

## ABSTRACT

ANDERSON, LAUREN ELIZABETH. Changes in Growth Performance, Cytokine Profile, and Behaviors of Growing Pigs Subjected to Heat Stress. (Under the direction of Dr. Jonathan Holt).

Heat stress is one of the most pervasive problems in animal agriculture, especially in swine production, and the rise in global temperature increases the prevalence of this threat. Due to the lack of functional sweat glands, pigs depend on other, often more detrimental, physiologic methods to diffuse heat. The induction of the stress response in swine results in welfare concerns and performance losses. The ability to non-invasively and objectively recognize heat stress before it becomes pathogenic could lead to more effective prevention and treatment. However, in order to achieve this, a non-invasive strategy such as video monitoring or the measurement of surface temperature must be correlated with measurable physiological changes. To evaluate possible physiologic parameters, two studies were conducted. The first project (Chapter II) assessed individually housed pigs exposed to a constant, low-grade heat stress (HS; mean 27.76°C) or thermoneutral temperatures (CON; mean 19.53°C) for 15 d, followed by a 7 d thermoneutral recovery period. Intermittent video monitoring as well as rectal temperatures, performance measurements, and serum samples for cortisol and cytokine analysis were collected. HS pigs had a lower ADFI and ADG compared to CON. Serum concentrations of IL1 $\beta$  and IL12 were greater in HS pigs, and IL8 was greater in CON pigs. HS pigs visited the feeder less frequently in the morning than CON pigs, and HS pigs visited the drinker more frequently on d 9 compared to CON. HS pigs laid down in both lateral and ventral postures more frequently than CON on d 2, d 6, and d 9. Overall, heat stress reduced performance, modulated cytokines in circulation, increased lying behavior frequency, and altered drinking frequency and daily time spent eating. The second project (Chapter III) trial evaluated group housed pigs exposed to a constant heat stress. Pigs (n = 64; 4 per pen) were exposed to a constant, low-grade heat stress

(HS; mean 28.63°C) or thermoneutral temperatures (CON; mean 24.75°C) for 18 d, followed by a thermoneutral period from d 19 to d 21, then a diurnal heat stress for both groups from d 22 to d 30. While unintended, the diurnal heat stress allowed for the assessment of acclimation following repeat exposure. Intermittent video monitoring, performance measurements, rectal and surface temperatures, and serum for cortisol and cytokine analysis were collected. In addition, subsets of pigs were euthanized on d 15, and d 30 for collection of lung, ileum, and jejunum epithelial samples. Samples were analyzed for cytokine profile and intestinal histology. The ADG for CON was greater than HS on week 3, but the GF for HS was greater on week 1 than CON. Surface temperature and respiration rate were greater in HS pigs between d 2 and d 15. Surface temperature for CON dropped below HS on d 18 and d 22. There was a significant time by day interaction for cortisol concentration, regardless of treatment. HS had a greater cortisol concentration on d 2, but the CON concentration was greater on d 22 and d 25. HS pigs had a greater systemic concentration of IL12. CON pigs had greater IL12 concentration in the jejunal mucosa. HS had greater concentrations of IL4 and IL12 in the BALF. Overall, low-grade, chronic heat stress reduces growth performance, increases systemic cortisol, and changes posturing, eating, and drinking behaviors. Additionally, circulating, lung, ileum, and jejunum cytokine profiles exhibit inflammatory activity occurring in tandem.

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Changes in Growth Performance, Cortisol Concentration, Cytokine Profile, and Behaviors of  
Growing Pigs Subjected to Heat Stress

by  
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## **BIOGRAPHY**

Lauren Anderson is from Quincy, Illinois where she grew up without much exposure to agriculture. She earned her Bachelor's in Animal Science at the University of Missouri, where she became interested in agriculture. During her undergraduate, she completed research projects in both animal functional genetics and plant biotechnology. After her graduation, she began her career at a plant biotechnology company in St. Louis, where she worked for 2 years. Following a move to North Carolina, she briefly worked for a human clinical lab company before returning to school and animal agriculture. She started her Master's in Animal Science with Dr. Jon Holt in 2018 at NC State. She plans to continue with her PhD with Dr. Holt, and she ultimately wants to work in an R&D position at a small or startup company.

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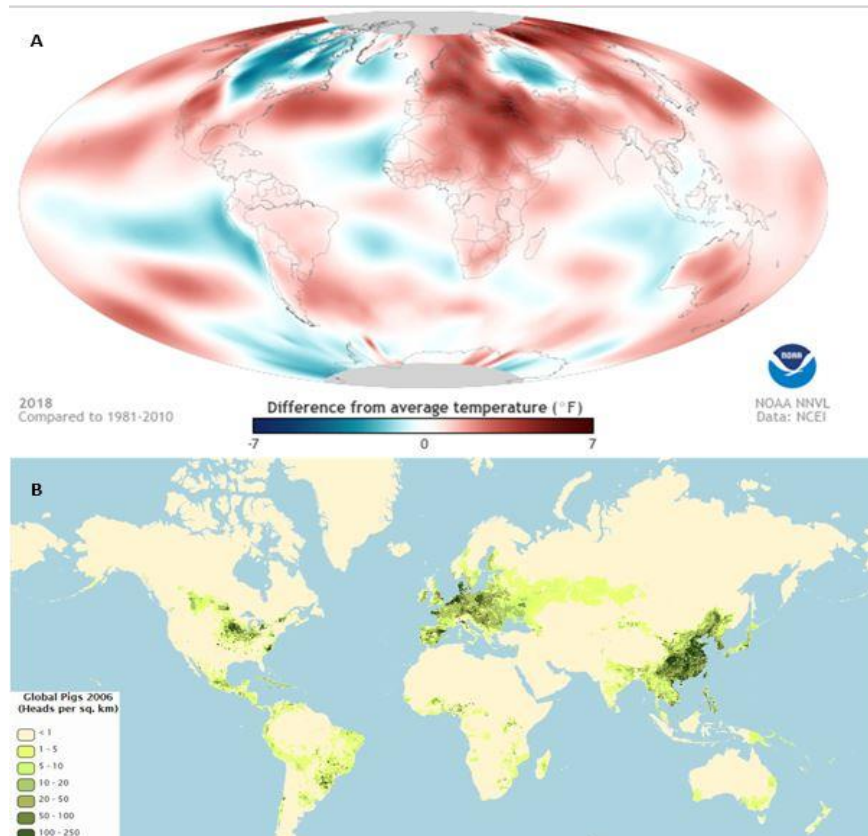
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## Chapter I: Literature Review

### Introduction to Heat Stress and Pork Production

High environmental temperatures pose a physiologic threat to livestock that can result in a reduction in welfare and performance for the animal and a loss of profitability for livestock producers. Among abiotic stressors, temperature is ubiquitous in its impact across species and regions (Horowitz, 2002). The National Oceanic and Atmospheric Administration (NOAA) reports that the average global temperature has been increasing, with 9 out of the 10 warmest recorded years occurring since 2005 (Lindsey and Dahlman, 2020). Much of the world's pork production occurs in regions that are impacted by rising temperatures (Gilbert et al., 2018) (Figure 1.1).

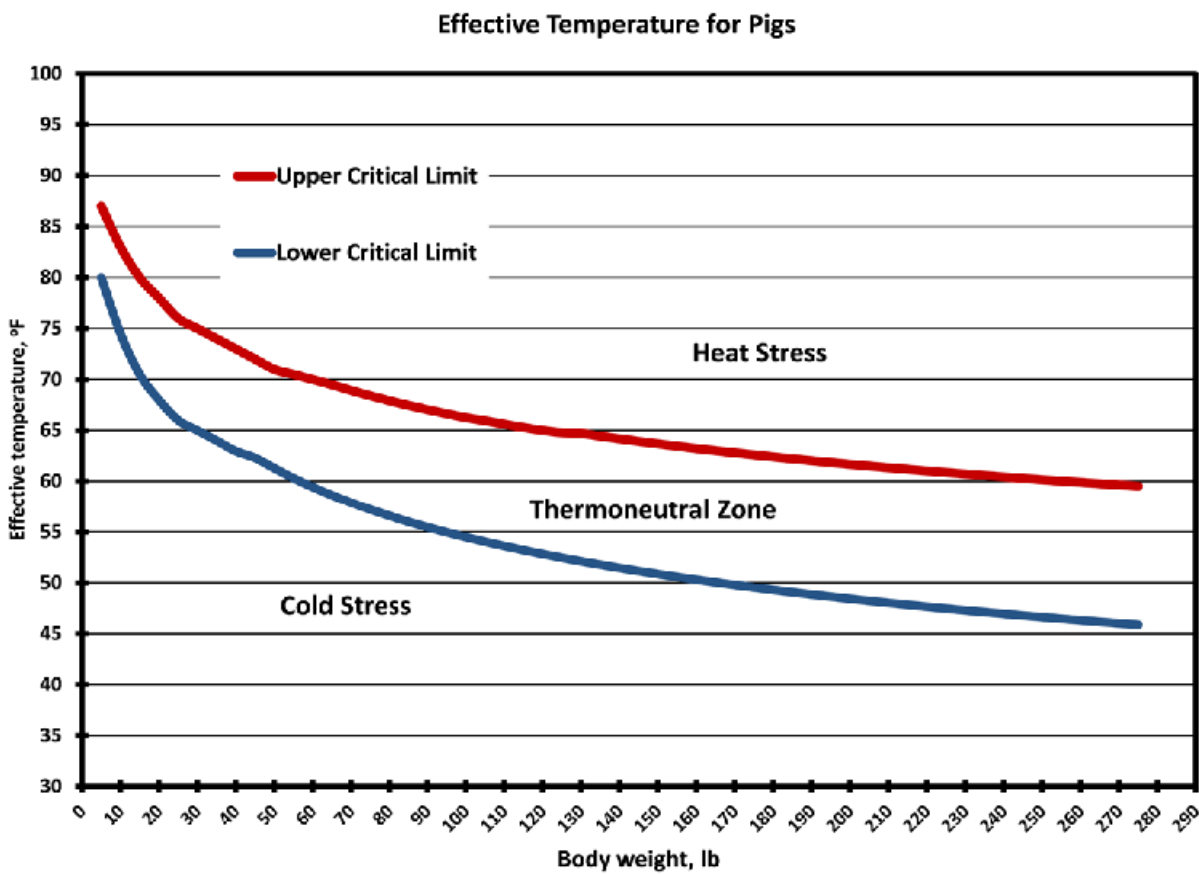


**Figure 1.1:** Rising Global Temperatures and Pig Production. (A) Global temperature change from 1981-2010 adapted from Lindsey and Dahlman (2020) and (B) global pigs per square km adapted from Livestock Geo-Wiki (2006) highlight the overlapping regions in which rising temperature could impact pig production.

The annual loss to the swine industry attributed to heat stress in the United States was about \$300 million (St-Pierre et al., 2003). Considering this was published in 2003, this number has likely increased over time. With both genetic advancement and global temperature on the rise, the economic threat of heat stress in swine production is an ever-growing problem. Energy expenditure to reduce heat load is especially prevalent in modern genetic lines that grow quickly and efficiently accrete more lean muscle tissue than has been seen historically (Rhoads et al., 2013; Ross et al., 2015). There is a positive relationship observed between basal heat production and growth performance in swine. The energy metabolism required for fast, lean growth produces more heat when compared to slower growing pigs, as demonstrated by the 30% increase in heat generation in production pigs in 1995 as compared to production pigs in 1959 (Brown-Brandl et al., 1998). During hyperthermia, energy is diverted from growth to temperature homeostasis, compromising performance (Brown-Brandl et al., 2001; Brown-Brandl et al., 2004). The setback of heat stress undermines the genetic and nutritional production efficiency progress that has been made in the swine production over the years (Ross et al., 2015), and the problem is likely to grow as selection for efficient pigs intensifies (Rhoads et al., 2013).

A negative correlation between atmospheric temperature and ADG can be observed in finishing pigs at temperatures exceeding their thermoneutral zone (Heitman et al., 1958). A loss in body weight can be observed within 24 hours of heat exposure (Gabler and Pearce, 2015). A study conducted in 1991 reported that pigs raised in environments ranging from 22.5°C to 35°C had a 16.3% slower growth rate and a 10.9% reduction in feed intake when compared to pigs raised at 20°C (Bridges et al., 1992). Another study noted that when temperatures increased from 20°C to 30°C, 45 kg and 85 kg pigs reduced feed intake 65-74 g/d per degree increase (Huynh et al., 2005). Reduction in nutrient intake during hyperthermia is a conserved response across many

species (Rhoads et al., 2013). Pigs rely on expending energy to dissipate heat in a hyperthermic environment (Lambert, 2009), reducing the production of heat metabolically by directly reducing feed intake and indirectly by altering the hierarchy of metabolism and tissue synthesis (Pearce et al., 2013a; Sanz Fernandez et al., 2015). Thermolytic capacity decreases with an increase in body weight (Figure 1.2), requiring even more energy utilization for heat dissipation during growth (Miller, 2012; Rhoads et al., 2013).



**Figure 1.2:** Effective Temperatures for Pigs at Different Weights (Miller, 2012).

Fertility for both males and females are impacted by heat stress. Boars experience reduced ejaculate volume, sperm count and motility, and reduced conception rates. Heat stressed gilts and sows lose body condition due to reduced feed intake, and as a result, they have

decreased litter sizes from early gestational losses, reduced milk production, and delayed return to estrus after farrowing. Heat is one of the main components of seasonal infertility, which is a prominent economic problem in the swine industry (Banhazi et al., 2008). Meat quality detriments have also been observed after pig exposure to heat stress (Gabler and Pearce, 2015). Physiologic heat abatement strategies employed by the pig have economic drawbacks in the form of physiologic dysfunction, reduced growth and potential death loss in production animals (St-Pierre et al., 2003).

While heat stress is a welfare and profitability concern, there is a challenging balance of optimizing the temperature for the pigs and for the economics of the facility housing them. Barn design, technological implementation, and management strategies have improved the alleviation of heat stress, however, understanding the underlying physiological mechanisms behind performance loss will lead to more effective abatement strategies and improvements in swine welfare and production.

