Robbins, L. A., A. R. Green-Miller, J. S. Johnson, and B. N. Gaskill. 2020. Early life thermal stress: impacts on future temperature preference in weaned pigs (3 to 15 kg). Journal of animal science. 98:skaa327.

Robbins, L., A. R. Green-Miller, J. S. Johnson, C. Gonzales, and B. N. Gaskill. 2018. Thermocline Design for Thermal Preference Testing in Piglets. In: 10th International Livestock Environment Symposium (ILES X). p 1. doi:10.13031/iles.18-154.

Roverud, R. C., and M. A. Chappell. 1991. Energetic and thermoregulatory aspects of clustering behavior in the neotropical bat Noctilio albiventris. Physiological Zoology 64: 1527-1541. doi:10.1086/physzool.64.6.30158228?journalCode=physzool.

Vasdal, G., I. Mogedal, K. E. Boe, R. Kirkden, and I. L. Andersen. 2010. Piglet preference for infrared temperature and flooring. Applied Animal Behaviour Science 122: 92-97. doi:10.1016/j.applanim.2009.12.008.

Vickery, W. L., and J. S. Millar. 1984. The energetics of huddling by endotherms. Oikos: 88-93. doi:10.2307/3544249.

Weary, D.M., Appleby, M.C., and Fraser, D. 1999. Responses of piglets to early separation from the sow. Appl. Anim. Behav. Sci. 63, 289-300. doi:10.1016/S0168-1591(99)00021-0.

White, C. R., and R. S. Seymour. 2003. Mammalian basal metabolic rate is proportional to body mass2/3. Proceedings of the National Academy of Sciences 100: 4046-4049. doi:10.1073/pnas.0436428100.

3.6 Tables and Figures

Table 3.1. Number of pigs and average weight exposed by weight category and group size.

Weight category, kg (n=12)	**Group size (n=4)**	**Average weight, kg (average ±SD)**
Small (3-7)	1	5.08 ± 1.22
	2	5.15 ± 0.73
	4	5.65 ± 1.34
Medium (7.1-11)	1	9.23 ± 1.78
	2	9.23 ± 1.11
	4	9.09 ± 2.36
Large (11.1-15)	1	12.79 ± 1.32
	2	14.67 ± 0.49
	4	14.29 ± 1.15

Table 3.2. Ethogram used for behavioral observations.

Category	Behavior	Description
Active	Active	Pig is walking about, can be seen actively engaged with the environment or with another pig. This includes fighting or head tossing with another piglet or interacting with troughs located on the long ends of the thermal apparatuses such as biting or chewing.
	Eating	Pigs head is in the feeding trough, located under heating elements (5 total), can only see back of head and ears while within in the feeding trough. All food troughs are located under the heating lamps.
	Drinking	Pigs head is in the watering trough, located opposite wall to heating elements (3 total), can only see back of head and ears while within in the watering trough. Water troughs are located on the opposite side of the thermal apparatus from the heating elements.
	Defecation	Pig is stationary or in a dog-sit position, can see fecal matter being excreted
Inactive		Pig is motionless and assumed to be sleeping. The animal may be inactive if sitting, standing or lying still and alert. An animal is stationary, slow and small head movements may be seen but their body is motionless.
Other		Pigs' behavior cannot be determined, camera angles or glare do not allow for accurate assessment

Table 3.3. Ethogram used for posture observations.

Posture	**Description**
Upright	Pigs' body is erect and top line (back) is to the camera, includes piglet standing on all four hoofs on ground and dog-sitting where piglet has rump on the floor
Sternal Laying	Pig lies up-right with stomach and chest touching the ground, top line is facing the camera. This includes when a piglet is sternal on her anterior body and lateral on her posterior body. Sternal includes the medial plane of the head and body being perpendicular to a 45-degree angle to the ceiling.
Lateral Laying	Pig lies on side with shoulder and rump touching the ground, top line is facing a wall. Medial plane of head and body are greater than 45 degrees and approximately 90 degrees to the ceiling.
Huddling	Pig is in contact with another piglet, with at least 50% of their bodies are touching. Pigt can be sternal or lateral laying
Other	Any other postures or those that cannot be determined, camera angles or glare do not allow for accurate assessment. When pig is in position transition and down on front knees but stays with hind end up for a while and may still be moving about.

Table 3.4. Statistical terms included in the cubic regression model which tested for differences in the percent of observations in various locations (temperatures) while pigs were inactive

Parameters	**F Value**	***P*-value**
GroupID(Sex*GroupSize*WeightCat)	$F_{18,127} = 0.05$	1.000
Sex	$F_{1,127} = 0.00$	0.949
GroupSize	$F_{2,127} = 4.05$	*0.020
WeightCat	$F_{2,127} = 1.22$	0.298
Location	$F_{1,127} = 2.47$	0.119
Sex*GroupSize	$F_{2,127} = 0.01$	0.989
Sex*WeightCat	$F_{2,127} = 0.11$	0.900
GroupSize*WeightCat	$F_{4,127} = 0.09$	0.986
Location*GroupSize	$F_{2,127} = 6.90$	*0.001
Location*WeightCat	$F_{2,127} = 15.01$	*<0.001
GroupSize*WeightCat*Location	$F_{4,127} = 2.36$	0.057
Sex*GroupSize*WeightCat	$F_{4,127} = 0.24$	0.914
Location*Location	$F_{1,127} = 1.98$	0.162
Location*Location*GroupSize	$F_{2,127} = 4.00$	*0.021
Location*Location*WeightCat	$F_{2,127} = 1.48$	0.232
Location*Location*Location	$F_{1,127} = 0.85$	0.357
Location*Location*Location*WeightCat	$F_{2,127} = 5.23$	*0.007

* denote a significant difference ($P < 0.05$).

Table 3.5. Peak temperature preference, °C by group size and weight category for inactive behaviors and posture (LSM ± SE) based on regression formula

	Parameter	**Peak temperature preference, °C**	**Temperature preference range, °C**
Behavior Data	Group Size		
	1	30.2*	N/A
	2	20.2+	18.6-21.0
	4	20.2+	18.6-21.2
	Weight Category		
	Small	30.2*	N/A
	Medium	28.4*	26.2-29.6
	Large	20.0+	19.0-21.0
Posture Data	Posture by Weight Category		
	Upright		
	Small	30.2¶	N/A
	Medium	30.2¶	N/A
	Large	19.8§	19.0-20.6
	Sternal		
	Small	30.2¶	N/A
	Medium	30.2¶	N/A
	Large	19.6§	18.8-20.6
	Lateral		
	Small	30.2¶	N/A
	Medium	29.4¶	28.4-30.2
	Large	20.6§	19.2-21.8
	Huddling	18.2	18.2-18.8

Different symbols denote a significant difference in peak temperature preference between parameters using Bonferroni corrected planned comparisons.

Table 3.6. Statistical terms included in the model which tested for differences in the percent of observations in various locations (temperatures) while pigs were huddling, groups tested as individuals has been excluded

Parameters	**F Value**	***P*-value**
GroupID(Sex*GroupSize*WeightCat)	$F_{12,90} = 0.23$	0.996
Sex	$F_{1,90} = 2.08$	0.152
GroupSize	$F_{1,90} = 0.28$	0.599
WeightCat	$F_{2,90} = 2.08$	0.131
Location	$F_{1,90} = 7.09$	*0.009
Sex*GroupSize	$F_{1,90} = 0.00$	0.959
Sex*WeightCat	$F_{2,90} = 1.76$	0.178
GroupSize*WeightCat	$F_{2,90} = 0.28$	0.759
Location*GroupSize	$F_{2,90} = 0.58$	0.447
Location*WeightCat	$F_{2,90} = 2.71$	0.072
Sex*GroupSize*WeightCat	$F_{1,90} = 0.24$	0.7865
GroupSize*WeightCat*Location	$F_{1,90} = 0.19$	0.827

* denote a significant difference ($P < 0.05$).

Table 3.7. Statistical terms included in the model which tested for differences in the percent of observations in various locations (temperatures) while piglets were in various postures, huddling has been excluded

Parameters	**F Value**	***P*-value**
GroupID(Sex*GroupSize*WeightCat)	$F_{18,459} = 0.63$	0.875
Sex	$F_{1,459} = 5.43$	*0.020
GroupSize	$F_{2,459} = 10.40$	*<0.001
WeightCat	$F_{2,459} = 7.37$	*0.001
Posture	$F_{2,459} = 47.31$	*<0.001
Location	$F_{1,459} = 12.17$	*0.001
Sex*GroupSize	$F_{2,459} = 4.73$	*0.009
Sex*WeightCat	$F_{2,459} = 0.09$	0.916
GroupSize*WeightCat	$F_{4,459} = 1.14$	0.339
GroupSize*Posture	$F_{4,459} = 0.39$	0.816
GroupSize*Location	$F_{4,459} = 19.53$	*<0.001
WeigtCat*Posture	$F_{4,459} = 1.33$	0.257
WeightCat*Location	$F_{2,459} = 36.44$	*<0.001
Posture*Location	$F_{2,459} = 1.62$	0.200
Sex*GroupSize*WeightCat	$F_{2,459} = 1.89$	0.111
GroupSize*WeightCat*Location	$F_{4,459} = 8.30$	*<0.001
GroupSize*Posture*Location	$F_{4,459} = 1.61$	0.170
WeightCat*Posture*Location	$F_{4,459} = 11.52$	*<0.001
GroupSzie*WeightCat*Posture	$F_{8,459} = 1.24$	0.272
Location*Location	$F_{1,459} = 9.47$	*0.002
Location*Location*GroupSize	$F_{2,459} = 11.52$	*<0.001
Location*Location*WeightCat	$F_{2,459} = 4.50$	*0.012
Location*Location*Location	$F_{1,459} = 6.26$	*0.013
Location*Location*Location*WeightCat	$F_{2,459} = 9.81$	*<0.001

* denote a significant difference ($P < 0.05$).

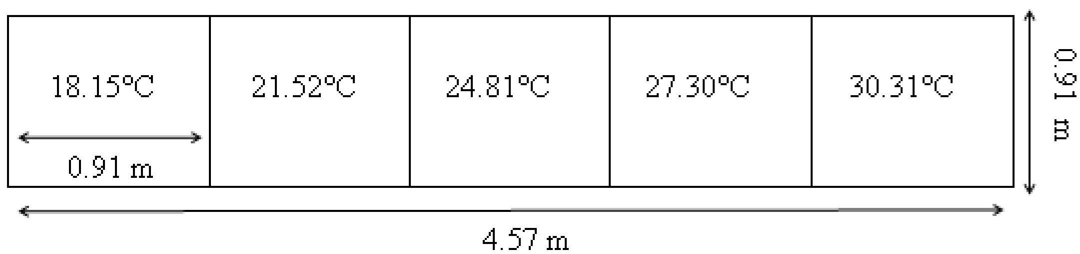

Figure 3.1. Scale drawing of one of two thermal apparatuses with thermal zones. Data loggers were placed 0.40 m and were in the middle of each thermal zone to calculate the average temperature of each zone.