### **Methods of Passive Heat Exchange with the Environment**

Thermoregulation is a process driven by the sympathetic nervous system that reads environmental stimuli and governs the appropriate response to maintain internal thermoneutrality (Ho et al., 2012). It is one of the primary tasks in the maintenance of homeostasis in endotherms. It is also among the most ecologically significant, as environmental variability is a prevalent abiotic stressor in production agriculture (Schmidt-Nielsen, 1981; Horowitz, 2002; Collier and Gebremedhin, 2015). In a thermoneutral setting, no energy expenditure is required to maintain homeostatic equilibrium in terms of body temperature. A thermoneutral environment increases potential energy availability for growth and reproduction, so it is a vital criterion to maintain in

order to maximize production capabilities (Collier and Gebremedhin, 2015). This is especially true in swine, which are not particularly efficient at heat dissipation due to their lack of functional sweat glands. Pigs are only able to produce sweat at 5% of the rate of humans (Morrison et al., 1967).

Sensible heat exchange with the environment occurs when the animal's temperature exceeds that of its surroundings, creating a temperature gradient. This allows for heat to be exchanged with the environment without the animal needing to utilize energy to equalize. Sensible heat exchange ensues in the form of conduction, convection, or radiation (Collier and Gebremedhin, 2015). These methods are most effective when used in tandem with one another (Banhazi et al., 2008). The efficiency of sensible heat exchange decreases as temperature increases (Collier and Gebremedhin, 2015). The primary strategy for many producers is indoor housing and confinement structures for the control of the pig's environment to maximize energy use for growth. Housing accommodations like temperature and humidity control, slatted flooring, strategic ventilation, and sprinklers can be employed to improve the capabilities of these heat exchange methods. Other management practices including optimal stocking density and nutrition decisions can also be layered with housing decisions to help keep pigs in stable thermal conditions (Banhazi et al., 2008). A total of 52.8% of the world's pork production is indoors, not only for environmental control, but it can reduce the carbon footprint, pollution management, and energy efficiency per unit of product produced (Schauberger et al., 2019). Environmental modification must be cost effective or have an economic return on investment for the producer in order for it to be practically applied (Pedersen and Ravn, 2008).

### *Conduction*

Conduction occurs by contact between the pig and a surface, and it can be facilitated by postures and behavior. Behavior alteration is the first indicator of heat stress (Huynh et al., 2005). A standing pig has very little contact with the floor, and there is minimal heat loss through the hooves. However, lying increases the skin contact with the floor, thus increasing the potential for the molecular mode of heat exchange with the environment (Collier and Gebremedhin, 2015). A heat stressed pig spends more time lying than a pig in thermoneutral housing (Banhazi et al., 2008). Pigs normally lie in contact with pen mates with their legs pulled close to their bodies, but a heat stressed pig will maximize its contact with the floor by lying laterally with its legs stretched out and not in contact with other pigs. This change in behavior can be observed at 25°C for a 25 kg pig and 20°C for a 100 kg pig (Nienaber and Hahn, 2004). However, lying time is increased more by shipping stress than heat stress (Hicks et al., 1998). Increased lying time during heat stress could be to facilitate heat exchange with the floor but lying following shipping stress may indicate that stress itself increases lying, not just the heat abatement effort (Hicks et al., 1998).

### *Convection*

Convection occurs when cooler gases or fluids remove heat as they move across a warmer surface, in this case, the animal. There are two types of convection, free and forced. Free convection occurs when the differing temperature itself causes movement of gasses. Air density decreases with the increase in air temperature acquired from the body heat of the animal, and gasses surrounding the animal move to carry heat away from the surface of the animal. Convection can also be supplemented by behaviors such as a pig spraying itself with water from

a drinker or orienting itself towards a fan (Banhazi et al., 2008). Heat stressed pigs increase their water to feed ratio with increasing temperature. At 20°C the water to feed ratio was reported to be 2.2, at 25°C it was 3.8, and at 30°C it was 6.3; however, much of the water used is for wetting the skin and floor to cool off, not drinking (Nienaber and Hahn, 2004). Pigs seek wet places to lie when heat stressed, and they may try to wet themselves by pen fouling and lying in excrement, which increases immune challenges within the herd, and this can also result in pigs lying beneath the drinker, causing guarding and fighting behavior near the water source (Huynh et al., 2005).

Forced convection requires exogenous energy use to move air. Natural ventilation in curtain sided barns or mechanical ventilation, such as a fan or pressure gradient, can remove an insulating boundary layer of air from around the skin that could be trapped by hair without energy use by the animal (Collier and Gebremedhin, 2015). Increasing air speed by forced convection is employed in barns both for the maintenance of air quality and to maximize convection in swine facilities. Convective heat transfer from the pig to the environment has many complex variables and depends on the size and surface area of the pig, air velocity, turbulence intensity, hair and surface roughness of the pig, the orientation of the animal, and the temperature difference between the pig and the environment. Collectively, an increase in air speed and turbulence immediately enveloping the animal will increase heat release from the pig to the surrounding moving air (Collier and Gebremedhin, 2015). When environmental temperature exceeds thermoneutrality and the temperature gradient is switched to environment-to-animal, free convective heat exchange can add more of a heat load to pigs unless forced convection is employed to remove hot air (Collier and Gebremedhin, 2015).

## *Radiation*

Radiation heat exchange occurs when energy in the form of heat is emitted by a source, for example, the sun, a heater, or the animal itself (Nakamura and Morrison, 2008). Swine housing is often equipped with radiant heaters to protect animals from their lower critical temperature and the onset of cold stress (Banhazi et al., 2008; Pedersen and Ravn, 2008). Pigs housed indoors are protected from solar radiant heat, but they can be impacted by the heat of other pigs in their surroundings. Appropriate stocking density can protect exacerbation of heat stress from the body heat of pen mates (Banhazi et al., 2008).

## **Acute Heat Stress Recognition: The Sympathetic-Adrenal-Medullary “First-Wave”**

### **Response**

If thermoneutrality cannot be maintained passively, latent heat exchange is employed in addition to modifying metabolic heat production in response to environmental temperatures outside of the thermoneutral comfort zone (Nienaber et al., 1999). This occurs when the environmental temperature of the pig reaches 27.6°C (Brown-Brandl et al., 2013). General activity of the pig is also reduced to prevent energy use and heat production (Nienaber and Hahn, 2004). This point is called the upper critical temperature (UCT), and it can be established when the core temperature and respiration of the pig increase or a reduction in voluntary feed intake is observed, indicating behavioral and metabolic heat abatement attempts (Nienaber et al., 1999; Kovács et al., 2005). Homeorhetic influence directs nutrients toward productive functions in the pig under thermoneutral conditions, but under acute heat stress, energy expenditure and activation of the autonomic nervous system is required for the return to homeostasis in the first-

wave stress response that occurs within seconds of the stressor (Sapolsky et al., 2000; Salak-Johnson and McGlone, 2007).

Sequentially, sensation of environmental stimulus and internal state are processed, this information is transmitted to the brain and sympathetic nervous system via afferent neural pathways, and the appropriate response for the return to homeostasis is initiated from the sympathetic-adrenal-medullary (SAM) system (Collier and Gebremedhin, 2015). Both epinephrine and norepinephrine, the core products of the SAM system, are greater in heat stressed animals than thermoneutral animals (Afsal et al., 2018). Their influence on the cellular and physiologic responses are critical for the survival of stress (Salak-Johnson and McGlone, 2007; Collier and Gebremedhin, 2015).

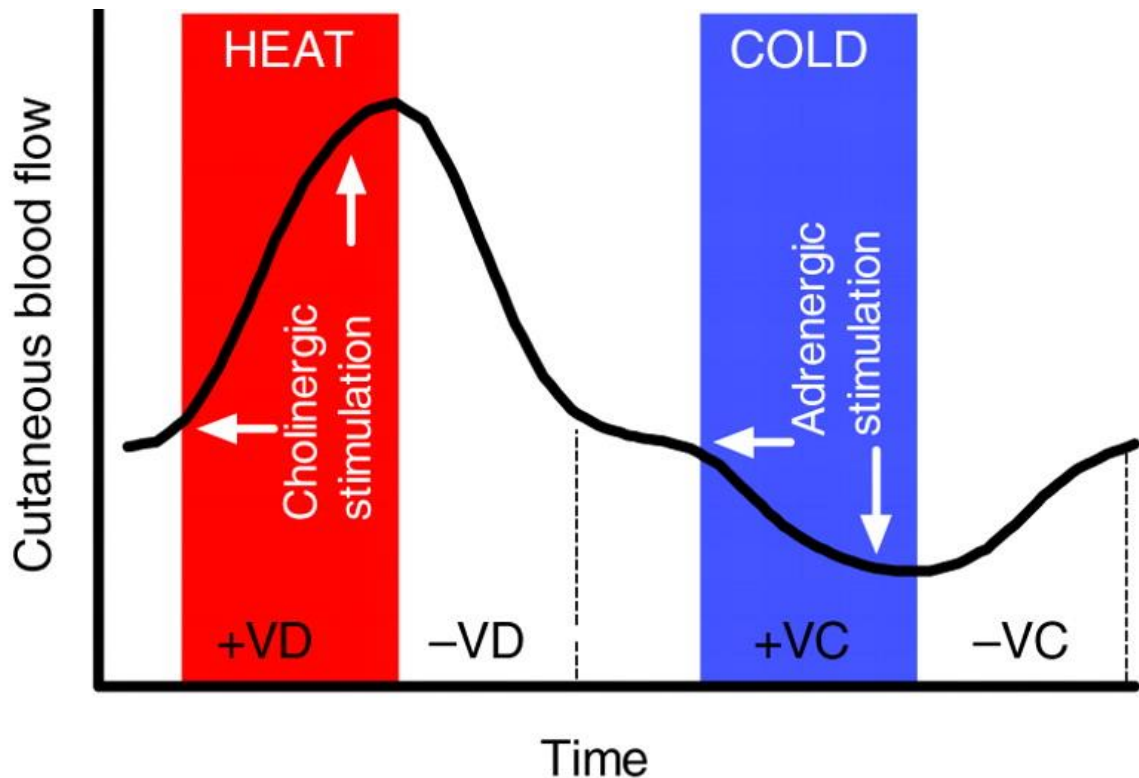
### *Cellular Response*

The paraventricular nucleus of the hypothalamus receives afferent periphery signals during heat stress and stimulates the production of corticotrophin releasing factor (CRF), which activates the production of norepinephrine in the locus coeruleus (LC) and norepinephrine and epinephrine in the adrenal medulla (Salak-Johnson and McGlone, 2007; Afsal et al., 2018). A key player in this response at very high temperatures is transient receptor potential cation channel subfamily V member 1 (TrpV1), which is capable of environmental nociception (Ho et al., 2012). In an experiment using mice with a TrpV1 knockout, they did not avoid injurious heat when given the opportunity (Caterina et al., 2000). TrpV1 was originally identified as a capsaicin receptor, a compound in chili peppers that causes pain and burning, but it has more recently been shown to respond to noxious heat and other stressors. Activation of TrpV1 can stimulate proinflammatory mediators, including substance P, prostaglandins, bradykinins, and cytokines (Collier and Gebremedhin, 2015).

Several transcription factors mediate the initial gene expression cellular response to heat stress, notably heat shock factor 1 (HSF1) among others (Collier and Gebremedhin, 2015). The activation of HSF1 was shown to influence the expression of genes involved in energy generation, carbohydrate metabolism, oxidative stress modulation, ubiquitination, proteolysis, and other cellular functions in a genome wide study (Hahn et al., 2004). The activation of HSF1 and hypoxia inducible factor (HIF1A), another transcription factor induced by heat stress, are essential to the induction of the cellular heat shock response, which is highly conserved across species (Collier and Gebremedhin, 2015; Gabler and Pearce, 2015).

### *Cardiovascular Response*

The rapid sympathetic premotor neurons response to heat stress that initiates the synthesis of norepinephrine and epinephrine is integral in initial thermolysis (Afsal et al., 2018). This results in the thermoregulatory cardiovascular response, increasing the heart rate, shifting vascular tone, and adjusting metabolic priority (Nakamura and Morrison, 2008; Afsal et al., 2018). Blood perfusion to the skin increases via noradrenergic stimulation of vasoconstriction in the core and cholinergic stimulation of cutaneous vasodilation in order to facilitate radiant heat exchange with the environment (Seebacher, 2009). The thermoreceptors at the core of the body are not responsible for thermoregulatory adjustments except in times of extreme heat stress when the initial response is not enough to protect the core body temperature from being impacted (Seebacher, 2009) (Figure 1.3). The thermoneutral surface temperature of pigs lies between 17.4°C and 23.2°C depending on the size and age of the animal, but it increases to 35.7°C to 40.5°C when the redistribution of blood flow for heat abatement begins (Brown-Brandl et al., 2013).



**Figure 1.3:** Cutaneous Redistribution of Blood Flow by Vasodilation (VD) and Vasoconstriction (VC) During Thermal Challenge. Adapted from Seebacher (2009).

Evaporative heat loss by the skin of a pig is relatively constant and ineffective without exogenous wetting, even with an increase in ambient temperature due to their lack of ability to produce enough sweat for evaporation (Morrison et al., 1967). The use of mist to assist with evaporative cooling is more effective than sweating and is used as supplemental evaporative cooling method for swine (Collier and Gebremedhin, 2015). Banhazi (2008) reported that spray cooling resulted in a 5.8% increase in feed intake, a 4.5% improvement in feed conversion, and an 11.2% increase in ADG. However, the rate at which water can evaporate from the surface of the pig is dependent on environmental factors such as temperature, humidity, and air movement acting simultaneously (Collier and Gebremedhin, 2015). The efficiency of this form of latent heat and moisture exchange between the pig and the environment can be improved when employed in tandem with evaporative cooling in the form of panting (Robertshaw, 2006).

## *Respiratory Response*

Panting is an autonomic response that requires increased blood flow and oxygen use by the respiratory muscles, a reduction of blood flow to muscles not involved in respiration, and a metabolism priority shift to respiratory muscles (Robertshaw, 2006). The net use of oxygen is the same due to this balance (Robertshaw, 2006), however, the shift from non-respiratory muscles is detrimental in swine production (Lambert, 2009). When breathing in a thermoneutral environment, sensible heat exchange occurs internally when the core body temperature exceeds the temperature of inspired air and water vapor (Collier and Gebremedhin, 2015). The inspired air is heated by the body passively and water in the breath and respiratory tract is actively vaporized and expelled during expiration, removing some internal heat (Robertshaw, 2006). This thermolytic strategy is bolstered by an active, controlled change in breathing rhythm and depth during panting (Collier and Gebremedhin, 2015). The frequency of respirations is increased, but there is a reduction in overall tidal volume (Robertshaw, 2006). This increases air movement in the upper respiratory tract, especially the dead volume of air not involved in gas exchange, without increasing alveolar ventilation, maximizing heat exchange while diminishing the risk of alveolar hyperventilation that could result in dysregulation of pH homeostasis (Robertshaw, 2006). A large part of the heat dissipation in panting occurs via moisture and heat exchange at the nasal epithelial lining, which can selectively cool the blood supply to the brain (Robertshaw, 2006). Initial change in rate of respiration can be observed at about 22.4°C in 60 kg pigs (Brown-Brandl et al., 2004). At temperatures above 23.1°C, an increase of 4.9 breaths/minute occur per degree temperature increase (Brown-Brandl et al., 2003; Nienaber and Hahn, 2004).

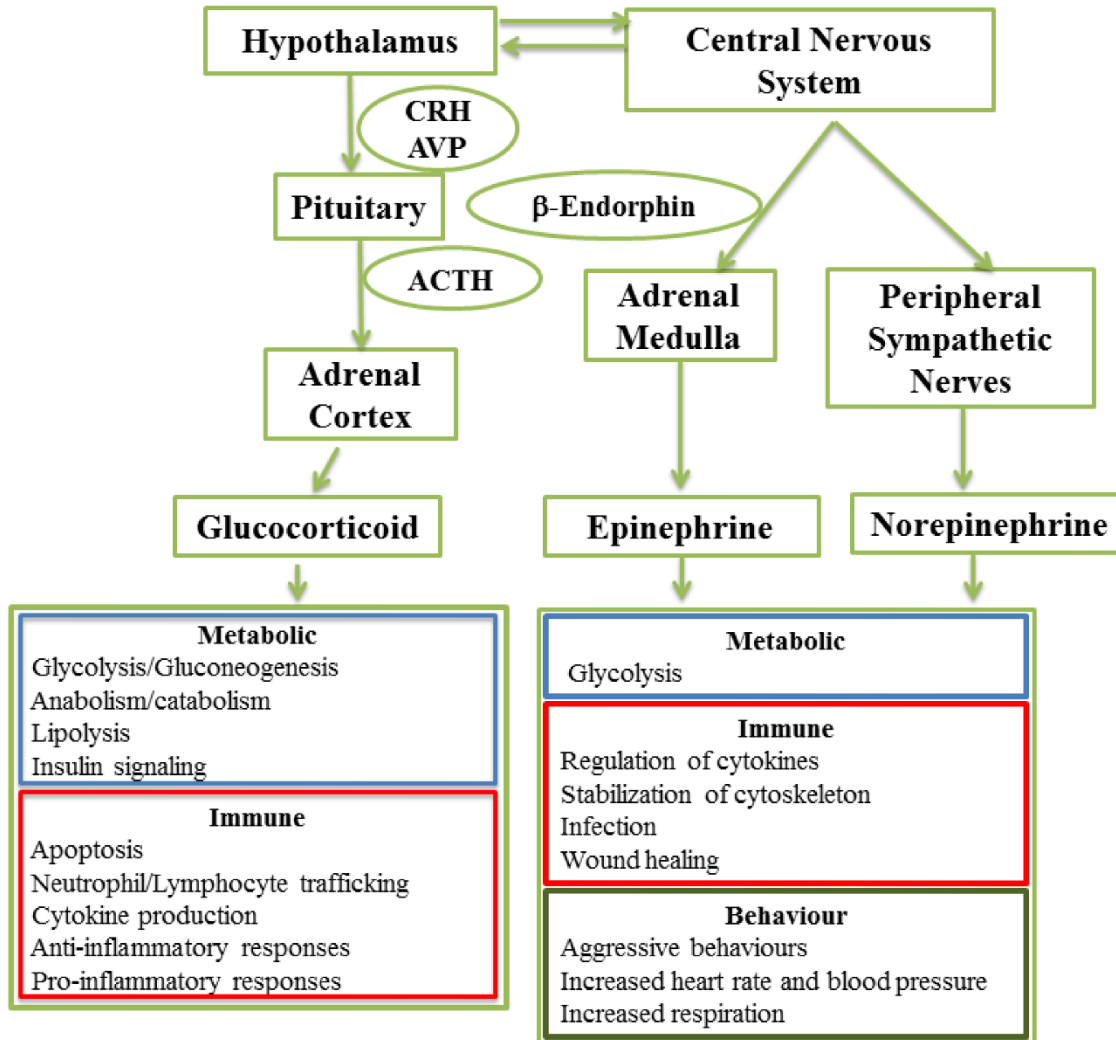
### *Metabolic Response*

During short term exposure to heat stress, changes can be observed including increase of water intake, direct reduction of feed intake, and indirect metabolic alterations to reduce heat production (Song et al., 2011; Pearce et al., 2014). Pearce et al. (2014) reported that gastric inhibitory polypeptide was lesser in heat stressed pigs, but ghrelin was greater, resulting in increased gastric motility and secretion. Pearce et al. (2014) also found that cholecystokinin was lower, reducing digestive enzyme secretion from the pancreas, and neuropeptides that affected appetite were suppressed in heat stressed pigs. Together, it is likely that these changes occur to reduce the production of metabolic heat during heat stress (Pearce et al., 2014). Increased blood glucose can be observed during heat stress despite the reduction in feed intake (Pearce et al., 2014), which could be due to increased HPA and SAM activation in the pig (Afsal et al., 2018). This shift can modify energy mobilization in circulation (Salak-Johnson and McGlone, 2007). During heat stress pigs have been observed eating the same number of meals and at the same rate as pigs in thermoneutral conditions, but they spend less time eating per meal (Nienaber and Hahn, 2004), which may be attributable to the modification in gastric peptides attenuating appetite and digestion, as observed by Pearce et al. (2014).

### **HPA Activation**

In addition to catecholamines released from the adrenal medulla during the initial SAM heat stress response (Salak-Johnson and McGlone, 2007), the release of CRF by the paraventricular nucleus of the hypothalamus also activates the second wave of the stress reaction (Fulford and Harbuz, 2005). CRF affects the anterior pituitary via the hypothalamic hypophyseal portal system, stimulating the release of adrenocorticotrophic hormone (ACTH) (Fulford and

Harbuz, 2005). ACTH travels in circulation to the zona fasciculata of the adrenal cortex, which releases glucocorticoids, most notably, cortisol (Fulford and Harbuz, 2005). Glucocorticoids participate in self-regulation of the HPA axis in a negative feedback loop impacting the paraventricular nucleus and the production of CRF and overall activation of the HPA axis (Fulford and Harbuz, 2005). In times of stress, the negative feedback loop can be overridden and converted into a feedforward system to stimulate the HPA axis further (Valentino and van Bockstaele, 2005). Both increased ACTH and cortisol has been observed in heat stressed pigs (Marple et al., 1974). Individuals have highly variable response intensity to the same stressor in terms of corticosteroid release (Sapolsky et al., 2000; Salak-Johnson and McGlone, 2007). Also, different stressors, such as heat, cold, dehydration, restraint, social stress, etc., activate different pathways, but most converge at the paraventricular nucleus and elicit a similar response (Kovács et al., 2005; Salak-Johnson and McGlone, 2007). Together, the SAM and HPA axis responses coordinate an attempted return to homeostasis in order to protect from the long term effects of stress (Chen et al., 2015) (Figure 1.4).



**Figure 1.4:** Activation and Effect of the HPA Axis. Stimulation of the hypothalamus activates the pituitary and CNS, which ultimately result in the production of glucocorticoids and catecholamines and the initiation of the stress response.

### *HPA and SAM and Immune Response*

The immune response to stress is partially regulated by the HPA axis and SAM system (Salak-Johnson and McGlone, 2007). The time and duration of the stressor could result in different HPA and SAM outputs, and thus, different measurable immune markers (Salak-Johnson and McGlone, 2007). Cortisol secretes in a diurnal pattern; the highest concentration of circulating cortisol can be observed early in the morning as the pig is waking up, and it is lowest

in the evening (Martínez-Miró et al., 2016). It is generally accepted that cortisol suppresses the immune system and epinephrine can stimulate or suppress, but the inflammation reaction within the immune system is not that definitive (Salak-Johnson and McGlone, 2007). Adrenalectomized animals deficient in glucocorticoids have pathological overreaction to inflammatory stimulus, supporting the idea of stress induced immune suppression (Sapolsky et al., 2000). However, different stressors stimulate the release of glucocorticoids and catecholamines differently. Catecholamines reduce the production of IL12 and stimulate IL10 production (Salak-Johnson and McGlone, 2007). Exogenous cortisol and ACTH limit lymphocyte proliferation and antibody production, and cortisol alone inhibits interleukin IL2 secretion, and neutrophil function (Mcglone et al., 1991; Wallgren et al., 1994), though there is evidence that IL2 can develop resistance to cortisol (Skjolaas and Minton, 2002). Glucocorticoids have been shown to inhibit the production of IL4, IL5, IL6, IL12, interferon gamma (IFN $\gamma$ ), and tumor necrosis factor alpha (TNF $\alpha$ ), which modulate inflammation and T-cell differentiation, among others (Wiegers and Reul, 1998; Sapolsky et al., 2000; Salak-Johnson and McGlone, 2007). The sensitivity to glucocorticoid suppression varies for the different cytokines (Skjolaas and Minton, 2002). The production of IL10, an anti-inflammatory cytokine, is stimulated by glucocorticoids (Blotta et al., 1997; Salak-Johnson and McGlone, 2007). Some cytokines, such as IL1, IL4, IL6, and TNF $\alpha$  work in synergy with the HPA axis, and in turn, suppress their own production to protect from immune overreaction and autoimmunity (Wiegers and Reul, 1998; Sapolsky et al., 2000; Salak-Johnson and McGlone, 2007). Cytokine reactions to heat stress observed in pigs (Pearce et al., 2013b; Pearce et al., 2013a; Pearce et al., 2015) differ from those observed in humans, chickens, rodents, and cattle (Leon et al., 2006; Salak-Johnson and McGlone, 2007; Lambert, 2009). Immune modulation associated with heat stress may be a contributing factor in slowed muscle

growth, as the energy cost of an immune response likely directs away from productive growth (Hicks et al., 1998).

Overall, it cannot be said simply that stress suppresses the immune response, more that it modulates it and molds it into a more precise response that expends less energy to deploy. Instead of a general response, a more precise response is primed in the acclimated animal.

### *Chronic Heat Stress Response*

The response to the homeostatic signaling applied during acute heat exposure alters the sensitivity of signaling and response when maintained over long periods of time without relief (Collier and Gebremedhin, 2015). The endocrine system is not able to respond as quickly as the autonomic nervous system, so it is not functional in the acute response (Fulford and Harbuz, 2005). Though it is not as quick, activation still occurs within 10-20 minutes of the induction of stress (Sapolsky et al., 2000). When the SAM system alone fails to reduce stress on the pig's body, both the autonomic nervous system and the HPA axis become involved in the effort to return to homeostasis (Salak-Johnson and McGlone, 2007).

Under chronic heat stress conditions, reprogramming occurs at the level of gene expression and endocrine involvement (Collier and Gebremedhin, 2015). Differential expression of transcription factors and over 200 genes has been observed in the small intestine during heat stress (Yu et al., 2010). Among them are genes involved in management of misaggregated and unfolded proteins and the regulation of cellular growth, proliferation, and migration (Yu et al., 2010). Many of the differentiated genes are involved in one or more of 5 pathways: linoleic acid metabolism, MAPK signaling, xenobiotic metabolism, and arachidonic acid metabolism (Liu et al., 2009; Yu et al., 2010). Glucose transport is also impacted; hyperglycemia can be observed

after 24 hours of heat stress, as well as an increase in GLUT2 expression in the intestinal membrane (Pearce et al., 2013a). The physiologic thermoregulatory reaction is bolstered by a feed forward response before environmental heat impacts core body temperature (Nakamura and Morrison, 2008). Chronic heat stress can result in “heat stress fatigue” over time as the pig grows (Nienaber and Hahn, 2004). Long term exposure and repeat exposure can have pathobiological effects (Liu et al., 2009), and larger pigs are impacted more strongly than smaller pigs (Nienaber and Hahn, 2004).

## **Pathobiology**

### *Intestine*

Severe or long term stressors can result in abatement responses becoming harmful to health, production, and welfare of the affected animal. Sustained reallocation of blood and energy in an effort to dissipate heat upsets homeostatic balance and normal function (Lambert, 2009). In shunting blood to the periphery to increase heat exchange with the environment, splanchnic blood flow is reduced (Nakamura and Morrison, 2008). Within 2 hours of severe heat stress in which the vascular tone is shifted, hypoxia of the intestinal epithelium can occur, which causes the dysfunction of tight junction and secretory proteins that normally prevent permeation of toxins into circulation (Lambert, 2009; Pearce et al., 2013a), resulting in reduced nutrient absorption, endotoxemia, inflammation, and oxidative stress (Hall et al., 2001). Expression of heat shock proteins (Liu et al., 2009) and intestinal damage can be observed before damage in other organs, indicating that vasoconstriction occurs here first (Liu et al., 2009; Ross et al., 2015). Enterocytes require a large amount of energy to maintain a high cellular turnover during normal conditions, and it is especially susceptible to injurious stress that causes cellular necrosis

(Lambert, 2009; Liu et al., 2009; Ross et al., 2015). Within 3 days of exposure to heat stress, the worst of the damage can be observed in the ileum and jejunum, but the jejunum begins recovery within 6 days of alleviation of the stressor (Liu et al., 2009; Yu et al., 2010). Desquamation of the villi tips exposes the lamina propria and compromises capillary exchange of nutrients during gastrointestinal ischemia (Liu et al., 2009). A reduction in ileal mucosal sucrase and maltase reduce digestive efficiency (Song et al., 2009).