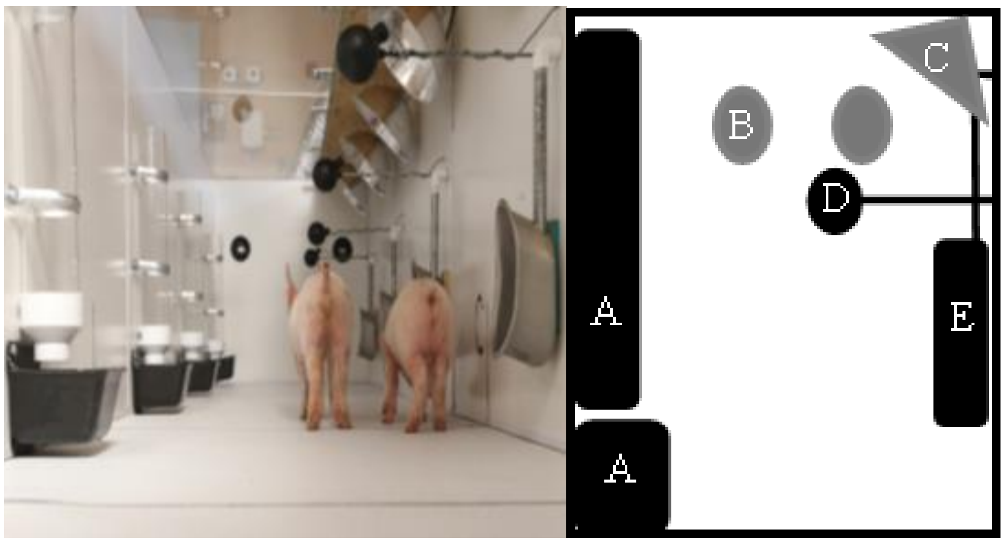

Figure 3.2. Image displayed of one thermal apparatus with a group of 2 piglets. On the left a diagram depicting what was inside the thermal apparatus: A) Feeders with PVC pipe to allow gravity feeding found within each thermal zone (0.40 m apart), B) two computer fans that ducted cold air into the thermal apparatus located 0.60 m from the ground, C) heating elements inside a heat guard, D) 3” black globe with datalogger probe measuring temperature every 15 min located in the middle of each thermal zone 0.40 m apart, E) waterers with ad lib access located 0.40 m apart and within each thermal zone. All feeders, waterers and black globes totaled 5, one per thermal zone.

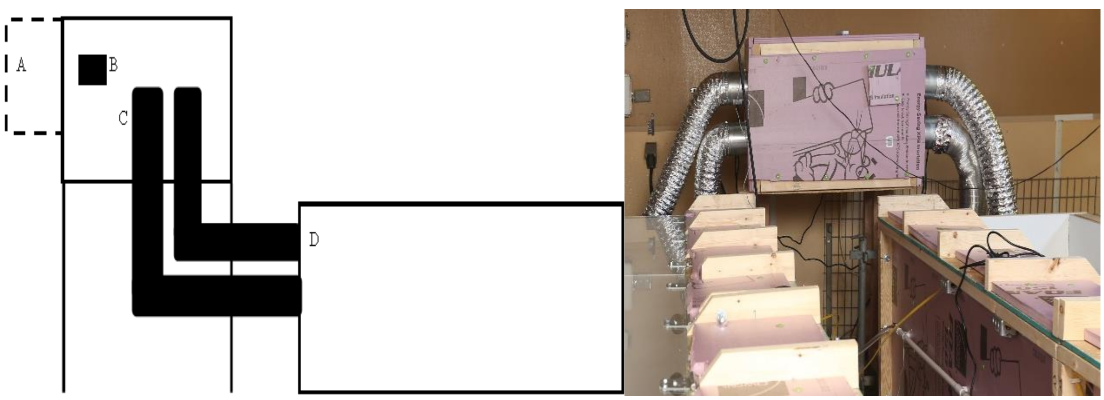

Figure 3.3. Image on the left displays the conditioning box used to push cold air into the thermal apparatuses, image on the right actual picture of the conditioning box. Here pictured is only one of the apparatuses. Legend: A) air conditioner, B) coolbot, C) ducts, D) thermal apparatus.

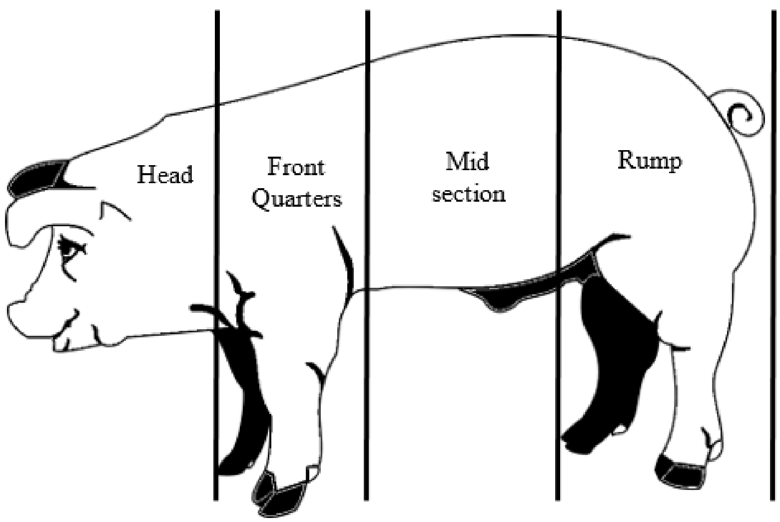

Figure 3.4. Diagram depicting sections of a pig used to assess percentage of body part located within a thermal section, each body section was equivalent to 25%. Head was considered from back of the ears to the snout, front quarters were considered back of the ears to behind the forelimbs, mid-section was from behind the forelimbs to front of the back limbs, and the rump was considered the front of the back limbs to base of the tail.

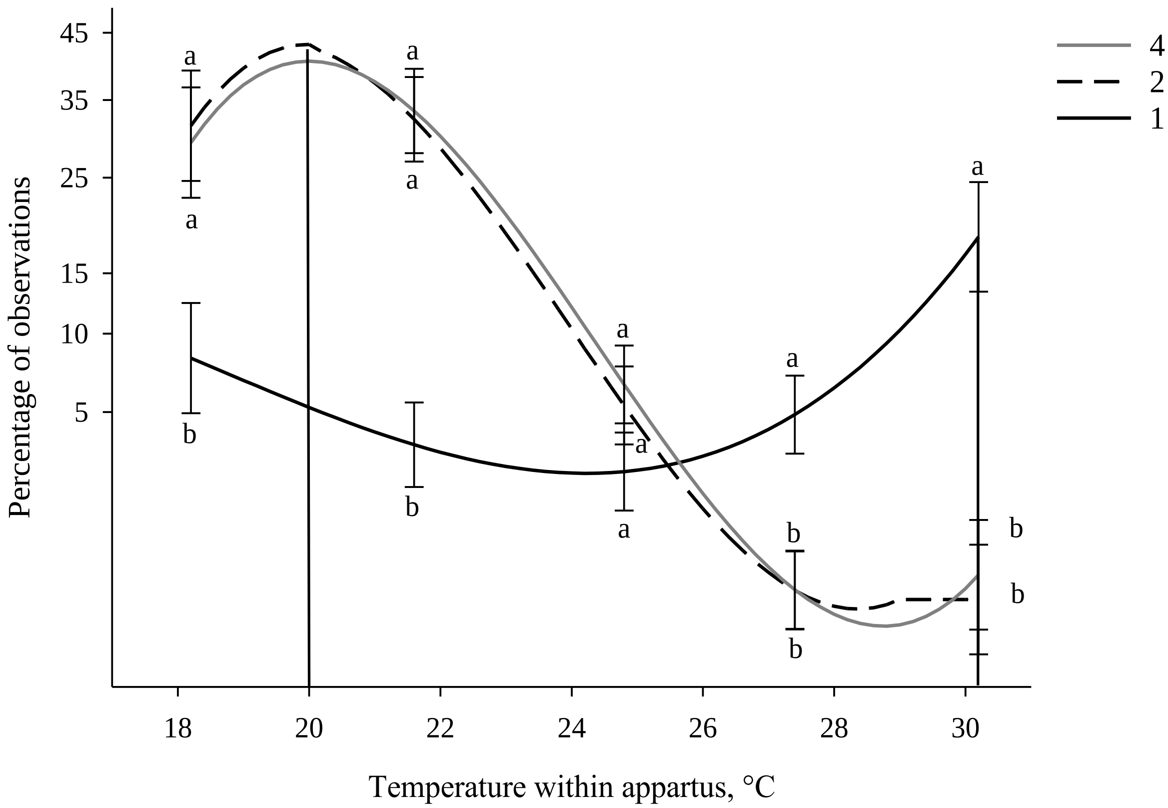

Figure 3.5. Percentage of observations in different temperatures within the thermal gradient while inactive based on group size. The effects of being tested as an individual (1), in a group of 2 or 4, on temperature preference. Temperature within the thermal apparatus is plotted on the x-axis and percentage of time spent in different temperatures are plotted on the y-axis as a square root scale. Cubic peaks are indicated by vertical lines corresponding to group size. Standard error bars are located at the temperatures of the five thermal zones (18.2°C, 21.5°C, 24.8°C, 27.3°C, and 30.3°C) and different letters denote significant Tukey pairwise comparisons (*P* < 0.01). The gray box indicates the recommended temperatures (26 to 32°C) for pigs between 3 to 15 kg (FASS, 2010).

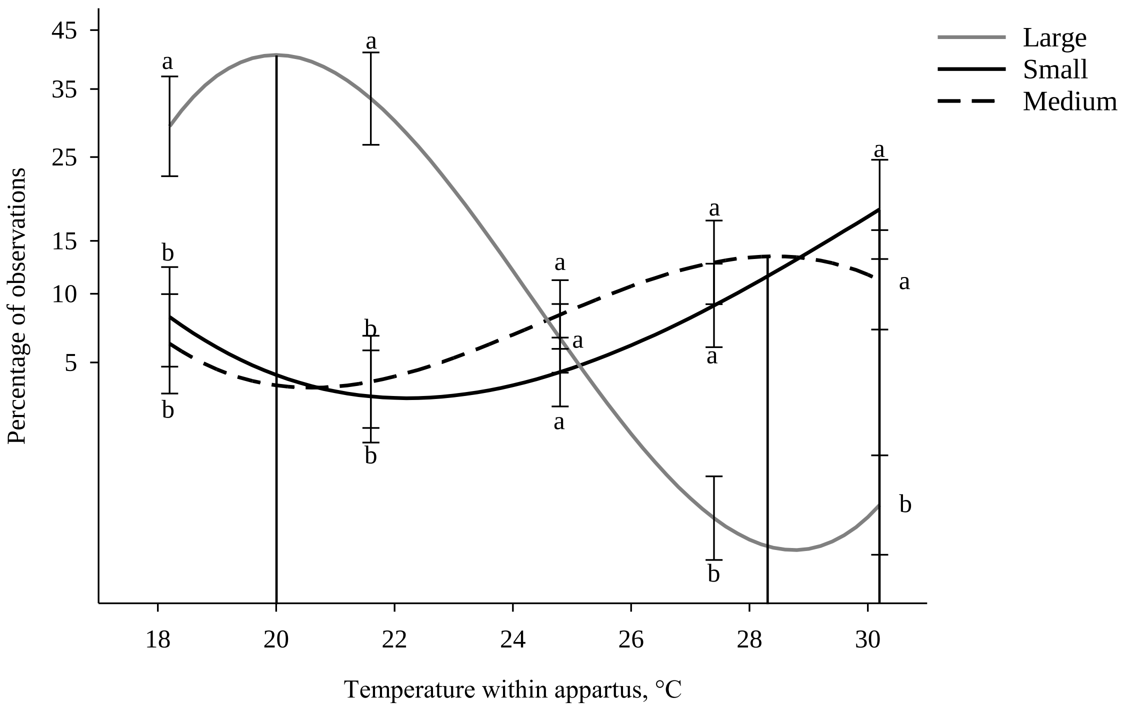

Figure 3.6. Percentage of observations in different temperatures within the thermal gradient while inactive based on weight category. The effects of being tested as a small group (3-7 kg), medium (7.1-11 kg) and large (11.1-15 kg), on temperature preference. Temperature within the thermal apparatus is plotted on the x-axis and percentage of time spent are plotted on the y-axis as a square root scale. Cubic peaks are indicated by vertical lines corresponding to group weight category. Standard error bars are located at the temperatures of the five thermal zones (18.2°C, 21.5°C, 24.8°C, 27.3°C, and 30.3°C) and different letters denote significant Tukey pairwise comparisons (*P* < 0.01). The gray box indicates the recommended temperatures (26 to 32°C) for pigs between 3 to 15 kg (FASS, 2010)