Intestinal damage can be observed in feed restriction without the application of heat stress, so the intestinal impact may be partially indirect. A pair-feeding study by Pearce et al., (2013a) of a heat stressed and thermoneutral group indicated that heat stressed pigs were able to gain more weight and had different biological markers in the post absorptive state. This could be due to HPA and SAM activation and altered mobilization of stored energy responding differently to heat stress and feed restriction stressors (Salak-Johnson and McGlone, 2007; Afsal et al., 2018). Increased sodium-potassium pump activity in the intestine of the heat stressed group was observed, but not the thermoneutral group, and this energetically expensive change was likely to maintain osmotic homeostasis (Pearce et al., 2013a).

Intestinal mucosa is integral for the protection against pathogenic bacteria, and its compromise is crucial to the pathobiology of heat stress (Pearce et al., 2013a). Heat stress results in increased circulating endotoxins and some acute phase proteins and reduced capability to clear or neutralize LPS (Pearce et al., 2013a). Heat stress also reduces haptoglobin, alkaline phosphatase and lysozyme, which would diminish protection against bacterial pathogens or LPS (Pearce et al., 2013a). If heat stress is sustained, core temperature will rise with surface temperature as the system fails to release heat and the vascular tone becomes dysfunctional, exacerbating heat related intestinal compromise (Ho et al., 2012). Overall, heat stress

compromises intestinal integrity, increases circulating endotoxins, suppresses intestinal glucose transport, and reduces digestive capacity and post-absorptive metabolism (Pearce et al., 2013b; Pearce et al., 2013a; Pearce et al., 2014).

### *Lung*

Panting aids in the dissipation of heat via evaporative cooling (Collier and Gebremedhin, 2015). However, after prolonged heat stress in which normal panting does not dissipate enough heat to regain thermoneutrality, dysfunction intensifies, panting switches from closed mouth to open mouth (likely to reduce air resistance), the respiration rate slows, and the tidal volume increases to exceed the dead volume of the upper respiratory tract (Robertshaw, 2006). The alveoli are not protected from hyperventilation as they are in normal panting, and there is an increased risk of respiratory alkalosis as the partial pressure requirements for gas exchange are compromised (Robertshaw, 2006). The effectiveness of evaporative heat exchange by panting is dependent on the relative humidity of the environment (Morrison et al., 1967). The expulsion of heated water vapor in the breath is not as efficient in high humidity situations, and if the water vapor in the environment is hot, evaporative cooling may not be effective at all (Morrison et al., 1967).

### **Acclimation to Heat Stress**

During chronic heat stress exposure, some of the efficiency of physiologic pathways are regained by acclamatory homeostasis (Horowitz, 2002; Afsal et al., 2018). Dynamic thermoregulatory span is expanded, permissive and preparatory signaling pathways and metabolic processes are optimized, and the physiological detrimental effects of stress are reduced

via morphological and biochemical changes (Horowitz, 2002). Behavior is modified, and the pig's endurance in hyperthermic conditions is improved (Horowitz, 2002; Afsal et al., 2018). Endocrine response is integral in this shift from stress response to stress management (Afsal et al., 2018).

The response to repeated exposure to stress is also modulated as certain responses become sensitized (Kovács et al., 2005; Collier and Gebremedhin, 2015). The threshold for the determination of heat stress is decreased upon repeat exposure (Kovács et al., 2005). Heat dissipation begins at an earlier state of stress, which better protects against thermal injury to the gastrointestinal tract (Horowitz, 2002). In addition, cardiovascular reserves increase, allowing for heat dissipation by vasodilation at the skin (Horowitz, 2002; Seebacher, 2009) while limiting the induction of hypoxia in the gastrointestinal tract by the excessive limitation of splanchnic blood flow (Horowitz, 2002; Collier and Gebremedhin, 2015).

Acclimation recovery to the production state of an unstressed animal is not normally possible (Afsal et al., 2018), though adapted animals do not have to expend as much energy as naive animals to cope with repeated stress (Horowitz, 2002; Collier and Gebremedhin, 2015). Pigs' acclimation capability may be primed in early life, as there is a difference in post-stress weight and ADG recovery based on social status (Hicks et al., 1998). Return to normal rate of production is especially hindered in modern fast-growing genetic lines (Rhoads et al., 2013), though there is evidence that heat stress adaptability may have genetic components (Afsal et al., 2018). Acclimated animals undergo epigenetic histone modifications, resulting in more abundant circulating heat shock proteins (Ross et al., 2015), which decrease the response time of the cytoprotective system (Horowitz, 2002; Collier and Gebremedhin, 2015). There is evidence that

heat stress driven epigenetic programming can occur in utero, but the exact mechanism is not well understood (Ross et al., 2015).

Carbohydrate metabolism and liver production of glucose increases relative to fatty acid oxidation (Lambert, 2009). This occurs independently of dietary nutrient intake or state of energy balance (Pearce et al., 2013a) and may occur to support increased energy consumption for immune response (Lambert, 2009). Overall, a tandem alteration of the availability and use of nutrients can be observed in different tissues in the heat stressed pig (Lambert, 2009; Pearce et al., 2013a; Collier and Gebremedhin, 2015). Intake of nutrients is also altered in that pigs that have acclimated to heat stress eat fewer meals, but they increase their meal size (Nienaber and Hahn, 2004). Heat stressed finishing pigs have lower backfat thickness and organ weight in comparison to pigs raised in thermoneutral conditions, indicating a smaller required digestive capacity and maintenance energy requirement (Nienaber and Hahn, 2004).

### **Variable Stress Response**

Due to the overlapping responses of the SAM system and HPA axis and the variability in physiologic response between stress and acclimation, the interpretation of changes in response to heat stress can be difficult (Salak-Johnson and McGlone, 2007). There is evidence that cross-tolerance and acclimation to other stressors can be achieved following chronic heat stress, as the signaling pathways for many stressors are similar or the same (Seebacher, 2009). Conflicting data have been reported as to whether stress inhibits or stimulates the immune system (Wrona et al., 2001). There are many sources of variation when it comes to stress reactions that could impact the measurement of it. These include timing at which the stressor is applied, the duration that the stressor is maintained before sampling, and the time of day that the sample is taken, all

of which effect of glucocorticoids on immune biomarkers in circulation (Salak-Johnson and McGlone, 2007). The sampling procedure itself could induce stress (Salak-Johnson and McGlone, 2007), as stressful restraint is often applied to take blood samples from pigs (Parrott and Lloyd, 1995). Some reactions are localized, so a blood sample may not represent the immune reaction taking place, and local tissue samples may be more appropriate (Ganesan et al., 2019). In addition, age, genetics, sex, and social standing of the animal in the herd also influence their response to stress (Salak-Johnson and McGlone, 2007).

Social status, such as dominant or submissive pigs in a pen, can impact the immunological stress response more than the stressor itself, as animals of different social status are primed to react differently (Hicks et al., 1998). Sutherland et al. (2006) reported that pigs recognized as the dominant pigs in the pen have a more robust natural killer cell cytotoxic response to shipping and cold stress than submissive pigs, but they have a suppressed response to mixing stress. Both dominant and submissive pigs react more strongly than pigs recognized as neither, in terms of cytotoxicity (Sutherland et al., 2006). Social status can result in differing basal levels of plasma cortisol (Hicks et al., 1998; Sutherland et al., 2006), which would result in differing stress and immune responses following exposure to a stressor (Salak-Johnson and McGlone, 2007). Differing levels of acute phase proteins, or proteins activated by inflammation have also been observed in pigs of different social status (Hicks et al., 1998; Sutherland et al., 2006).

The way that behavior changes in response to stress is unique to each stressor (Hicks et al., 1998), so compounding stressors such as social and heat stress could cause varied results (Hicks et al., 1998; Salak-Johnson and McGlone, 2007). Despite the varied complexity of stress responses, production pigs inevitably must endure stress during piglet processing, weaning,

mixing, handling, vaccination, restraint, transport, and adverse climates, so understanding the stress response is critical to limit welfare and performance losses throughout a pig's life (Hicks et al., 1998; Sutherland et al., 2006; Salak-Johnson and McGlone, 2007).

### *T-Cell Homeostasis*

An animal's previous immune experiences, such as vaccination or disease exposure, can also impact the variability of immune reaction to stress, especially in terms of a T-helper 1 (Th1) or T-helper 2 (Th2) differentiation balance at the time of stressor application (Salak-Johnson and McGlone, 2007). The Th1 and Th2 responses function differently in terms of host defense, and it can be stimulated or result in the secretion of different cytokine profiles (Janeway and Medzhitov, 2002). Th1 differentiated cells impact cellular immunity and clear viral challenges, but it can be hindered if the Th1/Th2 balance is shifted toward Th2 (Bot et al., 2004). Th2 is prominent in humoral immunity and protects against bacterial infections that the animal has been previously exposed to, but it can impair innate immunity, and autoimmunity becomes more likely if Th2 dominates. A balance of Th1/Th2 immunity is required for robust and appropriate reactions to physiologic challenges (Salak-Johnson and McGlone, 2007).

Stress can alter Th1/Th2 balance by stimulating glucocorticoid and/or catecholamine release and inducing or suppressing the production of cytokines produced by either Th1 or Th2 cells expressing glucocorticoid receptors, tipping the scale to the one that dominates (Elenkov et al., 1999). Cytokines bridge the innate and adaptive immune responses by cross-communication and balance T-cell homeostasis. Th1 immunity favors the production of IFN $\gamma$ , IL2, and TNF $\alpha$  (Kovács et al., 2005). IL4 expression is generally a strong indicator of Th2 immunity (Bot et al., 2004). IL5, IL10, and IL13 are also secreted in Th2 immunity (Kovács et al., 2005). IL4 and

IL10 inhibit Th1 immunity, whereas IFN $\gamma$  and IL12 inhibit Th2 immunity (Coffman, 2005). There is indication that catecholamines cause a shift from Th1 to Th2 immunity, though it is disputed (Elenkov and Chrousos, 2002). Glucocorticoids are thought to shift from Th1 to Th2 by reducing responsiveness to and production of IL12, which indirectly induces Th1 immunity (Wiegers and Reul, 1998; Salak-Johnson and McGlone, 2007). Different stressors create a different stress response and shift in T-cell differentiation. Cold and crowding stress shift the immune system toward Th1 (Skjolaas et al., 2002; Skjolaas and Minton, 2002). Restrain stress causes a Th1 shift within the first hour that rebounds to a Th2 shift after 4 hours (Wrona et al., 2001). It cannot be said that stress enhances or suppresses immunity, only that it alters it (Salak-Johnson and McGlone, 2007).

#### *Heat Stress and the Inflammatory Response*

There have been many conflicting reports of immune responses to heat stress in swine, specifically with regard to cytokines. Many cytokines stimulate or inhibit the release of other cytokines directly or indirectly through HPA axis modulation, so it is important to measure as many different cytokines as possible to get a wholistic picture of the inflammatory state. TNF $\alpha$ , IL1, and IL6 can stimulate CRF production and HPA axis activation (Kovács et al., 2005). Different species respond differently to stress in terms of cytokine release, making it difficult to compare swine to previous research in other species. Humans show a decrease in IL1, IL2, IL3, IL4, IL5, IL6, IL8, IL12, and IFN $\gamma$  whereas mice show an increase in IL1, IL2, IL3, and IL4 in response to stress (Sapolsky et al., 2000). In culture, catecholamines increase IL10 and IL4, and decrease IL12. Glucocorticoids also decrease IL12 as well as IL2 and TNF $\alpha$ , but they increase TGF $\beta$  (Kovács et al., 2005).

The inconsistency in cytokine concentrations reported during heat stress in swine is likely due to differences in age, sex, health, genetics, production status, heat stress intensity and duration, sampling timing, and systemic or local sampling strategies for the different experiments (Sapolsky et al., 2000; Salak-Johnson and McGlone, 2007). There is evidence that in the case of endotoxin challenge, which can be observed in intense heat stress, HPA regulation of immunity is circumvented (Carroll et al., 2001). Also, a lack of cytokines in circulation does not necessarily indicate a lack of inflammatory response (Salak-Johnson and McGlone, 2007). It is also possible that glucocorticoids act permissively within specific tissues, promoting transport from the bloodstream to target tissues (Sapolsky et al., 2000). Local immune responses to stress have been shown to increase TNF $\alpha$ , IL1, and IL8 and decrease TGF $\beta$  (Elenkov and Chrousos, 2002). The general idea that stress suppresses the immune system does not accurately encompass the adjustments in immunocompetence from different types and durations of stressor (Kovács et al., 2005).

## **Summary**

The economic relevance of heat stress can be observed across species and regions in animal agriculture. The complex physiologic changes the pig employs in an attempt to maintain homeostasis have an energy cost that compromise production efficiency and welfare. Duration, intensity, and repeat exposure to heat stress all attenuate the stress reaction differently, and the multiple facets and variation of heat as a stressor makes it difficult to tie all the responses together to create a complete picture of the heat stress response. The study of heat stress physiology is important for the understanding of production losses and welfare detriments in the pursuit of practical and effective measures of alleviation.