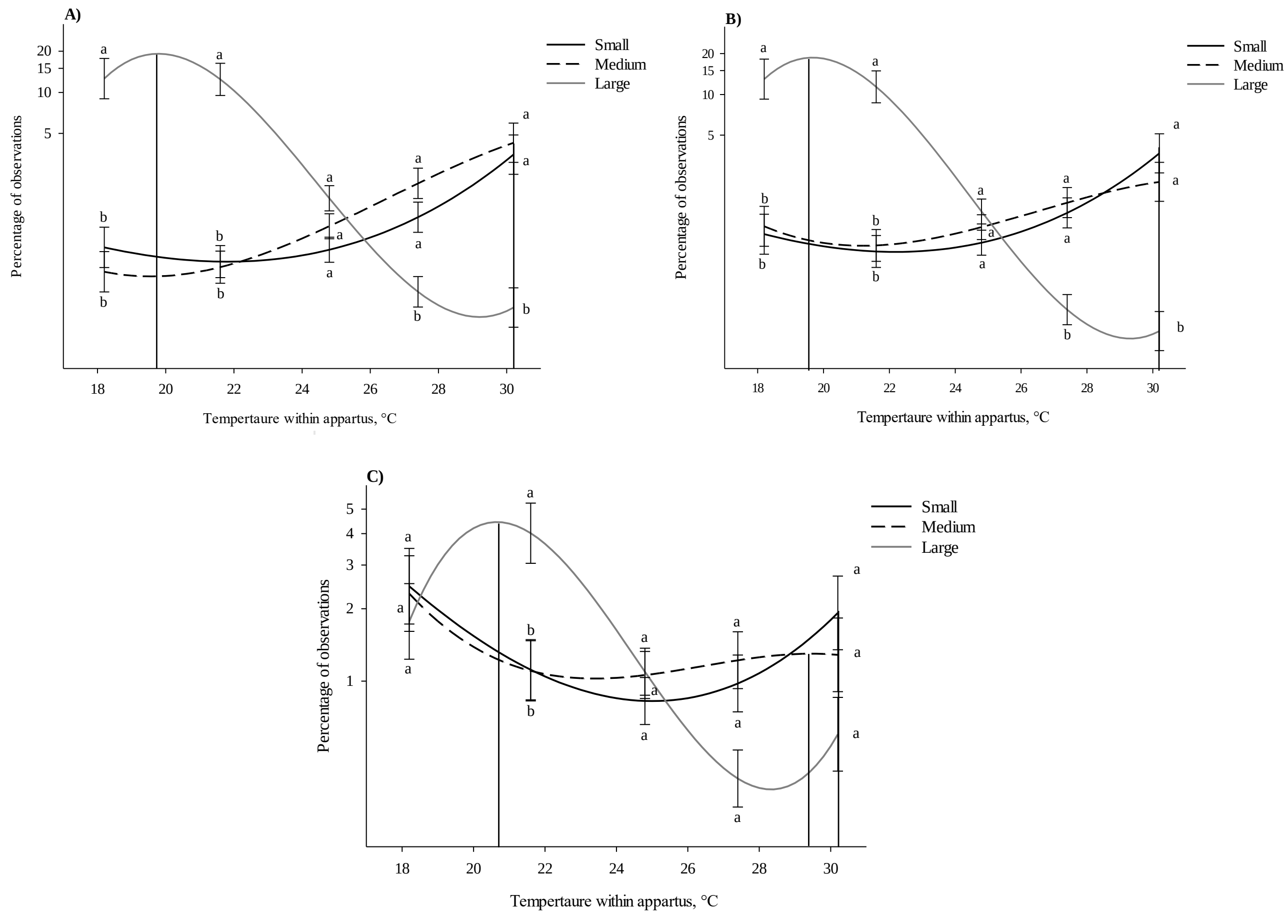

Figure 3.7. Percentage of observations in different temperatures within the thermal gradient while in different postures: A) Lateral laying, B) Sternal laying and C) Upright. The effects of being tested by weight category: small (3-7 kg), medium (7.1-11 kg) and large (11.1-15 kg), on temperature preference based on posture. Temperature within the thermal apparatus is plotted on the x-axis and percentage of time spent are plotted on the y-axis as a log10+0.001 scale. Cubic peaks are indicated by vertical lines corresponding to group weight category within posture. Standard error bars are located at the temperatures of the five thermal zones (18.2°C, 21.5°C, 24.8°C, 27.3°C, and 30.3°C) and different letters denote significant Tukey pairwise comparisons ($P < 0.01$). The gray box indicates the recommended temperatures (26 to 32°C) for pigs between 3 to 15 kg (FASS, 2010)

CHAPTER 4. EVALUATION OF SOW THERMAL PREFERENCE ACROSS THREE STAGES OF REPORDUCTION

Heat stress (**HS**) negatively affects swine well-being, resulting in greater morbidity and mortality, and reduced welfare continues to occur despite advances in management strategies. The first step in developing heat mitigation strategies is to identify the thermal comfort zone (**TCZ**) of sows. Currently, the Guide for the Care and Use of Agricultural Animals in Research and Teaching (Federation of Animal Science Societies, 2010) states that the recommended temperature range for sows and boars over 100 kg is between 10 to 25°C. Studies in humans and rodents have shown that thermal comfort can be dependent upon reproductive stage and current swine guidelines do not reflect this. Thus, the study objective was to evaluate whether different reproductive stages of sows altered their thermal preference. Twenty multi-parous sows (3.4 ± 1.2 parity) in different reproductive stages (open: not pregnant, n=7; mid-gestation: 58.5 ± 5.68 d, n=6; and late-gestation: 104.7 ± 2.8 d, n=7) were tested. Thermal preference, where individually tested sows freely choose a temperature, was determined using a thermal gradient of 10.4 to 30.5°C. Sows were given 24 h to acclimate to the thermal apparatus. Before testing began, sows were given daily feed allotment and returned to the apparatus. Video from the 24 h test period was used to record sow behavior (time spent inactive), posture (upright, sternal and lateral lying), and location using instantaneous scan samples every 10 min. Data were analyzed using PROC MIXED in SAS 9.4. A cubic regression model was used to calculate the sow's most preferred temperature based on the location, or temperature, in which they spent the most time. The preference range was calculated using peak temperature preference ±SE for each sow. Reproductive stage altered where sows spent their time within the thermal gradient ($P < 0.01$). Late-gestation sows preferred cooler temperatures (14.0°C) than mid-gestation (14.8°C; $P < 0.01$) and open sows (14.8°C; $P < 0.01$). In summary, sow thermal preferences were within the lower half of the current recommended range (10 to 25°C). This indicates that temperatures at the higher end of the recommended range could be uncomfortable to sows and that the TCZ of sows may be narrower than recommendations indicate.

4.1 Introduction

Heat stress (**HS**) causes infertility in sows, characterized by anestrus, increased wean-to-estrus interval, decreased farrowing rate, and a reduction in both litter size (e.g. number of piglets born per year) and litter weight (Bertoldo et al., 2012; Peltoniemi and Virolainen, 2006; Muns et al., 2016). Due to reduced heat tolerance from the high metabolic heat load during lactation, studies have focused on understanding HS during this period (Williams et al., 2013). However, the impact of HS is not limited to lactation, it also affects the sow during gestation. For example, HS during early stages of gestation can result in increased embryo mortality (Wildt et al., 1975; Edwards et al., 1968; Tompkins et al 1967), reduced farrowing rates, and litter size (Nardone et al., 2006). Additionally, exposure to HS in late-gestation can result in an increased number of stillborn piglets and reduced piglet birth weight (as reviewed by Lucy and Safranski, et al., 2017). Furthermore, HS during *in utero* development can permanently alter postnatal phenotypes and negatively affect future animal performance (Johnson and Baumgard, 2019; Johnson et al., 2020). Thus, HS has a profound impact on the swine industry both economically (St-Pierre et al, 2003) and in terms of animal health and welfare (as reviewed by Johnson, 2018).

Although HS can alter various reproductive measures, current temperature recommendations do not reflect thermal preference differences based on reproductive stage. The *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (hereon referred to as the *Ag Guide*; Federation for Animal Science Societies, 2010), indicates that sows or boars over 100 kg prefer temperatures between 10 to 25˚C. However, this may not be accurately reflecting preferences during different stages of reproduction since data indicates that physiological changes occur during maternal adaptation to pregnancy in mammals (pigs: Noblet and Etienne, 1987; humans: Hartgill et al., 2011; rodents: Fewell, 1995) that may increase HS sensitivity. Furthermore, sows have an increased body (rectal) temperature indicating increased heat production near parturition (King et al., 1972; Hendrix et al., 1978). Therefore, greater metabolic rate in late-gestation could result in a cooler temperature preference to increase heat loss.

In addition to not accounting for reproductive stage differences in recommended temperatures, previous studies took a theoretical approach to determining the thermoneutral zone (**TNZ**) of sows, using mathematical formulas to calculate the preferred temperature of sows based on previous work in pigs (Holmes and Close, 1977; Hahn, 1985). For example, Curtis (1983) estimated a sow's lower limit was between 18 to 20°C, based on calculations on still air conditions in barns and insulation of the pig. Since the publication of these studies, genetic selection of modern pigs has focused on larger litter sizes and a higher lean-growth pig which creates an animal with higher metabolic heat production (Brown-Brandl et al., 2014). Consequently, modern sows have likely become more sensitive to HS (Brown-Brandl et al., 2014) and as such, their TNZ has likely shifted.

An animal is likely to take behavioral action before the physiological mechanisms for thermoregulation. As such, researchers can determine an animal's thermal comfort zone (**TCZ**) by offering an animal a choice of temperatures that are comfortable to them by placing them inside a thermal gradient (pigs; Robbins et al., 2020; mice: Ogilvie and Stinson, 1966). This type of experiment utilizes an animal's innate motivation to seek an ambient temperature (**T_A**) where it does not have to utilize any physiological mechanisms for thermoregulation, thus heat loss equals heat production (i.e., thermopreferrendum; Gordon, 1993). This technique provides information about the animals' preferred temperatures, or TCZ, from the animal's perspective (Gordon, 1993). From this research, it can be extrapolated the preferred temperatures and set out the fundamental temperatures of an animal's TNZ.