## Literature Cited

- Afsal, A., V. Sejian, M. Bagath, G. Krishnan, C. Devaraj, and R. Bhatta. 2018. Heat Stress and Livestock Adaptation: Neuro-endocrine Regulation. *International Journal of Veterinary and Animal Medicine*. 1. doi:10.31021/ijvam.20181108. Available from: <https://www.boffinaccess.com/journals/international-journal-veterinary-animal-medicine/index.php>
- Banhazi, T. M., A. J. Aarnink, H. T. Thuy, S. Pedersen, J. Hartung, H. Payne, B. Mullan, and D. Berckmans. 2008. Review of Issues Related to Heat Stress in Intensively Housed Pigs. In: *Livestock Environment VIII*, 31 August - 4 September 2008, Iguassu Falls, Brazil. American Society of Agricultural and Biological Engineers, St. Joseph, MI. Available from: <http://elibrary.asabe.org/abstract.asp?JID=1&AID=25578&CID=lenv2009&T=1>
- Blotta, M. H., R. H. DeKruyff, and D. T. Umetsu. 1997. Corticosteroids inhibit IL-12 production in human monocytes and enhance their capacity to induce IL-4 synthesis in CD4+ lymphocytes. *Journal of immunology (Baltimore, Md. : 1950)*. 158:5589–95. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/9190905>
- Bot, A., K. A. Smith, and M. von Herrath. 2004. Molecular and Cellular Control of T1/T2 Immunity at the Interface between Antimicrobial Defense and Immune Pathology. *DNA and Cell Biology*. 23:341–350. doi:10.1089/104454904323145227. Available from: <http://www.liebertpub.com/doi/10.1089/104454904323145227>
- Bridges, T. C., R. S. Gates, L. W. Turner, and A. Asae. 1992. Stochastic Assessment Of Evaporative Misting for Growing-Finishing Swine in Kentucky.
- Brown-Brandl, T. M., R. A. Eigenberg, John A. Nienaber, Stephen D. Kachman, J. A. Nienaber, and S. D. Kachman. 2001. Thermoregulatory profile of a newer genetic line of pigs. Available from: <https://digitalcommons.unl.edu/usdaarsfacpubhttps://digitalcommons.unl.edu/usdaarsfacpub/178www.elsevier.com/locate/livprodsci>
- Brown-Brandl, T. M., R. A. Eigenberg, and J. L. Purswell. 2013. Using thermal imaging as a method of investigating thermal thresholds in finishing pigs. *Biosystems Engineering*. 114:327–333. doi:10.1016/j.biosystemseng.2012.11.015.
- Brown-Brandl, T. M., J. A. Nienaber, R. A. Eigenberg, G. L. Hahn, and H. Freetly. 2003. Thermoregulatory responses of feeder cattle. *Journal of Thermal Biology*. 28:149–157. doi:10.1016/S0306-4565(02)00052-9.
- Brown-Brandl, T. M., J. A. Nienaber, and L. W. Turner. 1998. Acute heat stress effects on heat production and respiration rate in swine. *Transactions of the American Society of Agricultural Engineers*. 41:789–793. doi:10.13031/2013.17216.
- Brown-Brandl, T. M., J. A. Nienaber, H. Xin, and R. S. Gates. 2004. A Literature Review of Swine Heat Production. *Transactions of the ASAE*. 47:259–270.

doi:10.13031/2013.15867. Available from:  
<http://elibrary.asabe.org/abstract.asp??JID=3&AID=15867&CID=t2004&v=47&i=1&T=1>

- Carroll, J. A., R. L. Matteri, C. J. Dyer, L. A. Beausang, and M. E. Zannelli. 2001. Impact of environmental temperature on response of neonatal pigs to an endotoxin challenge. *American Journal of Veterinary Research*. 62:561–566. doi:10.2460/ajvr.2001.62.561.
- Caterina, M. J., A. Leffler, A. B. Malmberg, W. J. Martin, J. Trafton, K. R. Petersen-Zeitz, M. Koltzenburg, A. I. Basbaum, and D. Julius. 2000. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science*. 288:306–313. doi:10.1126/science.288.5464.306.
- Chen, Y., R. Arsenault, S. Napper, and P. Griebel. 2015. Models and methods to investigate acute stress responses in cattle. *Animals*. 5:1268–1295. doi:10.3390/ani5040411.
- Coffman, R. L. 2005. Origins of the T<sub>H</sub>1-T<sub>H</sub>2 model: a personal perspective. Available from: <http://www.nature.com/natureimmunology>
- Collier, R. J., and K. G. Gebremedhin. 2015. Thermal Biology of Domestic Animals. *Annu. Rev. Anim. Biosci.* 3:513–545. doi:10.1146/annurev-animal-022114-110659. Available from: [www.annualreviews.org](http://www.annualreviews.org)
- Elenkov, I. J., and G. P. Chrousos. 2002. Stress Hormones, Proinflammatory and Antiinflammatory Cytokines, and Autoimmunity.
- Elenkov, I. J., E. L. Webster, D. J. Topry, and G. P. Chrousos. 1999. Stress, Corticotropin-Releasing Hormone, Glucocorticoids, and the Immune/Inflammatory Response: Acute and Chronic Effects. *Annals of the New York Academy of Sciences*. 876:1–13. doi:10.1111/j.1749-6632.1999.tb07618.x. Available from: <http://doi.wiley.com/10.1111/j.1749-6632.1999.tb07618.x>
- Fulford, A. J., and M. S. Harbuz. 2005. Chapter 1.3 An introduction to the HPA axis. In: *Techniques in the Behavioral and Neural Sciences*. Vol. 15. Academic Press. p. 43–65.
- Gabler, N. K., and S. C. Pearce. 2015. The impact of heat stress on intestinal function and productivity in grow-finish pigs. *Animal Production Science*. 55:1403. doi:10.1071/AN15280. Available from: <http://www.publish.csiro.au/?paper=AN15280>
- Gilbert, M., G. Nicolas, G. Cinardi, T. P. van Boeckel, S. O. Vanwambeke, G. R. W. Wint, and T. P. Robinson. 2018. Global distribution data for cattle, buffaloes, horses, sheep, goats, pigs, chickens and ducks in 2010. *Scientific Data*. 5. doi:10.1038/sdata.2018.227.
- Hahn, J.-S., Z. Hu, D. J. Thiele, and V. R. Iyer. 2004. Genome-Wide Analysis of the Biology of Stress Responses through Heat Shock Transcription Factor. *Molecular and Cellular Biology*. 24:5249–5256. doi:10.1128/mcb.24.12.5249-5256.2004.

- Hall, D. M., G. R. Buettner, L. W. Oberley, L. Xu, R. D. Matthes, and C. v. Gisolfi. 2001. Mechanisms of circulatory and intestinal barrier dysfunction during whole body hyperthermia. *American Journal of Physiology - Heart and Circulatory Physiology*. 280. doi:10.1152/ajpheart.2001.280.2.h509.
- Heitman, H., C. F. Kelly, and T. E. Bond. 1958. Ambient Air Temperature and Weight Gain in Swine. *Journal of Animal Science*. 17:62–67. doi:10.2527/jas1958.17162x.
- Hicks, T. A., J. J. McGlone, C. S. Whisnant, H. G. Kattesh, and R. L. Norman. 1998. Behavioral, Endocrine, Immune, and Performance Measures for Pigs Exposed to Acute Stress. *Journal of Animal Science*. 76:474–483. doi:10.2527/1998.762474x.
- Ho, K. W., N. J. Ward, and D. J. Calkins. 2012. TRPV1: A stress response protein in the central nervous system. *American Journal of Neurodegenerative Diseases*. 1:1–14.
- Horowitz, M. 2002. From molecular and cellular to integrative heat defense during exposure to chronic heat. *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology*. 131:475–83. doi:10.1016/s1095-6433(01)00500-1. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11867273>
- 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. doi:10.1016/j.applanim.2004.10.020.
- Janeway, C. A., and R. Medzhitov. 2002. Innate Immune Recognition. *Annu. Rev. Immunol.* 20:197–216. doi:10.1146/annurev.immunol.20.083001.084359. Available from: [www.annualreviews.org](http://www.annualreviews.org)
- Kovács, K. J., I. H. Miklós, and B. Bali. 2005. Chapter 6.1 Psychological and physiological stressors. In: *Techniques in the Behavioral and Neural Sciences*. Vol. 15. Academic Press. p. 775–792.
- Lambert, G. P. 2009. Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. *Journal of animal science*. 87. doi:10.2527/jas.2008-1339.
- Leon, L. R., M. D. Blaha, and D. A. DuBose. 2006. Time course of cytokine, corticosterone, and tissue injury responses in mice during heat strain recovery. *Journal of Applied Physiology*. 100:1400–1409. doi:10.1152/jappphysiol.01040.2005.
- Lindsey, R. L., and L. A. Dahlman. 2020. Climate Change: Global Temperature. Climate. Available from: <https://www.climate.gov/news-features/understanding-climate/climate-change-global-temperature>

- Liu, F., J. Yin, M. Du, P. Yan, J. Xu, X. Zhu, and J. Yu. 2009. Heat-stress-induced damage to porcine small intestinal epithelium associated with downregulation of epithelial growth factor signaling. *Journal of animal science*. 87:1941–1949. doi:10.2527/jas.2008-1624.
- Livestock Geo-Wiki. 2006. Geo-Wiki. Available from: <https://livestock.geo-wiki.org/Application/>
- Marple, D. N., D. J. Jones, C. W. Alliston, and J. C. Forrest. 1974. Physiological and endocrinological changes in response to terminal heat stress in swine. *Journal of animal science*. 39:79–82. doi:10.2527/jas1974.39179x.
- Martínez-Miró, S., F. Tecles, M. Ramón, D. Escribano, F. Hernández, J. Madrid, J. Orengo, S. Martínez-Subiela, X. Manteca, and J. J. Cerón. 2016. Causes, consequences and biomarkers of stress in swine: An update. *BMC Veterinary Research*. 12. doi:10.1186/s12917-016-0791-8.
- Mcglone, J. J., E. A. Lumpkin, and R. L. Norman. 1991. Adrenocorticotropin Stimulates Natural Killer Cell Activity\*. Available from: <https://academic.oup.com/endo/article-abstract/129/3/1653/2535580>
- Miller, T. G. 2012. Swine Feed Efficiency: Influence of Temperature Swine Feed Efficiency: Influence of Temperature. Available from: [http://lib.dr.iastate.edu/ipic\\_factsheets](http://lib.dr.iastate.edu/ipic_factsheets)
- Morrison, S. R., T. E. Bond, and H. Jr. H. Hubert. 1967. Skin and Lung Moisture Loss from Swine. *Transactions of the ASAE*. 10:0691–0692. doi:10.13031/2013.39762. Available from: <http://elibrary.asabe.org/abstract.asp??JID=3&AID=39762&CID=t1967&v=10&i=5&T=1>
- Nakamura, K., and S. F. Morrison. 2008. A thermosensory pathway that controls body temperature. *Nature Neuroscience*. 11:62–71. doi:10.1038/nn2027.
- Nienaber, J. A., and G. L. Hahn. 2004. Engineering and Management Practices to Ameliorate Livestock Heat Stress.
- Nienaber, J. A., G. L. Hahn, and R. A. Eigenberg. 1999. Quantifying livestock responses for heat stress management: A review. *International Journal of Biometeorology*. 42:183–188. doi:10.1007/s004840050103.
- Parrott, R. F., and D. M. Lloyd. 1995. Restraint, but not frustration, induces prostaglandin-mediated hyperthermia in pigs. *Physiology and Behavior*. 57:1051–1055. doi:10.1016/0031-9384(94)00343-4. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/7652024>

- Pearce, S. C., N. K. Gabler, J. W. Ross, J. Escobar, J. F. Patience, R. P. Rhoads, and L. H. Baumgard. 2013a. The effects of heat stress and plane of nutrition on metabolism in growing pigs. *Journal of Animal Science*. 91:2108–2118. doi:10.2527/jas.2012-5738.
- Pearce, S. C., V. Mani, R. L. Boddicker, J. S. Johnson, T. E. Weber, J. W. Ross, R. P. Rhoads, L. H. Baumgard, and N. K. Gabler. 2013b. Heat Stress Reduces Intestinal Barrier Integrity and Favors Intestinal Glucose Transport in Growing Pigs. G. López-Lluch, editor. *PLoS ONE*. 8:e70215. doi:10.1371/journal.pone.0070215. Available from: <https://dx.plos.org/10.1371/journal.pone.0070215>
- Pearce, S. C., M. v. Sanz-Fernandez, J. H. Hollis, L. H. Baumgard, and N. K. Gabler. 2014. Short-term exposure to heat stress attenuates appetite and intestinal integrity in growing pigs. *Journal of Animal Science*. 92:5444–5454. doi:10.2527/jas.2014-8407.
- Pearce, S. C., M.-V. Sanz Fernandez, J. Torrison, M. E. Wilson, L. H. Baumgard, and N. K. Gabler. 2015. Dietary organic zinc attenuates heat stress–induced changes in pig intestinal integrity and metabolism. *Journal of Animal Science*. 93:4702–4713. doi:10.2527/jas.2015-9018. Available from: <https://academic.oup.com/jas/article/93/10/4702/4701604>
- Pedersen, S., and P. Ravn. 2008. Characteristics of Floors for Pig Pens: Friction, shock absorption, ammonia emission and heat conduction. *CIGR eJournal*.
- Rhoads, R. P., L. H. Baumgard, J. K. Suagee, and S. R. Sanders. 2013. Nutritional Interventions to Alleviate the Negative Consequences of Heat Stress. *Advances in Nutrition*. 4:267–276. doi:10.3945/an.112.003376.
- Robertshaw, D. 2006. Mechanisms for the control of respiratory evaporative heat loss in panting animals. *Journal of Applied Physiology*. 101:664–668. doi:10.1152/jappphysiol.01380.2005. Available from: <https://www.physiology.org/doi/10.1152/jappphysiol.01380.2005>
- Ross, J. W., B. J. Hale, N. K. Gabler, R. P. Rhoads, A. F. Keating, and L. H. Baumgard. 2015. Physiological consequences of heat stress in pigs. *Animal Production Science*. 55:1381–1390. doi:10.1071/AN15267. Available from: <http://dx.doi.org/10.1071/AN15267>
- Salak-Johnson, J. L., and J. J. McGlone. 2007. Making sense of apparently conflicting data: stress and immunity in swine and cattle. *Journal of animal science*. 85. doi:10.2527/jas.2006-538.
- Sanz Fernandez, M. V., J. S. Johnson, M. Abuajamieh, S. K. Stoakes, J. T. Seibert, L. Cox, S. Kahl, T. H. Elsasser, J. W. Ross, S. Clay Isom, R. P. Rhoads, and L. H. Baumgard. 2015. Effects of heat stress on carbohydrate and lipid metabolism in growing pigs. *Physiological Reports*. 3. doi:10.14814/phy2.12315.