The study objective was to determine the preferred T_A of sows at three stages of reproduction (open, mid-gestation, and late-gestation). Based on previous research, this study hypothesized that reproductive stage would alter thermal preference in sows. Due to an increase in metabolic heat production during gestation and an increase in mass from growing piglets (Noblet et al., 1997), it is expected that the preferred ambient temperatures will be cooler for sows later in gestation compared to open sows. Specifically, both late- and mid-gestation sows will prefer cooler temperatures compared to open sows, and late gestation sows will prefer cooler temperatures compared to mid gestation sows.

4.2 Methods and Materials

All procedures involving animal care and use were approved by the Institutional Animal Care and Use Committee at Purdue University (protocol # 1712001652), and animal care and use standards were based upon the *Ag Guide* (Federation of Animal Science Societies, 2010).

4.2.1 Animals and housing

Twenty multi-parous (3.4 ± 1.2 parity) sows (Yorkshire x Landrace) were selected for temperature preference testing based on reproductive stage (open: not pregnant; mid-gestation: 58.5 ± 5.7 d pregnant; and late-gestation: 104.7 ± 2.8 d pregnant; table 4.1). Before being brought to the thermal preference testing location, all sows were housed in a 3-sided enclosure on concrete, outside, at the Animal Sciences Research and Education Center at Purdue (ASREC: West Lafayette IN). During this study, sows were housed in groups of 8 to 10 under thermoneutral temperatures (according to the *Ag Guide*) of a similar reproductive stage, and limit fed based on recommendations for gestating sows (NRC, 2012). Prior to being moved to the preference testing location, sows were fed their 24 h ration (approximately 1.82 kg) and had access to water *ad libitum*.

4.2.2 Experimental design

Mead's resource equation was used *a priori* to determine the number of sows required for this study (Mead, 1990). Twenty sows were randomly assigned (via random integer generator, random.org) to be tested individually in one of two thermal apparatuses; such that the testing of each reproductive stage would be relatively balanced between the two apparatuses. Testing began on February 8, 2018 and ran until April 4, 2018. Body weight was documented prior to acclimation to the thermal gradient.

For temperature preference testing, two sows at a time were transported from ASREC to the USDA-ARS Farm Animal Behavior Laboratory (FABL: West Lafayette, IN; 3.38 km from ASREC) where the thermal apparatuses were located. Sows were fed and weighed prior to transport and individually placed inside their assigned thermal apparatus upon arrival. Sows were allowed 24 h to acclimate to the new enclosure and thermal gradient. During acclimation and temperature preference testing, sows were able to explore the entirety of the thermal apparatus. Each apparatus was cleaned in between acclimation, testing, and prior to new sows being delivered. Between acclimation and testing, sows were removed from the thermal apparatus and individually housed in an adjacent pen (5.5 m^2) for approximately 3 h to clean and re-establish the thermal gradient. Furthermore, sows were given their daily food ration upon entering the pen, which took them approximately 30 min to consume. Waste was removed from within the thermal apparatus with a pressure washer and disinfected (LYSOL disinfectant all-purpose cleaner, Reckitt Benckiser LLC, NJ, US). After cleaning, the sows were returned to their assigned thermal apparatus. During experimental set-up and testing, researchers were not blinded to the sows' reproductive stage. However, during video coding, observers (LR and MR) were blinded to this information.

Prior to placement inside the thermal apparatus, a calibrated Thermochron temperature recorder (iButton model 1921H, calibrated accuracy ± 0.10 °C; resolution = 0.125 °C; Dallas Semi-conductor, Maxim, Irving, TX) attached to a blank controlled internal drug-releasing device (Eazi-Breed; Zoetis, New York, NY) was inserted intravaginally into each sow. This allowed for continuous recoding of a sow's vaginal temperature (method previously described by Johnson and Shade, 2017). Unfortunately, data was lost from 14 sows due to the Thermochrons being dislodged and started part-way through the study. Thus, data from the Thermochrons are not reported here but was used where available as an exclusion criterion if any sows had a vaginal temperature that appeared abnormal (> 39.0°C).

4.2.3 Thermal apparatus

The materials and methods were adapted in part from Robbins et al. (2018 and 2020). Two thermal gradient apparatuses were built (12.2 m x 1.52 m x 1.86 m; L x W x H) to provide the required space per sow and create a desired temperature gradient (Fig. 4.1). To create the thermal gradient (10.35 ± 0.42˚C to 30.49 ± 0.45˚C), ceramic heating lamps (herein referred to as heating elements (Floureon 100 to 250W Multi Basking IR Heat Bulb) were placed at strategic locations 1.80 m above the floor (Fig. 4.2). Heating elements were connected to thermostats that would turn on/off specific heating elements at different T_A to maintain a constant gradient. Temperature was recorded every hour for two weeks prior to sows being placed inside the apparatus and every two hours while the sow was inside by the observer using a digital thermometer (table 4.2). Light, humidity, and temperature were monitored using data loggers (HOBO Data Logger; U12-013, Onset Computer Corporation, MA USA; temperature range of -20°C to 70°C with accuracy of ± 0.35°C and RH range 5% to 95% with accuracy of ± 2.5% to max 3.5%) to assure that the 2 apparatuses were as identical as possible. The data loggers were placed on the wall of each apparatus approximately 0.94 m above the floor and 0.61 m apart to calculate the average temperature within each thermal zone. An additional set of data loggers were placed inside a black globe, at a similar location on the opposite wall.

The thermal apparatus was fully enclosed with overhead lighting. Led lighting strips (10500 lumen, 12.19 m length, CBconcept, California, US) were used to illuminate the apparatuses with a light:dark cycle similar to their housing conditions at ASREC (10:14 light:dark cycle). To create the cool end of both thermal apparatuses, a conditioning box (1.52 m x 1.52 m x 0.76 m, L x W x H; Fig. 4.3) was designed with an air conditioning unit (10,000 BTU, LG Electronics, Seoul, South Korea) attached to a Coolbot (Store It Cold LLC, Florida, US) to circulate 5°C air into the apparatuses. Both apparatuses had *ad libitum* water supplied using waterers (AquaChief™, Hog Slat, Inc. NC, US) located within each thermal zone.

4.2.4 Behavior and posture observations

The sows were video recorded continuously over the 24 h testing period for behavior, location, and posture using infrared cameras (Sony Corporation, Tokyo, Japan) and video surveillance software (GeoVision, Taiwan). The location, behavior and posture were recorded for each sow using instantaneous scan samples every 15 min. The scan interval used for recording data from video was determined by comparing proportion of observations at each location with different sub-sampling intervals (5, 10, 15, 20, 25, 30 min). Data from each subsample were compared pairwise (Cochran's comparison test) and considered to accurately estimate the behavior or posture if the intervals were not significantly different from each other compared to the 5 min interval (Ledgerwood et al., 2010). Based on this data, a 15 min sampling interval was selected for this study. The ethogram contained 3 simple behavior categories: active, inactive, and other (table 4.3). If sows were observed in more than 1 thermal zone (location), the proportion of the sow in each zone was documented in 25% increments (head, front quarter, mid-section and rump; Fig. 4.4). Postures can provide an indication of thermal comfort (Mount, 1960); therefore, posture was also documented at each scan sample (table 4.4).

The proportion of behaviors was calculated for each sow by counting the total number of times each behavior was observed in each location during testing. This calculation was repeated separately for posture data. Since sows were observed inactive in ~85% of observations, only inactivity was included in the behavioral analysis. For the postural data, any observations of sows documented in the "other" category were similarly excluded from the analysis. Thus, the time budgets do not total 100% and the independent variables are not co-linear and a change in one category will not directly influence the level of another category.

4.2.5 Analyses

All analyses were performed using the PROC MIXED (GLM) procedure in SAS 9.4 (SAS Institute INC., Cary, NC). The assumptions of the GLM (normality of error, homogeneity of variance, and linearity) were confirmed post-hoc, and data were transformed when necessary to meet these assumptions (Grafen and Hails, 2002). The threshold for significance $P < 0.05$ was used and Bonferroni corrected for multiple tests.

4.3.5.1 Body weight, number of piglets and peak thermal preference

Originally, sow was nested with reproductive stage, weight category (classified as above or below the mean split of all sows' weight), and parity. However, when parity and weight category were included as blocking factors, the AIC was greater and the R2 was reduced indicating a better model was to remove these factors. Further, these were found to be non-significant. Thus, the final Analyses were blocked by Sow, nested within reproductive stage, and was treated as a random effect. A cubic regression model was used for both behavior and posture data and both were log10+0.001 transformed to meet the assumption of a GLM. Main effects plus second order interactions of reproductive stage, time spent inactive and posture (where applicable), and location were tested. Location was used as a cubic variable. Since the data were not orthogonal, non-significant higher order interactions were dropped from the final analysis.

Data from three sows (not reported here) were also excluded from the analyses. Complications within the thermal gradient required us to drop data from two sows and another due to a 1.2°C warmer vaginal temperature than normal. It was assumed that the increased temperature indicated illness, which, would alter thermal preference.

The cubic curve from the final model above was generated in 0.2°C increments starting with the coldest thermal zone temperature (10.4°C) and increased to the warmest temperature (30.4°C). Peak temperatures for inactive behavior and posture were calculated by identifying the temperature with the greatest proportion of time spent in each location. Thermal preference range was then calculated from the peak temperature ± SE. Tukey tests for differences in LSM between reproductive stages were run in each thermal zone. Since Tukey tests were run 5 times (for each thermal zone), the alpha was Bonferroni corrected for the multiple tests ($\alpha = 0.05/5 = 0.01$).

4.3.5.2 Behavior and posture by location

First, we wanted to determine if number of piglets gestating (that is number of piglets the sow had in total, including mummified and stillborn, referred to hereon as piglets) and stage of reproduction altered overall sow body weight (**BW**). Only the main effect of reproductive stage and piglets were tested in this GLM analysis.

Body weight is such an influential variable on thermal comfort; it was necessary to see if it affected peak thermal preference. To test this, the peak thermal preference for each sow was identified from the raw inactive behavior data, by determining the location (temperature) where sows spent most of their time. For this GLM, only the main effect of reproductive stage and BW were included in the model. Number of piglets was tested initially as a covariate, but it was not significant, thus it was removed from the model.

4.3 Results

4.3.1 Body weight and peak thermal preference

No significant differences in BW were detected based on the main effect of reproductive stage ($P = 0.199$: table 4.5). Piglet numbers did affect BW, where sows with increasing number of piglets had increased BW (Fig. 4.5).

Body weight ($P = 0.001$) and stage of reproduction ($P = 0.005$) affected peak thermal preference (table 4.6). Late-gestation sows had a cooler peak thermal preference compared to mid- and open sows (table 4.6). Sows, regardless of reproductive stage, with a higher BW preferred cooler temperatures (Fig. 4.6).

4.3.2 Behavior by location

Reproductive stage altered the amount of time sows spent in the different thermal zones (*P* = 0.015; table 4.7). Late gestation sows had a peak thermal preference of 14.0°C (spending 32.59% of their time at this temperature) with a range between 12.6 to 15.6°C. This peak temperature was cooler than mid-gestation sows with a peak preference of 14.8°C (22.31%, α/3: $F_{1,75} = 6.83$; $P = 0.011$: Fig. 4.7) and ranged between 13.2 to 16.4°C. Peak thermal preference was also different between late and open reproductive sows (α/3: $F_{1,75} = 6.37$; $P = 0.014$: Fig. 4.7), with open sows having the same peak thermal preference (14.8°C, 22.96%) and range as mid-gestation sows (α/3: $F_{1,75} = 0.04$; $P = 0.852$).

4.3.3 Posture by location

The percentage of time spent in various postures differed across the thermal gradient ($P < 0.001$; table 4.8). Lateral and sternal lying were observed most often at 14.8°C (spending 19.74 and 8.04% of their time, respectively) and had a preference range between 10.8 to 19.8°C and 10.6 to 20.0°C, respectively. Upright posture was observed most often at 14.0°C (9.61%) with a range between 10.4 to 20.2°C, this peak differed from sternal (α/3: $F_{1,267} = 11.19$; $P < 0.001$) and lateral lying (α/3: $F_{1,267} = 24.56$; $P < 0.001$: Fig. 4.8).

4.4 Discussion

This study examined the preferred T_A of sows, by reproductive stage, to the best of our knowledge, for the first time by utilizing the animal's innate motivation to seek out their preferred T_A. Reproduction is an energetically demanding process, with the greatest energetic costs occurring during late pregnancy and lactation (Kaczmarski, 1966; Millar, 1978). Therefore, this study hypothesized that reproductive stage would alter thermal preference of sows.

Reproductive stage affected the temperature preference of sows when inactive. Late-gestation sows preferred a temperature that was 0.8°C cooler than both open sows and mid-gestation sows. Additionally, late-gestation sows spent less time at the hot end of the thermocline (27.5°C and 30.5°C) than both mid-gestation and open sows, indicating an aversion to these temperatures. Interestingly, mid- and open sows preferred similar temperatures while inactive indicating that their TCZ is like each other despite the potential increased metabolic heat production in mid-gestation compared to open sows. Although metabolic rate was not directly measured in this study, it is known that late-gestation sows have increased total heat production (**THP**) due to rapidly developing fetuses and increased energetic demands (Kaczmarski, 1966; Millar, 1978; Noblet et al., 1997). Therefore, this THP increase likely explains the late-gestation sows' preference for cooler temperatures relative to mid-gestation and open sows whereas the THP differences between mid-gestation and open sows was likely minimal resulting in similar temperature preferences.

During times of HS, pigs alter their posture and increase lateral lying to increase skin contact with the floor (Mount, 1979; Huynh et al., 2005) and increase heat loss through the skin. Typically, there is a linear relationship between lateral lying and environmental temperature in pigs (Huynh et al., 2005). Alternatively, a decrease in T_A increases sternal lying because this posture reduces exposed skin surface area to the floor and helps reduce heat loss (Mount, 1960). In the present study, sows spent most of their time in the lateral posture (20%) compared to the sternal posture (9%), both of which were observed at a temperature within the *Ag Guide* recommendations (FASS, 2010). However, both behaviors were observed in a narrower temperature range (10.6 to 19.8°C for sternal and 10.6 to 20.0°C for lateral laying) compared to 10 to 25°C as outlined in the *Ag Guide* (FASS, 2010). The fact that the sows chose cooler temperatures than expected and were lying in a posture that indicates comfort, supports that the temperatures selected fall within their TCZ.

Sows were individually tested in this study, which does not accurately reflect normal living conditions on commercial farms. As such, solitary conditions are less likely to represent a typical sow's thermoregulatory environment. In group housing, sows tend to spend a considerable amount of time near each other, often huddling together (Kittawornrat & Zimmerman, 2011). Although no data related to thermal preference in group housed sows exists, groups of mice typically prefer a cooler temperature than those housed individually (Gordon et al., 1998). Thus, the temperature preference ranges observed here might be warmer than if sows were tested in groups. Although it is currently unknown to what extent temperature preference may be reduced for group-housed sows, future research should investigate the effects of group size and temperature preference to provide TCZ guidelines for various housing scenarios.

Additionally, this study did not acknowledge the radiant temperature load from the heat lamps and how that may have contributed to the reported T_A, this potential increase might make the sows feel a bit warmer than the same temperature achieved a different way. Examining the potential differences in T_A based on different formulas might yield different results. This study is the first to look at temperature preference differences based on reproductive stage of sows. The temperature preferences found in this study demonstrates that further research is required to produce accurate guidelines on preferred temperature ranges. The results of this study indicate that late-gestation sows prefer a temperature range of 12.6 to 15.6°C and may have a cooler upper critical limit than what is currently recommended (approximately, 25°C; FASS, 2010). This indicates that sows might be experiencing discomfort and HS at cooler temperatures than expected based on stage of reproduction. Based on the research conducted in this study, individual sows should be housed in temperatures between 12.6 to 16.4°C to provide their TCZ and could optimize production and improve overall well-being.

4.5 References

Bertoldo, M., P. Holyoake, G. Evans, and C. Grupen. 2012. Seasonal variation in the ovarian function of sows. Reproduction, Fertility and Development 24: 822-834. doi:10.1071/RD11249

Brown-Brandl, T. M., M. D. Hayes, H. Xin, J. A. Nienaber, and H. Li. 2014. Heat and moisture production of modern swine. ASHRAE Trans. 120: 469–489. doi:10.13031/ trans.57.1071

Curtis, S. E. 1983. Environmental management in animal agriculture. Ames, Iowa: Iowa State University Press. p. 6-96

Edwards,R.L., I. T. Omtvedt, E. J. Tuesman, D. F. Stephens, G. W. A. Mahoney, 1968. Reproductive Performance of Gilts following Heat Stress Prior to Breeding and in Early Gestation. Journal of Animal Science 27: 1634-1637. doi:10.2527/jas1968.2761634x

Federation of Animal Science Societies. (FASS). 2010. Guide for the care and use of agricultural animals in research and teaching. 3rd ed. Champaign (IL): Fed. Anim. Sci. Soc. Chap. 11.

Fewell, J. E. 1995. Body temperature regulation in rats near term of pregnancy. Canadian journal of physiology and pharmacology 73: 364-368. doi:10.1139/y95-046

Gordon, C. J. 1993. Temperature regulation in laboratory rodents. Cambridge University Press, Cambridge, NY. xii: 276.

Gordon, C. J., P. Becker, and J. S. Ali. 1998. Behavioral thermoregulatory responses of single- and group-housed mice. Physiology & behavior 65: 255-262. doi:10.1016/S0031-9384(98)00148-6

Grafen, A., and R. Hails. 2002. Modern statistics for the life sciences. Oxford University Press, Oxford, NY. p. 155-163.

Hahn, G. 1985. Managing and housing of farm animals in hot environments in Stress Physiology in Livestock. Vol II: Ungulates. M. K. Yousef, ed. CRC Press, Boca Raton, FL. p. 151–174.

Hartgill, T., T. Bergersen, and J. Pirhonen. 2011. Core body temperature and the thermoneutral zone: a longitudinal study of normal human pregnancy. Acta physiologica 201: 467-474. doi:10.1111/j.1748-1716.2010.02228.x

Hendrix, W. F., K. W. Kelley, C. T. Gaskins, and R. B. Bendel. 1978. Changes in respiratory rate and rectal temperature of swine near parturition. Journal of animal science 47: 188-191. doi:10.2527/jas1978.471188x

Holmes, C., W. Close, W. Haresign, H. Swan, and D. Lewis. 1977. Nutrition and the climatic environment. Eds W. Haresign, H. Swan and D. Lewis. London, UK. p. 51-74

Huynh, T. T. T. et al. 2005. Thermal behaviour of growing pigs in response to high temperature and humidity. Applied Animal Behaviour Science 91: 1-16. doi:10.1016/j.applanim.2004.10.020

Johnson, J. S. 2018. Heat stress: impact on livestock well-being and productivity and mitigation strategies to alleviate the negative effects. Animal Production Science 58: 1404-1413. doi:10.1071/AN17725

Johnson, J. S., and L. H. Baumgard. 2019. PHYSIOLOGY SYMPOSIUM: Postnatal consequences of in utero heat stress in pigs. Journal of animal science 97: 962-971. doi:10.1093/jas/sky472

Johnson, J. S., and K. A. Shade, 2017. Characterizing body temperature and activity changes at the onset of estrus in replacement gilts. *Livestock Science, 199*, 22-24. doi:10.1016/j.livsci.2017.03.004

Johnson, J. S., K. R. Stewart, T. J. Safranski, J. W. Ross, and L. H. Baumgard. 2020. In utero heat stress alters postnatal phenotypes in swine. Theriogenology. doi:10.1016/j.theriogenology.2020.05.013

Kaczmarski, F. 1966. Bioenergetics of pregnancy and lactation in the bank vole. Acta Theriologica 11: 409-417. doi:10.4098/AT.arch.66-19

King, G., R. Willoughby, and R. Hacker. 1972. Fluctuations in rectal temperature of swine at parturition. The Canadian Veterinary Journal 13: 72

Kittawornrat, A., and J. J. Zimmerman. 2011. Toward a better understanding of pig behavior and pig welfare. Animal Health Research Reviews 12: 25-32. doi:10.1017/S1466252310000174

Ledgerwood D. N., Winckler C., Tucker C. B. 2010. Evaluation of data loggers, sampling intervals, and editing techniques for measuring the lying behavior of dairy cattle. J. Dairy Sci. 93:5129–5139. doi:10.3168/jds.2009-2945.

Lucy, M. C., and T. J. Safranski. 2017. Heat stress in pregnant sows: thermal responses and subsequent performance of sows and their offspring. Molecular Reproduction and Development 84: 946-956. doi:10.1002/mrd.22844

Mead, R. 1990. The design of experiments: statistical principles for practical applications. Cambridge University Press, Cambridge, NY. p. 620.

Millar, J. S. 1978. Energetics of reproduction in Peromyscus leucopus: the cost of lactation. Ecology 59: 1055-1061. doi:10.2307/1938558

Mount, L. 1960. The influence of huddling and body size on the metabolic rate of the young pig. The Journal of Agricultural Science 55: 101-105. doi:10.1017/S0021859600021651

Mount, L. 1975. The assessment of thermal environment in relation to pig production. Livestock Production Science 2: 381-392. doi:10.1016/0301-6226(75)90121-9

Mount, L. E. 1979. Adaptation to thermal environment. Man and his productive animals. Edward Arnold (Publishers) Ltd.

Muns, R., Malmkvist, J., Larsen, M. L. V., Sørensen, D., & Pedersen, L. J. (2016). High environmental temperature around farrowing induced heat stress in crated sows. *Journal of animal science*, *94*(1), 377-384. doi.org/10.2527/jas.2015-9623

Nardone, A., B. Ronchi, N. Lacetera, and U. Bernabucci. 2006. Climatic effects on productive traits in livestock. Veterinary Research Communications 30: 75-81. doi:10.1007/s11259-006-0016-x

Noblet J, and Etienne M. 1987. Metabolic utilization of energy and maintenance requirements in pregnant sows. Livestock Prod Sci 16: 243-257. doi. 10.1016/0301-6226(87)90042-X.