- Sapolsky, R. M., L. M. Romero, and A. U. Munck. 2000. How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions\*. Available from: <https://academic.oup.com/edrv/article-abstract/21/1/55/2423840>
- Schauberger, G., C. Mikovits, W. Zollitsch, S. J. Hörtenhuber, J. Baumgartner, K. Niebuhr, M. Piringer, W. Knauder, I. Anders, K. Andre, I. Hennig-Pauka, and M. Schönhart. 2019. Global warming impact on confined livestock in buildings: efficacy of adaptation measures to reduce heat stress for growing-fattening pigs. *Climatic Change*. 156:567–587. doi:10.1007/s10584-019-02525-3. Available from: <https://doi.org/10.1007/s10584-019-02525-3>
- Schmidt-Nielsen, K. 1981. Adaptation to Thermal Environment: Man and His Productive Animals . Laurence E. Mount . *The Quarterly Review of Biology*. 56:214–214. doi:10.1086/412251.
- Seebacher, F. 2009. Responses to temperature variation: Integration of thermoregulation and metabolism in vertebrates. *Journal of Experimental Biology*. 212:2885–2891. doi:10.1242/jeb.024430.
- Skjolaas, K. A., D. M. Grieger, C. M. Hill, and J. E. Minton. 2002. Glucocorticoid regulation of type 1 and type 2 cytokines in cultured porcine splenocytes. *Veterinary Immunology and Immunopathology*. 87:79–87. doi:10.1016/S0165-2427(02)00122-8.
- Skjolaas, K. A., and J. E. Minton. 2002. Does cortisol bias cytokine production in cultured porcine splenocytes to a Th2 phenotype? In: *Veterinary Immunology and Immunopathology*. Vol. 87. p. 451–458.
- Song, R., D. N. Foster, and G. C. Shurson. 2011. Effects of feeding diets containing bacitracin methylene disalicylate to heat-stressed finishing pigs. *Journal of Animal Science*. 89:1830–1843. doi:10.2527/jas.2010-3218. Available from: <http://academic.oup.com/jas/article/89/6/1830/4764439>
- Song, X., J. Xu, T. Wang, and F. Liu. 2009. Chinese medicine granule affects the absorption and transport of glucose in porcine small intestinal brush border membrane vesicles under heat stress. *Asian-Australasian Journal of Animal Sciences*. 22:246–253. doi:10.5713/ajas.2009.80358.
- St-Pierre, N. R., B. Cobanov, and G. Schnitkey. 2003. Economic losses from heat stress by US livestock industries 1. *Journal of Dairy Science*. 86. doi:10.3168/jds.S0022-0302(03)74040-5.
- Sutherland, M. A., S. R. Niekamp, S. L. Rodriguez-Zas, and J. L. Salak-Johnson. 2006. Impacts of chronic stress and social status on various physiological and performance measures in pigs of different breeds 1. Available from: <https://academic.oup.com/jas/article-abstract/84/3/588/4778470>

- Valentino, R. J., and E. J. van Bockstaele. 2005. Chapter 4.4 Functional interactions between stress neuromediators and the locus coeruleus-norepinephrine system. In: *Techniques in the Behavioral and Neural Sciences*. Vol. 15. Academic Press. p. 465–486.
- Wallgren, P., I. L. Wilén, and C. Fossum. 1994. Influence of experimentally induced endogenous production of cortisol on the immune capacity in swine. *Veterinary Immunology and Immunopathology*. 42:301–316. doi:10.1016/0165-2427(94)90075-2.
- Wiegers, G. J., and J. M. H. M. Reul. 1998. Induction of cytokine receptors by glucocorticoids: Functional and pathological significance. *Trends in Pharmacological Sciences*. 19:317–321. doi:10.1016/S0165-6147(98)01229-2.
- Wrona, D., W. Trojnar, A. Borman, Z. Ciepielewski, and J. Tokarski. 2001. Stress-induced changes in peripheral natural killer cell cytotoxicity in pigs may not depend on plasma cortisol. *Brain, Behavior, and Immunity*. 15:54–64. doi:10.1006/brbi.2000.0583.
- Yu, J., P. Yin, F. Liu, G. Cheng, K. Guo, A. Lu, X. Zhu, W. Luan, and J. Xu. 2010. Effect of heat stress on the porcine small intestine: A morphological and gene expression study. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*. 156:119–128. doi:10.1016/j.cbpa.2010.01.008.

## **Chapter II:**

**Changes in Growth Performance, Cytokine Profile, and Behaviors of Individually Housed**

**Growing Pigs Subjected to Constant Induced Heat Stress**

## Abstract

This study was conducted to determine effects of induced heat stress on growth performance, cytokine profile and eating and drinking behaviors of growing pigs. Pigs were housed individually in thermoneutral conditions (CON; mean 19.53 °C) or subjected to constant high ambient temperatures (mean 27.76°C) for 15 d (HS), followed by a 7 d recovery period (mean 20.33°C). Pig weights and feed disappearance were recorded weekly for ADG, ADFI and GF calculation. Blood samples were taken via venipuncture, and serum was analyzed for cytokine profile. Four focal pigs per treatment were video-recorded. The following behaviors were recorded: frequency and duration at the feeder, at the drinker, sitting, standing/walking, lying ventrally, and lying laterally. HS pigs had lower ADFI (2.32 kg/d;  $P = 0.0022$ ) and ADG (1.00 kg/d;  $P < 0.0001$ ) compared to CON pigs (2.70 and 1.14 kg/d, respectively). HS pigs had increased IL1 $\beta$  (616.85 pg/mL) and IL12 (1379.91 pg/mL) compared to CON pigs (247.46 and 1126.45 pg/mL, respectively). IL8 was increased for CON (1339.48 pg/mL) compared to HS (521.69 pg/mL;  $P < 0.001$ ). HS pigs ate less frequently in the morning (5.89) compared to CON pigs (8.40; time x treatment interaction,  $P = 0.0037$ ). HS pigs had more instances of lying laterally ( $P = 0.0006$ ) and ventrally ( $P = 0.0018$ ) on d 2 (22.67 and 25.15), d 6 (18.00 and 22.17), and d 9 (16.67 and 20.58) as compared to CON on d 2 (6.50 and 9.58), d 6 (7.33 and 11.58), and d 9 (10.92 and 14.25). HS pigs visited the drinker more frequently than CON on d 9 (13.5 and 5.92, respectively;  $P = 0.0361$ ). Heat stress reduces performance, increases circulating cytokines, and increases lying behavior frequency while altering drinking frequency and daily time of eating.

## **Introduction**

Heat stress is a prevalent welfare and profitability issue that impacts most species and regions of livestock production globally (Horowitz, 2002). This is especially true in swine, as they are not able to produce enough sweat for efficient evaporative cooling at the level of the skin (Morrison et al., 1967). High atmospheric temperature has a negative impact on feed intake and the ADG of pigs (Heitman et al., 1958), resulting in marked economic losses (St-Pierre et al., 2003). Environmental control was one of the driving forces that moved most of hog production indoors in the United States (Banhazi et al., 2008). It is important that housing accommodation decisions for the abatement of heat stress are both effective in alleviating heat stress for the animal and practical for producers to utilize (Pedersen and Ravn, 2008).

In order to optimize intervention to prevent hyperthermic reactions, or to support animals already experiencing heat stress, a more thorough understanding of the associated physiologic changes are needed. Challenges arise in the achievement of this understanding due to the variables related to heat stress severity that result in disparate physiologic reactions.

Temperature, duration of stress, genetics, age, health history, production status, diet, housing, and many other elements of pig production produce different physiologic changes (Salak-Johnson and McGlone, 2007). The stress response itself is difficult to characterize, as many stressors such as environmental stress and social stress interact (Sutherland et al., 2006).

This research was performed in attempt to layer behavior modification in individually housed heat stressed pigs with inflammatory and stress biomarkers in the blood. The goal of the project was to explore physiologic reasoning behind performance losses in heat stress.

## Materials and Methods

### *Experimental Design*

A protocol for these experiments was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at North Carolina State University (NCSU; Raleigh, NC, USA).

Barrows ( $n = 48$ ;  $42.27 \pm 3.06$  kg) used for this study were obtained from NCSU Swine Educational Unit. Prior to the start of the study, pigs were weighed and randomly assigned to their treatment groups. Pigs were placed into individual pens contained within two rooms, 24 in a heat stressed (HS) environment and 24 in a thermoneutral (CON) group. The pigs were offered a common diet and water was provided *ad libitum*.

An environmental control system (GT-5124LW Grower Direct, Monitrol Inc., Boucherville, Quebec, Canada) was used to maintain constant temperatures in each of the rooms. The CON room temperature was maintained at an average of  $19.53^{\circ}\text{C} \pm 1.53^{\circ}\text{C}$  (relative humidity  $65.23\% \pm 12.24\%$ ). The HS room temperature was set to produce a chronic heat-stressed environment at a constant average of  $27.76^{\circ}\text{C} \pm 1.50^{\circ}\text{C}$  (relative humidity  $35.96\% \pm 9.67\%$ ). Room temperature was recorded by data loggers (iButtonLink LLC, Whitewater, WI) every hour. Two loggers were placed in each room at approximately the same height of the pigs. There was a single speed exhaust fan that ran continuously in both rooms to remove odors, moisture, and provide fresh air for normal respiration. Heat stress conditions began on d 1 of the trial and lasted until d 15. After d 15, the HS room was returned to a thermoneutral average of  $20.33^{\circ}\text{C} \pm 1.01^{\circ}\text{C}$  (relative humidity  $72.75\% \pm 6.10\%$ ) until d 28. Lights in both rooms were on for the duration of the trial.

### *Performance Measurement*

Pig weights were recorded to the nearest 0.05 kg. Feed consumption was calculated from feed added to the feeder minus the feed left in the feeder at the end of the feeding phase. Feeders and pigs were weighed on d 0 (start of the trial) and d 7, 15 (end of heat stress), 21, and 23 (conclusion of the trial). Rectal temperatures were measured weekly using an iProven digital thermometer (9450 SW Gemini Dr. Beaverton, OR).

### *Cortisol and Cytokine Assays*

Blood samples were taken from pigs on d 2, 8, 15 and d 23 by venipuncture into 10 mL vacuum tubes (BD Vacutainer Systems, Franklin Lakes, NJ). Blood was centrifuged at 900 x g for 20 minutes at 25°C to collect serum. HPA axis activation was determined by quantitatively measuring serum concentrations of cortisol (Saint Louis Zoological Park Endocrinology Laboratory, Saint Louis, Missouri). Systemic inflammation status was determined by quantitatively measuring serum concentrations of GM-CSF, IFN $\gamma$ , IL1 $\alpha$ , IL1Ra, IL1 $\beta$ , IL2, IL4, IL6, IL8, IL10, IL12, IL18, and TNF $\alpha$  (Eve Technologies Corporation, NW, Calgary, AB Canada).

### *Behavioral Analysis*

M1065-L Fixed Network video recording cameras (Axis Communications, Lund, Sweden) were placed on each side of the room so that 4 pigs from each room were recorded for observation of drinking and feeding behaviors using Viso™ video recording software and Observer annotation software (Noldus Information Technology Inc., Leesburg, VA). Behaviors were recorded for 2 hour time blocks from 0800 to 1000 (Morning), 1200 to 1400 (Afternoon),

and 1600 to 1800 (Evening) on d 2, 6, 9, 16, and 21. Recording sessions were started on an automatic schedule as to avoid interference with behaviors. Number of times seen at the waterer and at the feeder, as well as duration of sitting, ventral lying, lateral lying, and standing/walking were recorded and analyzed between treatments.

### *Statistical Analysis*

Data were analyzed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). Pig was used as the experimental unit. Rectal temperature, serum measurements, and performance parameters were analyzed with repeated measures by day. Behaviors were analyzed with repeated measures with the variable time nested within day. Main effects and interactions were considered statistically significant at  $P \leq 0.05$ , and tendencies were considered when  $0.05 \leq P \leq 0.10$ . Least squared means were reported with the SEM.

## **Results and Discussion**

### *Performance Measurement*

ADG of CON ( $1.14 \pm 0.03$  kg) pigs was significantly greater than HS pigs ( $1.00 \pm 0.03$  kg/d;  $P \leq 0.05$ ). The ADFI of CON ( $2.70 \pm 0.05$  kg/d) was significantly greater than HS ( $2.32 \pm 0.05$  kg/d). This was consistent with previous research indicating performance loss during heat stress (Heitman et al., 1958). Gain to feed ratio (GF) was not significantly different between HS ( $0.43 \pm 0.02$ ) and CON ( $0.46 \pm 0.02$ ), so it can be inferred that the HS pigs gained less because they ate less than the CON group and not due to metabolic inefficiencies.

AFDI was significantly different at all weeks regardless of treatment, increasing from week 1 ( $2.18 \pm 0.06$  kg/d) to week 2 ( $2.87 \pm 0.06$  kg/d) and decreasing from week 2 to week 3 ( $2.49 \pm 0.06$  kg/d; Figure 2.5).

There was a tendency ( $P \leq 0.10$ ) for rectal temperatures to differ with HS group ( $39.81 \pm 0.05^\circ\text{C}$ ) being greater than CON ( $39.68 \pm 0.05^\circ\text{C}$ ), indicating a potential disruption in homeostasis in the HS group (Nienaber et al., 1999).

Figure 2.6 depicts the significant difference in rectal temperature by day, where d 2 ( $40.08 \pm 0.07^\circ\text{C}$ ) was significantly greater than d 8 ( $39.57 \pm 0.07^\circ\text{C}$ ), d 15 ( $39.72 \pm 0.07^\circ\text{C}$ ), and d 23 ( $39.60 \pm 0.07^\circ\text{C}$ ), regardless of treatment. Transport stress has been demonstrated to have an impact on body temperature (Geers et al., 1997), which could explain the greater rectal temperatures at the beginning of the trial.

### *Cytokine Analysis*

Systemic interleukin (IL)1 $\beta$  concentration was significantly ( $P \leq 0.05$ ) greater in HS ( $616.85 \pm 97.78$  pg/mL) than in CON ( $247.46 \pm 97.78$  pg/mL). IL12 concentration was significantly higher in HS ( $1379.91 \pm 67.90$  pg/mL) than CON ( $1126.45 \pm 67.90$  pg/mL). IL8 concentration was significantly higher in CON ( $1139.48 \pm 147.96$  pg/mL) than HS ( $521.69 \pm 147.96$  pg/mL). IL1 receptor antagonist (IL1Ra), IL4, IL6, and IL10 concentrations tended ( $P \leq 0.1$ ) to be higher in HS (IL1Ra  $663.09 \pm 60.84$  pg/mL, IL4  $1604.31 \pm 397.36$  pg/mL, IL6  $40.68 \pm 7.42$  pg/mL, and IL10  $649.34 \pm 117.87$  pg/mL) compared to CON (IL1Ra  $515.59 \pm 60.84$  pg/mL, IL4  $573.46 \pm 397.36$  pg/mL, IL6  $19.86 \pm 9.50$  pg/mL, and IL10  $342.93 \pm 117.87$  pg/mL; Figure 2.7).