Noblet, J., J.-Y. Dourmad, M. Etienne, and J. Le Dividich. 1997. Energy metabolism in pregnant sows and newborn pigs. Journal of Animal Science 75: 2708-2714. doi:10.2527/1997.75102708x

NRC. 2012. Nutrient requirements of swine. 11th rev. ed. Natl. Acad. Press, Washington, DC.

Ogilvie, D., and R. Stinson. 1966. The effect of age on temperature selection by laboratory mice (Mus musculus). Canadian Journal of Zoology 44: 511-517. doi:10.1139/z66-055

Peltoniemi, O., & Virolainen, J. V. (2006). Control of Pig Reproduction: Seasonality of reproduction in gilts and sows. In C. J. Ashworth , & R. R. Kraeling (Eds.), *Control of pig reproduction VII: proceedings of the seventh International Conference on Pig Reproduction, Kerkrade, The Netherlands, June 2005* (pp. 205-218). Nottingham University Press.

Robbins, L., A. R. Green-Miller, J. S. Johnson, C. Gonzales, and B. N. Gaskill. 2020. Early life thermal stress: Impacts on future temperature preference in weaned pigs (3 to 15 kg) Journal of Animal Science. doi: 10.1093/jas/skaa327.

Robbins, L., A. R. Green-Miller, J. S. Johnson, and B. N. Gaskill. 2018. Thermocline Design for Thermal Preference Testing in Piglets. In: 10th International Livestock Environment Symposium (ILES X). p 1. American Society of Agricultural and Biological Engineers. doi:10.13031/iles.18-154

St-Pierre, N., B. Cobanov, and G. Schnitkey. 2003. Economic losses from heat stress by US livestock industries. Journal of dairy science 86: E52-E77. doi:10.3168/jds.S0022-0302(03)74040-5

Tompkins, E., C. Heidenreich, and M. Stob. 1967. Effect of post-breeding thermal stress on embyronic mortality in swine. Journal of Animal Science 26: 377-380. doi:10.2527/jas1967.262377x

Wildt, D., G. Riegle, and W. Dukelow. 1975. Physiological temperature response and embryonic mortality in stressed swine. American Journal of Physiology-Legacy Content 229: 1471-1475. doi:10.1152/ajplegacy.1975.229.6.1471

Williams, A. et al. 2013. Effects of a controlled heat stress during late gestation, lactation, and after weaning on thermoregulation, metabolism, and reproduction of primiparous sows. Journal of animal science 91: 2700-2714. doi:10.2527/jas.2012-6055

4.6 Tables and Figures

Table 4.1. Number of sows based on reproductive stage with average body weight, kg prior to placement in a thermal apparatus.

Parameter	Number of sows	Average weight, kg (Mean ± SD)
Open	7	202.69 ± 19.19
Mid	6	233.83 ± 28.08
Late	7	237.94 ± 33.65

Table 4.2. Temperatures, for the thermal apparatus, averaged over the course of the entire study with relative humidity (**RH**).

Location within the thermal apparatus	Average Temperature, °C (± SD)	Average RH% (± SD)
A	10.35 ± 0.42	33.86 ± 9.20
B	19.22 ± 0.48	30.10 ± 7.85
C	23.55 ± 0.46	23.87 ± 7.28
D	27.47 ± 0.42	30.18 ± 16.85
E	30.49 ± 0.45	16.42 ± 3.23

Table 4.3. Ethogram used for behavioral observations.

Category	**Behavior**	**Description**
Active	Active	Sow is walking about, can be seen actively engaged with the environment. Sow can be observed interacting with water drinkers located in each thermal zone, such as biting, scratching, or chewing on.
	Drinking	Sow's head is in the water drinker, located opposite wall to lighting strips (10 total), can only see back of head and ears while within in the water drinker.
Inactive	Inactive	Sow is motionless and assumed to be sleeping. The animal may be inactive if sitting, standing or lying still and alert. Animal is stationary, slow and small head movements may be seen but their body is motionless.
Other	Other	Sow's behavior cannot be determined, camera angles or glare do not allow for accurate assessment
	Defecation	Sow is stationary or in a dog-sit position, can see fecal matter being excreted

Table 4.4. Ethogram used for posture observations.

Posture	Description
Upright	Sow's body is erect and top line (back) is to the camera, includes sow standing on all four hoofs on ground and dog-sitting where sow has rump on floor
Sternal Laying	Sow lies up-right with stomach and chest touching the ground, top line is facing the camera. This includes when a sow is sternal on her anterior body and lateral on her posterior body. Sternal includes the medial plane of the head and body being perpendicular to a 45-degree angle to the ceiling.
Lateral Laying	Sow lies on side with shoulder and rump touching the ground, top line is facing a wall. Medial plane of head and body are greater than 45 degrees and approximately 90 degrees to the ceiling.
Other	Any other postures or those that cannot be determined, camera angles or glare do not allow for accurate assessment. When sow is in position transition and down on front knees but stays with hind end up for a while and may still be moving about.

Table 4.5. Statistical terms included in the model which tested for differences in the percentage of observations in sows at peak temperature with BW as a covariate.

Effect	F Ratio	P-value
Reproductive stage	$F_{2,16} = 1.79$	0.199
		Body weight, kg (LSM ± SE)
Open		152.65 ± 51.27
Mid		185.91 ± 22.08
Late		161.06 ± 34.15
Effect	**F Ratio**	**P-value**
Piglet numbers	$F_{1,16} = 5.47$	0.033*

* denote a significant difference ($P < 0.05$).

Table 4.6. Reproductive stage and body weight influence peak thermal preference (LSM ± SE)

Effect	Estimate	SE	P-value
Reproductive stage			**0.001***
Open	18.77^{*}	1.12	
Mid	20.65^{*}	1.11	
Late	13.91^{+}	1.05	
Weight, kg			**0.005***

Different symbols and * denote a significant difference in peak thermal preference between parameters Tukey tests ($P < 0.01$).

Table 4.7. Statistical terms included in the inactive behavior model which tested for differences in the percentage of observations.

Effect	**F**	***P*-value**
Reproductive stage	$F_{2,17} = 1.33$	0.290
Location	$F_{1,75} = 74.36$	< 0.001*
Location*Reproductive stage	$F_{2,75} = 4.46$	0.015*
Location*Location	$F_{1,75} = 4.25$	0.043*
Location*Location*Location	$F_{1,75} = 30.67$	< 0.001*

* denote a significant difference ($P < 0.05$).

Table 4.8. Statistical terms included in the model which tested for differences in the percentage of observations in sows at various locations (temperatures) while in different postures.

Effect	**F**	***P*-value**
Reproductive stage	$F_{2,17} = 1.90$	0.179
Posture	$F_{2,267} = 8.06$	< 0.001*
Location	$F_{1,267} = 115.22$	< 0.001*
Location*Reproductive stage	$F_{2,267} = 11.73$	< 0.001*
Location*Posture	$F_{2,267} = 12.79$	< 0.001*
Location*Location	$F_{1,267} = 24.25$	< 0.001*
Location*Location*Posture	$F_{2,267} = 0.438$	0.646
Location*Location*Location	$F_{1,267} = 65.27$	< 0.001*
Location*Location*Location*Posture	$F_{2,267} = 3.21$	0.042*

* denote a significant difference ($P < 0.05$).

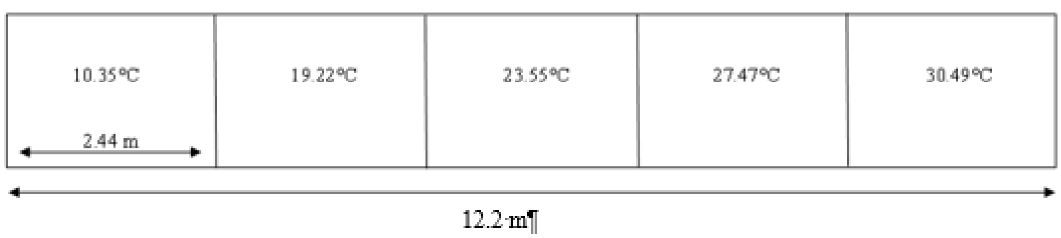

Figure 4.1 Top view of a single thermal apparatus showing average temperature per thermal zone

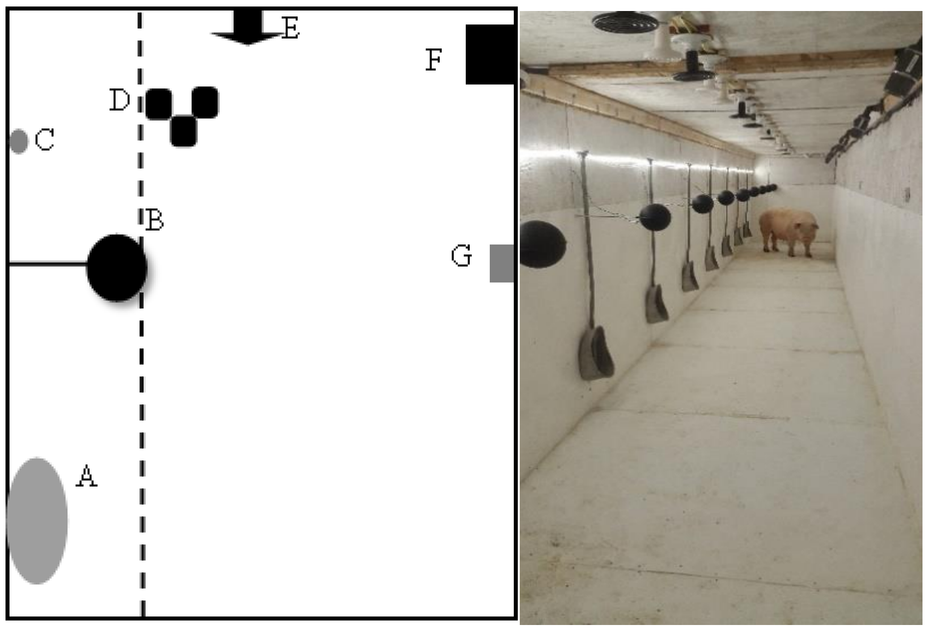

Figure 4.2 Depiction of a single thermocline and location of various elements, on the left: The dashed line indicates a doorway used to gain entrance to the back of the thermocline to clean, A) water drinker, located within each thermal zone, B) black globe, C) LED lighting strip that was attached to a timer to provide a Light:Dark cycle of 0800 on 1800 off, D) thermostats, located at the back end of the thermocline, that had a probe outside of the thermocline to shut on/off elements when outside ambient temperatures rose above or below set temperatures, E) indicates various heating elements (150 to 250W ceramic heating bulbs), these elements were attached to, F) CCTV cameras recorded continuously while sow was inside the apparatus, and G) Data loggers located at the same height as the black globes used to measure humidity, radiant temperature, light, and dew point. The right image displays an actual photo inside the thermocline.