Overall, the HS group had a greater concentrations of both pro-inflammatory and anti-inflammatory cytokines in circulation than CON, except for IL8, which appears lesser in HS than CON. This indicates inflammatory activity in the HS group, which is consistent with studies done in humans (Bouchama et al., 1991). IL1 $\beta$  and IL6 have been shown to reduce feed intake and attenuate intermediary metabolism in rodents (Johnson, 1997). This response has been observed in pigs, however, it was in response to LPS challenge and the cytokines may not be directly responsible in swine (Johnson and von Borell, 1994; Webel et al., 1997; Frank et al., 2005; Mani et al., 2013). IL1 $\beta$  and IL6 may have been contributors to the reduction in ADFI and ADG in the HS group.

Pearce et al. (2013b) measured IL1 $\beta$ , IL8, and TNF $\alpha$  in response to a 24 h 35°C heat stress and noted a reduction in circulating concentrations. Pearce also reported no change or a reduction in those cytokines in a 2-6 h 37°C heat stress (Pearce et al., 2015), 12 h 37°C heat stress (Pearce et al., 2013a), and 3-7 h 35°C day heat stress. Song et al. (2011) measured TNF $\alpha$  and IL1 $\beta$  in response to heat stress and reported no significant difference in TNF $\alpha$  as a result of heat stress. In the present study, IL1 $\beta$  was only detectable in 8 of 32 pigs. The reported reductions in porcine cytokines during heat stress contrast with humans, chickens, lab rodents, and cattle in which inflammatory cytokines in circulation were increased (Leon et al., 2006; Lambert, 2009). IL18 has been considered as a stress biomarker in swine, as it reacts to immobilization stress and is measurable in saliva (Muneta et al., 2011).

IL8 and IL12 concentrations were significantly different by day, regardless of treatment. The concentration of IL8 was greater on d 15 ( $1253.20 \pm 209.25$  pg/mL) and d 23 ( $122.16 \pm 209.25$  pg/mL) as compared to d 2 ( $395.36 \pm 209.25$  pg/mL), but not different on d 8 ( $846.62 \pm$

208.25 pg/mL). The concentration of IL12 was greater on d 8 ( $1448.15 \pm 96.02$  pg/mL) than d 2 ( $1058.50 \pm 96.02$  pg/mL), d 15 ( $1298.13 \pm 96.02$  pg/mL), or d 23 ( $1207.93 \pm 96.02$  pg/mL).

### *Cortisol Assays*

Cortisol concentration tended ( $P \leq 0.10$ ) to be different by day, increasing from d 2 ( $30.79 \pm 4.17$  ng/mL) to d 8 ( $38.46 \pm 4.17$  ng/mL), then again from d 8 to d 15 ( $43.39 \pm 4.17$  ng/mL) and d 23 ( $46.37 \pm 4.17$  ng/mL; Figure 2.8). These results may indicate a stress response to the sampling procedure (Salak-Johnson and McGlone, 2007) as pigs began to recognize the snare.

There was no significant treatment effect for cortisol concentration in this experiment (CON  $42.24$  ng/mg  $\pm 2.95$  ng/mL; HS  $37.27$  ng/mL  $\pm 2.95$  ng/mL). Considering increased cortisol has been recorded in the literature (Marple et al., 1974) future projects should include a more granular sampling schedule in order to better capture changes in cortisol concentration related to heat stress.

### *Behavioral Analysis*

The duration of lateral lying and standing/walking behaviors were significantly ( $P \leq 0.05$ ) different at different times of day, regardless of treatment. Lateral lying time was shorter in the evening ( $57.911 \pm 3.10$  min) compared to the afternoon ( $68.96 \pm 3.10$  min), but not different in the morning ( $64.41 \pm 3.10$  min; Figure 2.9). Standing and walking behaviors were the longest in the evening ( $18.69 \pm 2.23$  min) compared to the morning ( $4.47 \pm 2.18$  min) and the afternoon ( $3.87 \pm 2.23$  min; Figure 2.9). There was a tendency ( $P \leq 0.10$ ) for differences in ventral lying and sitting duration by time. Ventral lying time was longer in the morning ( $35.02 \pm 2.71$  min)

than the evening ( $27.22 \pm 2.71$  min), but afternoon ( $28.64 \pm 2.71$  min) was not different from any other time measured (Figure 2.9). Sitting time was shortest in the afternoon ( $2.39 \pm 0.60$  min) and longest in the evening ( $4.20 \pm 0.61$  min), but not different in the morning ( $2.50 \pm 0.60$  min; Figure 2.9). Active behavior duration is longer when lying behavior is shorter. Overall activity is increased in the evening, despite constant temperature and light in both rooms.

Time at the drinker increased significantly throughout the day,  $0.85 \pm 0.23$  min,  $1.72 \pm 0.24$  min, and  $2.53 \pm 0.22$  min, respectively. The temperatures in the rooms and the lights were held constant, so they were not likely motivators for evening drinking behavior (Figure 2.10).

HS ( $1.98 \pm 0.19$  min) pigs spent significantly more time at the drinker than CON pigs ( $1.42 \pm 0.19$  min) (Figure 2.11). While increased water disappearance has been observed in heat stressed pigs, not all can be considered consumed. Pigs use drinkers to spray themselves with water to improve evaporative cooling (Nienaber and Hahn, 2004; Banhazi et al., 2008).

Figure 2.12 depicts a tendency ( $P \leq 0.10$ ) for greater time sitting for CON ( $3.71 \pm 0.48$  min) over HS ( $2.35 \pm 0.49$  min) pigs. Sitting was often observed as a transition between postures and could indicate decreased overall activity in HS pigs.

There were no significant differences by treatment for duration at the feeder (CON  $15.74$  min  $\pm 0.95$  min; HS  $14.29$  min  $\pm 0.97$  min), lying laterally (CON  $62.30$  min  $\pm 2.53$  min; HS  $65.22$  min  $\pm 2.53$  min), or lying ventrally (CON  $29.25$  min  $\pm 2.21$  min; HS  $31.33$  min  $\pm 2.21$  min).

Figure 2.13 depicts significant ( $P \leq 0.05$ ) differences in the frequency of pigs visiting the feeder by day, regardless of treatment. There were fewer feeding instances on d 9 ( $5.18 \pm 0.77$ ), 16 ( $5.80 \pm 0.83$ ), and 21 ( $4.87 \pm 0.83$ ) as compared to d 2 ( $7.76 \pm 0.81$ ) and d 6 ( $8.63 \pm 0.79$ ). The reduction in feeding frequency and the increase in cortisol concentration occurred on similar

days (Figure 2.8, Figure 2.13). The activation of the HPA axis has been linked with appetite regulation in humans and other species (Fulford and Harbuz, 2005).

There was a significant ( $P \leq 0.05$ ) difference in sitting frequency by day, regardless of treatment. Sitting frequency increased between d 2 ( $4.98 \pm 0.57$ ) and d 6 ( $6.58 \pm 0.56$ ), but they dropped over d 9 ( $5.58 \pm 0.56$ ), d 16 ( $4.35 \pm 0.59$ ), and d 21 ( $3.47 \pm 0.58$ ).

Behavior frequencies are shown in Figures 2.9 and 2.14. Frequency of sitting, lying laterally, and lying ventrally were significantly different at different times of day regardless of treatment. Frequency of sitting ( $6.61 \pm 0.44$ ), lying ventrally ( $17.38 \pm 1.07$ ), and lying laterally ( $14.15 \pm 1.02$ ) were greater in the evening as compared to the morning ( $3.74 \pm 0.45$ ,  $10.98 \pm 1.07$ , and  $8.15 \pm 1.02$ , respectively) and afternoon ( $4.63 \pm 0.44$ ,  $13.7 \pm 1.07$ , and  $11 \pm 1.02$ , respectively). Pigs laid down ventrally and laterally more frequently, but for less time in the evening. They also sat more often and longer in the evening. Overall, instances of behavior changes occurred most frequently in the evening, indicating increased activity.

Frequency at the drinker was significantly different at different times of day, regardless of treatment. Drinker frequency increased from the morning ( $4.14 \pm 0.87$ ) to the afternoon ( $8.34 \pm 0.90$ ) and the afternoon to the evening ( $11.5 \pm 0.84$ ; Figure 2.15).

Figure 2.16 depicts a significant interaction between treatment and time of day on number of visits to the feeder. CON pigs visited the feeder more frequently in the morning ( $8.4 \pm 0.86$ ) than in the afternoon ( $5.92 \pm 0.89$ ) and evening ( $6.15 \pm 0.87$ ), which is consistent with feeding frequency data from group housed pigs (Hyun and Ellis, 2001; Hyun and Ellis, 2002). HS pigs had more visits to the feeder in the evening ( $7.83 \pm 0.87$ ) than in the morning ( $4.15 \pm 0.91$ ), but the afternoon ( $6.23 \pm 0.89$ ) was not different. CON pigs visited the feeder more frequently than HS pigs in the morning which is consistent with feeding behavior in group

housed pigs. HS and CON pigs highest feeding frequency occurred at opposite times of the day despite constant temperatures and continuous light, which typically dictates feeding patterns (Feddes et al., 1989).

Figure 2.17 indicates the significant interaction of lateral and ventral lying behaviors for the HS and CON treatments by day of the trial. HS pigs laid down more frequently in both lateral and ventral postures on d 2 ( $22.67 \pm 1.87$  and  $25.17 \pm 1.95$ ), d 6 ( $18 \pm 1.87$  and  $22.17 \pm 1.95$ ), and d 9 ( $16.67 \pm 1.87$  and  $20.58 \pm 1.95$ ) than CON pigs on d 2 ( $6.5 \pm 1.87$  and  $9.58 \pm 1.95$ ), d 6 ( $7.33 \pm 1.87$  and  $11.58 \pm 1.95$ ), and d 9 ( $10.92 \pm 1.87$  and  $14.25 \pm 1.95$ ). There was no difference in lying frequency in the lateral or ventral posture for HS and CON at the cessation of heat stress as observed on d 16 (HS  $8.67 \pm 1.87$  and  $10.58 \pm 1.95$ . CON  $7.17 \pm 1.87$  and  $9.58 \pm 1.95$ ) and d 21 (HS  $7.92 \pm 1.87$  and  $9.75 \pm 1.95$ . CON  $5.17 \pm 1.87$  and  $6.92 \pm 1.95$ ). Other studies show that pigs housed in thermoneutral conditions spend a majority of their time lying in a lateral posture in comparison to other behaviors (Ekkel et al., 2003), but heat stressed pigs spend more total time lying laterally (Nienaber and Hahn, 2004; Banhazi et al., 2008). Heat stressed pigs maximize contact with the floor in an effort to dissipate heat by convection (Nienaber and Hahn, 2004; Collier and Gebremedhin, 2015). Overall, activity level is decreased in heat stressed pigs, as is evident in the disparity in both lying postures during the heat stress phase. The reduction in activity for the HS group may have also led to decreased maintenance requirements and a subsequent reduction in feed intake.

Figure 2.18 depicts the significant interaction of treatment over days for drinking frequency. HS pigs visit the drinker more frequently on d 9 ( $13.5 \pm 1.53$ ) than on d 2 ( $9.42 \pm 1.53$ ), d 6 ( $9.92 \pm 1.53$ ), d 16 ( $6.01 \pm 1.88$ ), or d 21 ( $6.77 \pm 1.59$ ). CON pigs drinking frequency did not change over d 2 ( $8.00 \pm 1.53$ ), d 6 ( $8.17 \pm 1.53$ ), d 9 ( $5.92 \pm 1.53$ ), d 16 ( $6.01 \pm 1.59$ ), or

d 21 ( $6.77 \pm 1.68$ ). HS pigs visit the drinker more frequently than CON pigs on d 9. Pigs drank for longer durations and more frequently while experiencing heat stress (Figure 2.11, Figure 2.18). While heat stressed pigs were visiting the drinker more frequently and for longer, they could be using drinkers to spray themselves with water to cool down as well as drinking (Nienaber and Hahn, 2004; Banhazi et al., 2008).

## **Implications**

The inflammatory response observed in this trial cannot be compared to other heat stress trials using different stressor parameters. It is difficult to compare a long term, low grade heat stress to a shorter term, intense heat stress, as stressor type and duration variables impact physiologic response. The current trial should be considered a mild heat stress. However, it may be more influential on the pig's health than was previously considered, as behavioral changes and increased inflammatory activity was observed. Sampling timing similarity between experiments is also critical in order to compare them. Future projects should include a more granular sampling schedule in order to better capture changes in cortisol and cytokine concentration related to heat stress. It would also be worth considering localized tissues that are impacted by heat stress as a sampling site for cytokine response. Overall, this research addresses some of the potential physiologic responses induced by chronic heat stress in terms of systemic inflammatory response that occurs in tandem with eating, drinking, and postural changes. However, more research is needed to understand the impact of the systemic cytokine reaction to stress in a functional capacity. Furthermore, additional physiologic parameters need to be assessed in order to solidify the relationship between behavioral changes and internal pathogenic reactions in order to determine a non-invasive method for assessing stress state.