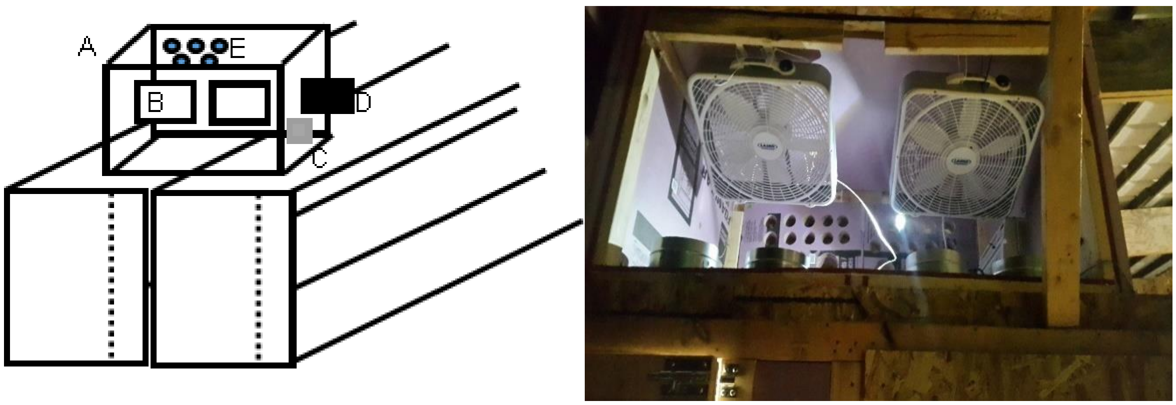

Figure 4.3 Depiction of conditioning box which sat on top of the thermoclines on the left: A) conditioning box, B) box fans (depicted as squares with black lining) used to circulate the air, C) depicted in grey solid box was the CoolBot which sat outside the conditioning box but had a thermal probe inside the box which was used to maintain a 5°C T_A inside the conditioning box, D) depicted as a black box the LG air conditioning unit), E) partially shown 5 out of 20 exhaust holes depicted as blue circles. On the right, the image displays an actual photo of the inside the conditioning box.

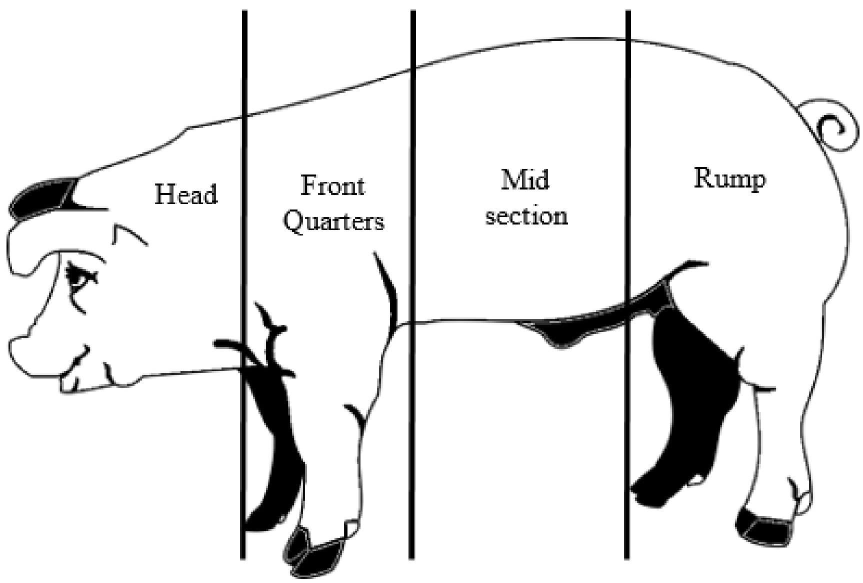

Figure 4.4. Depiction of sections of the sow used to assess percentage of body part, if a sow were recoded over a line rather in a single thermal zone. Each body part was equivalent to 25%. Head was considered from back of the ears to the snout, front quarters were considered back of the ears to behind the forelimbs, mid-section was from behind the forelimbs to front of the back limbs, and the rump was considered the front of the back limbs to base of the tail.

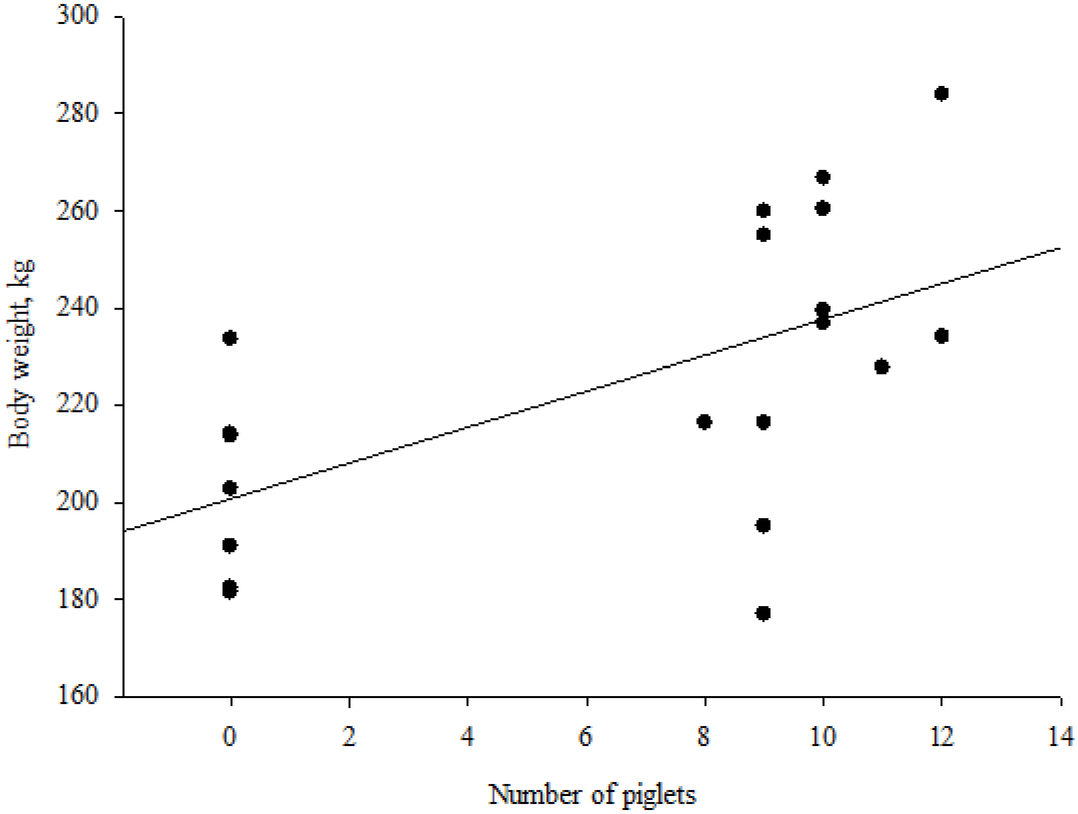

Figure 4.5 Body weight (kg) per sow based on number of piglets. Symbols represent individual sows.

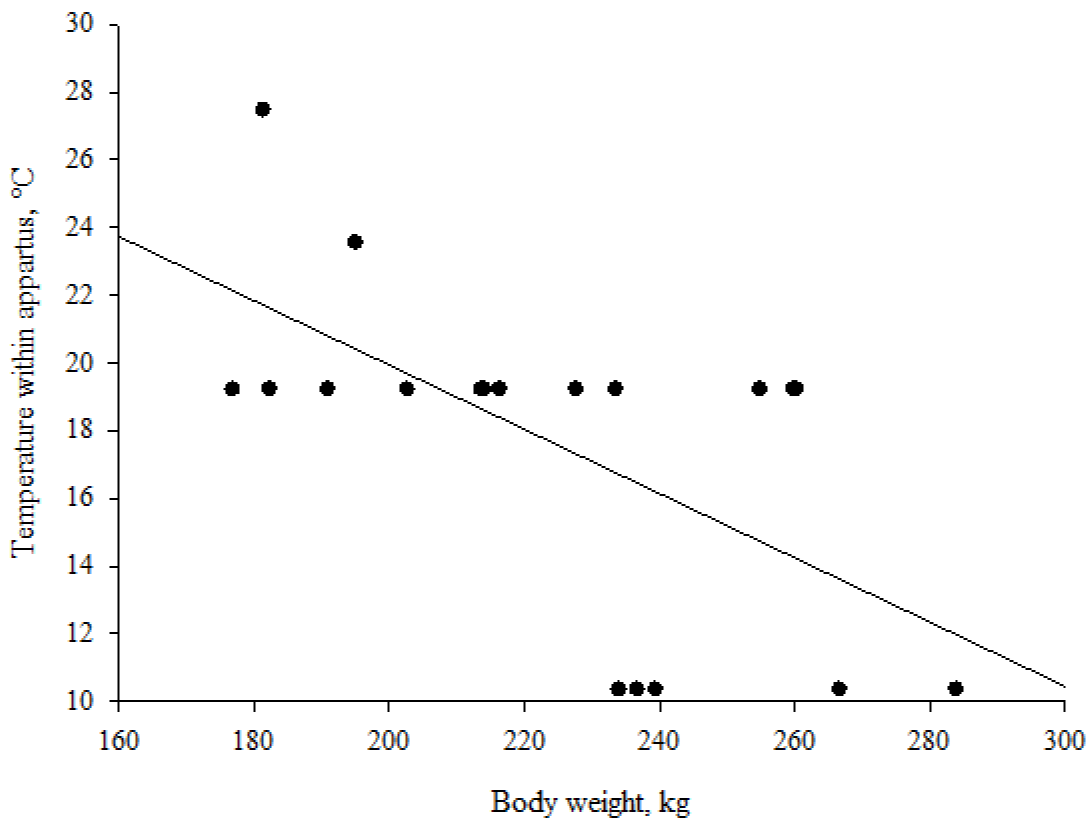

Figure 4.6 Body weight (kg) per sow and preferred peak thermal preference based on amount of time spent within the 5 thermal zones (10.4°C, 19.2°C, 23.6°C, 27.5°C, and 30.5°C) during inactivity. Symbols represent individual sow weights.

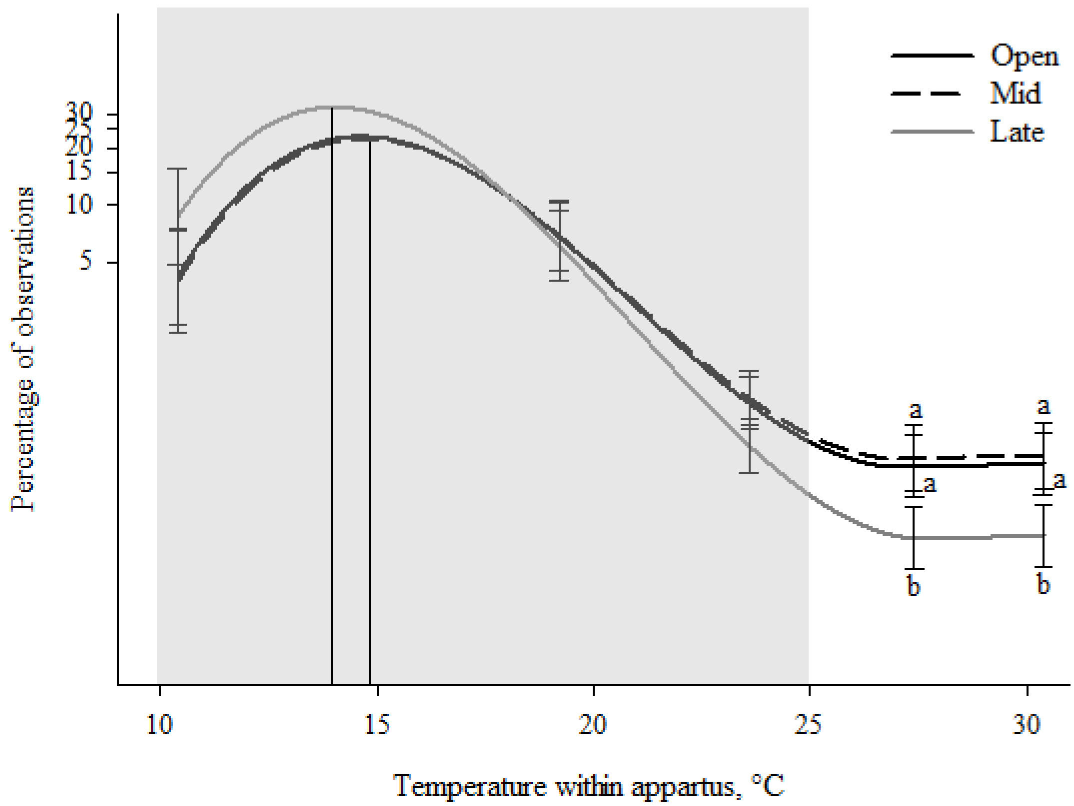

Figure 4.7 Percentage of observations in different temperatures within the thermal gradient based on reproduction stage and during inactive behaviors. The effects of reproductive stage (open: not pregnant; mid-gestation: 58.5 ± 5.68 d; and late gestation: 104.7 ± 2.8 d) on thermal preference. Temperature within the thermal apparatus is plotted on the x-axis and percentage of time observed during inactive behaviors are plotted on the y-axis as a log10+0.001 scale. Cubic peaks are indicated by vertical lines corresponding to reproductive stage. Standard error bars are located at the temperatures of the five thermal zones (10.4°C, 19.2°C, 23.6°C, 27.5°C, and 30.5°C) and different letters denote significant Tukey pairwise comparisons (*P* < 0.01), no letters given where no significance was found between the three reproductive stages. The gray box indicates the recommended temperatures (10 to 25°C) for sows or boars > 100 kg (FASS, 2010).

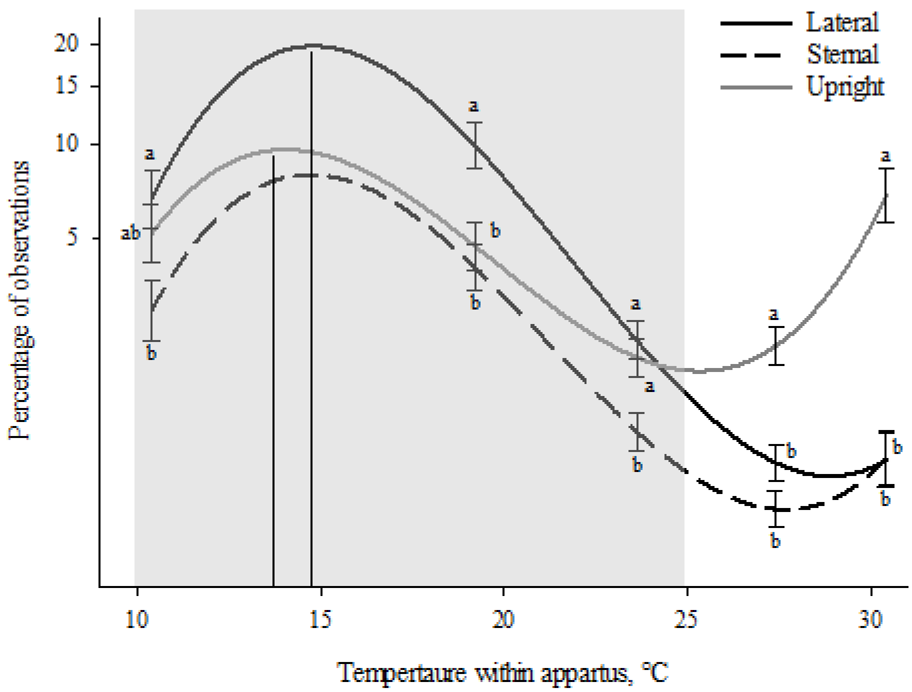

Figure 4.8 Percentage of observations in different temperatures within the thermal gradient based on posture. Data are plotted by postures: lateral and sternal lying, and upright. Temperature within the thermal apparatus is plotted on the x-axis and percentage of time observed are plotted on the y-axis as a log10+0.001 scale. Cubic peaks are indicated by solid vertical lines. Standard error bars are located at the temperatures of the five thermal zones (10.4°C, 19.2°C, 23.6°C, 27.5°C, and 30.5°C). Different letters denote significant Tukey pairwise comparisons (*P* < 0.01). The gray box indicates the recommended temperatures (10 to 25°C) for sows or boars > 100 kg (FASS, 2010).

CHAPTER 5. OVERALL CONCLUSIONS

5.1 Summary and Overall Conclusions

Housing pigs within their thermal comfort zone maximizes productivity and performance. Outside of these temperatures pigs can experience thermal stress resulting in increased production costs and decreased welfare. To determine when a pig is experiencing thermal stress there are several indices available; however, some indices extrapolate the thermal indices used for dairy cattle to be applied to pigs (Hahn et al., 2009). Unfortunately, this work is likely to be flawed due to differences in various factors that influence thermoregulation between production species (i.e., surface area to mass ratio, hair cover, and ability to sweat). In addition, the *Ag Guide* recommendations is outdated and based on theoretical modeling rather than utilizing a thermal preference choice presented to a pig. Furthermore, the *Ag Guide* does not take into consideration different parameters that may alter thermal preference such as exposure to early life thermal stress, group numbers, or pregnancy and thus may require updating. Therefore, the goal of this research was to determine the thermal comfort zone of pigs based on a preference, and how this preference might be altered by a variety of factors.

The focus of the second chapter was to test if early life thermal stress altered preference of pigs later in life. Early life heat stress (ELHS) piglets preferred similar temperatures as early life thermoneutral (ELTN) and early life cold stress piglets (ELCS). However, ELCS piglets demonstrated a warmer temperature (+2.2°C) preference when compared to ELTN piglets. These data supporting that early exposure to ELCS can alter piglets' temperature preference later in life. The only difference between the treatments of ELCS and ELTN was the lack of a heating lamp, indicating that heating lamps are a crucial source for piglets that alters thermal comfort later in life. This research also demonstrated the need to update the *Ag Guide* recommendations as the thermal preference of ELTN pigs was cooler compared to the recommendations that are outlined. However, due to a lack of a bell curve for ELTN pigs we were unable to determine their truce thermal preference. Thus, the focus of chapter 3 was to elucidate this information and determine how group size alters thermal preference.

Chapter 3 sought to eliminate the issue of not blocking for weight in the early life thermal stress study and account for group size influences on thermal preference. In short, this study found that increased group size and BW resulted in a cooler thermal preference probably due to the additional thermoregulation provided by proximity to a littermate and decreased surface area to volume ratio. However, no interaction between both size and weight category appeared to affect thermal preference during times of inactivity. However, when looking at posture this interaction can be observed perhaps due to only analyzing inactive time for behavior. These data provide guidance on temperature recommendations for housing individual piglets for research purposes and support that a group of 2 and 4 piglets have similar benefits when housed within their thermal comfort zone. Finally, since there was demonstrated need to update the *Ag Guide* for young pigs and to account for lack of information on reproductive stages of sow the goal of the final study was to determine the thermal preference for various reproductive stage of sows.

Chapter 4 sought to determine the thermal preference of sows. In short, reproductive stage did alter preference and in agreement with our prediction, late-gestation sows preferred the coolest temperatures compared to open and mid-gestation sows. Although thermal preference was within the *Ag Guide* recommendations (10 to 25°C: FASS, 2010), all sows preferred temperatures between 12.6 to 16.4°C. This indicates a need for further research to create temperature recommendations on modern pigs. This research demonstrated what temperatures sows prefer based on reproductive stage and that these temperatures are close to the average of the recommended range. Albeit a noticeably narrower range than the current guidelines. Housing sows within their TCZ will likely yield better production and improved well-being. Conducting research that utilizes an animal's innate motivation to seek out their preferred temperatures allows for data collection from non-stressed animals and yield improved results.

Overall, these studies demonstrate that thermal preference can be influenced by different factors: early life cold stress, increasing group size and body weight, and reproductive stage. This research indicates that thermal preference not only should look at different stages of production or different factors that may influence thermal preference, but also regularly be conducted to ensure the most up to date guidelines exist for both producers and researchers.

5.2 Future Research

This research remains incomplete, in that, these studies demonstrated a need to update the Ag Guide for other productive stage of pig such as board, grow and finish stage as well as preweaning piglets. Additional research into the other stages of production can be valuable for producers to ensure their animals remain within their thermal comfort zone to be highly productive animals and have positive welfare. Further, this research, indicates that thermal preference is a continuum, changing with improved genetic selection of swine and resulting in animals that have an increased metabolic heat production. Thus, research on thermal preference should be conducted regularly to provide up to date guidelines for researchers and producers and further work is needed to determine the thermal preference of grow-finish stage pigs and boars.

Previous research on fish (Zhang et al., 2018; Santi et al., 2017) and iguanas (Deen and Hutchison, 2001) indicates that thermal preference is altered after a Lipopolysaccharide (LPS) challenge. An LPS challenge evaluates an animal's ability to respond to an inflammatory stimulus by mounting an acute phase response. Individuals that were administered an LPS challenge preferred warmer temperatures compare to healthy individuals. This same research could be applied to pigs, and thermal preference could be used to determine the health status of an individual that might be seeking warmer temperatures compared to the expected temperature preference.

5.3 References

Deen, C. M., and V. H. Hutchison. 2001. Effects of lipopolysaccharide and acclimation temperature on induced behavioral fever in juvenile *Iguana iguana*. Journal of Thermal Biology 26: 55-63. doi:10.1016/S0306-4565(00)00026-7

Federation of Animal Science Societies. (FASS). 2010. Guide for the care and use of agricultural animals in research and teaching. 3rd ed. Champaign (IL): Fed. Anim. Sci. Soc. Chap. 11.

Hahn, G. L., Gaughan, J. B., Mader, T. L., & Eigenberg, R. A. (2009). Thermal indices and their applications for livestock environments. In *Livestock energetics and thermal environment management* (pp. 113-130). American Society of Agricultural and Biological Engineers.

Santi, S. et al. 2017. Temperature preference and sex differentiation in African catfish, *Clarias gariepinus*. Journal of Experimental Zoology Part A: Ecological and Integrative Physiology 327: 28-37. doi:10.1002/jez.2066

Zhang, Q., M. Kopp, I. Babiak, and J. M. Fernandes. 2018. Low incubation temperature during early development negatively affects survival and related innate immune processes in zebrafish larvae exposed to lipopolysaccharide. Scientific reports 8: 1-14