## Literature Cited

- Banhazi, T. M., A. J. Aarnink, H. T. Thuy, S. Pedersen, J. Hartung, H. Payne, B. Mullan, and D. Berckmans. 2008. Review of Issues Related to Heat Stress in Intensively Housed Pigs. In: *Livestock Environment VIII*, 31 August - 4 September 2008, Iguassu Falls, Brazil. American Society of Agricultural and Biological Engineers, St. Joseph, MI. Available from: <http://elibrary.asabe.org/abstract.asp?JID=1&AID=25578&CID=lenv2009&T=1>
- Bouchama, A., R. S. Parhar, A. El-Yazigi, K. Sheth, and S. Al-Sedairy. 1991. Endotoxemia and release of tumor necrosis factor and interleukin 1 $\alpha$  in acute heatstroke. *Journal of Applied Physiology*. 70:2640–2644. doi:10.1152/jappl.1991.70.6.2640.
- Collier, R. J., and K. G. Gebremedhin. 2015. Thermal Biology of Domestic Animals. *Annu. Rev. Anim. Biosci.* 3:513–545. doi:10.1146/annurev-animal-022114-110659. Available from: [www.annualreviews.org](http://www.annualreviews.org)
- Ekkel, E. D., H. A. M. Spoolder, I. Hulsege, and H. Hopster. 2003. Lying characteristics as determinants for space requirements in pigs. *Applied Animal Behaviour Science*. 80:19–30. doi:10.1016/S0168-1591(02)00154-5.
- Feddes, J. J. R., B. A. Young, and J. A. DeShazer. 1989. Influence of temperature and light on feeding behaviour of pigs. *Applied Animal Behaviour Science*. 23:215–222. doi:10.1016/0168-1591(89)90112-3.
- Frank, J. W., M. A. Mellencamp, J. A. Carroll, R. D. Boyd, and G. L. Allee. 2005. Acute feed intake and acute-phase protein responses following a lipopolysaccharide challenge in pigs from two dam lines. *Veterinary Immunology and Immunopathology*. 107:179–187. doi:10.1016/j.vetimm.2005.04.014.
- Fulford, A. J., and M. S. Harbuz. 2005. Chapter 1.3 An introduction to the HPA axis. In: *Techniques in the Behavioral and Neural Sciences*. Vol. 15. Academic Press. p. 43–65.
- Ganesan, S., C. Reynolds, K. Hollinger, S. C. Pearce, N. K. Gabler, L. H. Baumgard, R. P. Rhoads, and J. T. Selsby. 2016. Twelve hours of heat stress induces inflammatory signaling in porcine skeletal muscle. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*. 310:R1288–R1296. doi:10.1152/ajpregu.00494.2015. Available from: <https://www.physiology.org/doi/10.1152/ajpregu.00494.2015>
- Geers, R., R. Puers, and V. Goedseels. 1997. Electronic identification and monitoring of pigs during housing and transport. *Computers and Electronics in Agriculture*. 17:205–215. doi:10.1016/S0168-1699(96)01305-1.
- Heitman, H., C. F. Kelly, and T. E. Bond. 1958. Ambient Air Temperature and Weight Gain in Swine. *Journal of Animal Science*. 17:62–67. doi:10.2527/jas1958.17162x.

- Hyun, Y., and M. Ellis. 2001. Effect of group size and feeder type on growth performance and feeding patterns in growing pigs. *Journal of Animal Science*. 79:803–810. doi:10.2527/2001.794803x. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11325183>
- Hyun, Y., and M. Ellis. 2002. Effect of group size and feeder type on growth performance and feeding patterns in finishing pigs. *Journal of Animal Science*. 80:568–574. doi:10.2527/2002.803568x. Available from: <https://academic.oup.com/jas/article/80/3/568/4789410>
- Johnson, R. W. 1997. Inhibition of Growth by Pro-Inflammatory Cytokines: An Integrated View 1,2. Available from: <https://academic.oup.com/jas/article-abstract/75/5/1244/4625002>
- Johnson, R. W., and E. von Borell. 1994. Lipopolysaccharide-induced sickness behavior in pigs is inhibited by pretreatment with indomethacin. *Journal of Animal Science*. 72:309–314. doi:10.2527/1994.722309x. Available from: <https://academic.oup.com/jas/article/72/2/309-314/4632534>
- Lambert, G. P. 2009. Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. *Journal of animal science*. 87. doi:10.2527/jas.2008-1339.
- Leon, L. R., M. D. Blaha, and D. A. DuBose. 2006. Time course of cytokine, corticosterone, and tissue injury responses in mice during heat strain recovery. *Journal of Applied Physiology*. 100:1400–1409. doi:10.1152/jappphysiol.01040.2005.
- Mani, V., A. J. Harris, A. F. Keating, T. E. Weber, J. C. M. Dekkers, and N. K. Gabler. 2013. Intestinal integrity, endotoxin transport and detoxification in pigs divergently selected for residual feed intake1. *Journal of Animal Science*. 91:2141–2150. doi:10.2527/jas.2012-6053. Available from: <https://academic.oup.com/jas/article/91/5/2141/4717110>
- McDonald T. P., Jones D. D., Barrett J. R., Albright J. L., Miles G. E., Nienaber J. A., and Hahn G. L. 1988. Measuring the Heat Increment of Activity in Growing-Finishing Swine. *Transactions of the ASAE*. 31:1180–1186. doi:10.13031/2013.30841. Available from: <http://elibrary.asabe.org/abstract.asp??JID=3&AID=30841&CID=t1988&v=31&i=4&T=1>
- Muneta, Y., Y. Minagawa, T. Nakane, T. Shibahara, T. Yoshikawa, and Y. Omata. 2011. Interleukin-18 expression in pig salivary glands and salivary content changes during acute immobilization stress. *Stress*. 14:549–556. doi:10.3109/10253890.2011.565392.
- Nienaber, J. A., and G. L. Hahn. 2004. Engineering and Management Practices to Ameliorate Livestock Heat Stress.
- Nienaber, J. A., G. L. Hahn, and R. A. Eigenberg. 1999. Quantifying livestock responses for heat stress management: A review. *International Journal of Biometeorology*. 42:183–188. doi:10.1007/s004840050103.

- Pearce, S. C., N. K. Gabler, J. W. Ross, J. Escobar, J. F. Patience, R. P. Rhoads, and L. H. Baumgard. 2013a. The effects of heat stress and plane of nutrition on metabolism in growing pigs. *Journal of Animal Science*. 91:2108–2118. doi:10.2527/jas.2012-5738.
- Pearce, S. C., V. Mani, R. L. Boddicker, J. S. Johnson, T. E. Weber, J. W. Ross, R. P. Rhoads, L. H. Baumgard, and N. K. Gabler. 2013b. Heat Stress Reduces Intestinal Barrier Integrity and Favors Intestinal Glucose Transport in Growing Pigs. G. López-Lluch, editor. *PLoS ONE*. 8:e70215. doi:10.1371/journal.pone.0070215. Available from: <https://dx.plos.org/10.1371/journal.pone.0070215>
- Pearce, S. C., M.-V. Sanz Fernandez, J. Torrison, M. E. Wilson, L. H. Baumgard, and N. K. Gabler. 2015. Dietary organic zinc attenuates heat stress–induced changes in pig intestinal integrity and metabolism<sup>12</sup>. *Journal of Animal Science*. 93:4702–4713. doi:10.2527/jas.2015-9018. Available from: <https://academic.oup.com/jas/article/93/10/4702/4701604>
- Salak-Johnson, J. L., and J. J. McGlone. 2007. Making sense of apparently conflicting data: stress and immunity in swine and cattle. *Journal of animal science*. 85. doi:10.2527/jas.2006-538.
- Song, R., D. N. Foster, and G. C. Shurson. 2011. Effects of feeding diets containing bacitracin methylene disalicylate to heat-stressed finishing pigs. *Journal of Animal Science*. 89:1830–1843. doi:10.2527/jas.2010-3218. Available from: <http://academic.oup.com/jas/article/89/6/1830/4764439>
- Webel, D. M., B. N. Finck, D. H. Baker, and R. W. Johnson. 1997. Time course of increased plasma cytokines, cortisol, and urea nitrogen in pigs following intraperitoneal injection of lipopolysaccharide. *Journal of Animal Science*. 75:1514. doi:10.2527/1997.7561514x. Available from: <https://academic.oup.com/jas/article/75/6/1514-1520/4625013>

**Chapter III:**  
**Changes in Growth Performance, Cytokine Profile, and Behaviors of Group Housed**  
**Growing Pigs Subjected to Heat Stress**

## Abstract

This study was conducted to determine effects of heat stress on growth performance, cytokine profile and eating and drinking behaviors of growing pigs. Pigs ( $n = 64$ ) were housed 4 per pen in thermoneutral conditions (CON; mean  $24.75^{\circ}\text{C} \pm 2.90^{\circ}\text{C}$ ) or subjected to constant high ambient temperatures (mean  $28.63^{\circ}\text{C} \pm 1.42$ ) for 16 d (HS). Pig weights and feed disappearance were recorded weekly and used to calculate ADG, ADFI and GF. Blood samples were taken via venipuncture, and serum was analyzed for cytokine profile and cortisol concentration. Eight focal pigs (two pens per treatment) were video-recorded, and frequency and duration at the feeder, at the drinker, sitting, standing/walking, lying ventrally, and lying laterally was observed. On d 15 and d 30, 13 pigs per treatment were euthanized, and ileum, jejunum, and lung samples were excised. The ADG during week 3 was greater for CON (1.13 kg/d) than HS pigs (1.00 kg/d;  $P = 0.0201$ ). The ADFI for HS (2.14 kg/d) tended to be lesser than CON (2.24 kg/d;  $P = 0.0847$ ). The GF was greater for HS (0.62) during week 1 than CON pigs (0.52;  $P = 0.0059$ ). Surface temperature and respiration rate were greater in HS than CON from d 2 through d 15. Surface temperature was lesser for HS ( $36.41^{\circ}\text{C}$ ) than CON ( $37.77^{\circ}\text{C}$ ) on d 18 and d 22 (CON =  $38.56^{\circ}\text{C}$ , HS =  $39.34^{\circ}\text{C}$ ). A significant time by day interaction ( $P = 0.0022$ ) for cortisol concentration showed that HS pigs (40.12 ng/mL) were greater than CON (27.26 ng/mL) on d 2, however, on d 22 and d 25 CON pigs (48.85 ng/mL, 41.95 ng/mL) were greater than HS (32.93 ng/mL, 25.38 ng/mL). HS pigs tended to have greater systemic IL1 $\beta$  concentration (190.08 pg/mL) and had significantly higher IL12 concentration (914.89 pg/mL) compared to CON pigs (143.42 pg/mL;  $P = 0.0582$  and 785.37 pg/mL;  $P = 0.0024$ , respectively). HS (50.40 pg/mL) tended to have greater concentrations of IL6 in the ileal mucosa than CON (30.88 pg/mL;  $P = 0.0765$ ). The jejunal mucosa of the CON pigs (13.54 pg/mL) tended ( $P = 0.1014$ ) to have a

greater IL1A concentration than HS pigs (9.00 pg/mL) and CON pigs had a significantly greater IL12 concentration (18.13 pg/mL) compared to HS pigs (11.84 pg/mL;  $P = 0.0172$ ). BALF of the HS pigs tended ( $P = 0.1051$ ) to have a greater GMCSF concentration (6.92 pg/mL) compared to CON pigs (5.27 pg/mL) and had significantly greater concentration of IL4 (7.66 pg/mL) and IL12 (34.83 pg/mL) compared to CON pigs (4.17 pg/mL;  $P = 0.0185$ , 24.48 pg/mL;  $P = 0.0101$ , respectively). A significant treatment x day x time of day interaction was observed for the following; sitting frequency ( $P = 0.0182$ ), feeding duration ( $P = 0.0032$ ), ventral lying duration ( $P = 0.0251$ ), and lateral lying duration ( $P = 0.0001$ ). HS pigs visited the feeder more frequently in the evening (14.40;  $P = 0.0288$ ). Overall, heat stress reduces performance, impacts cortisol, increases circulating, ileum, and lung cytokines, and decreases jejunum cytokines. These physiologic changes occur simultaneously with changes in the frequency and duration of posture, eating, and drinking behaviors, indicating a potential relationship between them.

## **Introduction**

Heat stress in swine is a widespread problem that cost the industry about \$300 million annually (St-Pierre et al., 2003). A reduction in nutrient intake during heat stress (Rhoads et al., 2013), energy expenditure for cooling (Lambert, 2009), and behavior modification (Nienaber and Hahn, 2004) work in tandem to cool the pig, but they undermine genetic and nutritional efficiency advancements that have been made throughout the years (Ross et al., 2015). Most hog production has moved indoors in the United States and throughout the world in order to mitigate these losses via environmental control (Banhazi et al., 2008). Practicality and economics are important considerations for housing accommodation decisions for the abatement of heat stress

(Pedersen and Ravn, 2008), and a mechanistic understanding of the heat stress response is crucial for determining how to alleviate it.

The thermolytic response encompasses multiple organ systems. The cardiovascular system shunts blood from the core to the skin in order to facilitate heat exchange with the environment (Seebacher, 2009). The respiratory system alters the respiration rate and depth in order to maximize heat expulsion (Robertshaw, 2006). The digestive tract is both directly impacted through disparate expression of gastric substances (Pearce et al., 2014) and indirectly impacted by decreased feed intake (Heitman et al., 1958) and reduced splanchnic blood flow (Nakamura and Morrison, 2008). There are also many adjustments at the molecular level in response to heat stress. Stress activation of the hypothalamic-pituitary-adrenal axis can be monitored by free cortisol in the blood circulation (Salak-Johnson and McGlone, 2007). Inflammation and immune modulation can be assessed by systemic and local cytokines (Sutherland et al., 2006).

The current research was performed in an effort to expand the understanding of the cytokine response, both systemically and in specific organ systems, in the heat stressed pig. It was also a goal to associate stress biomarkers to changes in behavior so that heat stress pathogenesis can be measured objectively but non-invasively.

## **Materials and Methods**

### *Experimental Design*

A protocol for these experiments was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at North Carolina State University (NCSU; Raleigh, NC, USA).

Pigs (barrows n = 60; gilts n = 4) used for this study were obtained from the NCSU Swine Educational Unit at an average of  $24.57 \pm 3.69$  kg of body weight. The pigs are known to be PED and PRRS-negative. Prior to the start of the study, pigs were weighed, blocked by weight, and randomly assigned within weight block and sex to their treatment groups. Pigs were placed into pens (4 pigs per pen, 8 replicates per treatment) for a total of 32 pigs per treatment. . Two pens per treatment contained 1 gilt, and no gilts were used for temperature, respiration, blood, or tissue sampling. The pigs were offered a common diet and water was provided *ad libitum*.

An environmental control system was used to maintain constant temperatures in each of the rooms. Room temperature was recorded by data loggers (iButtonLink LLC, Whitewater, WI) every hour. Two loggers were placed in each room at approximately the same height of the pigs. The CON room temperature was set to at an average of 20°C. The HS room temperature was set to mimic a heat-stressed environment at an average of 27°C. There was a single speed exhaust fan that ran continuously in both rooms to remove odors, moisture, and provide fresh air for normal respiration. Ammonia content of the air was measured by a Basic NH<sub>3</sub> detector (Forensics Detectors, Los Angeles, California) twice per day. The heat stress began on d 1 of the trial and lasted until d 15. After d 15, the HS room setting was returned to a thermoneutral average of 19°C until d 30.

### *Performance Measurement*

Feed consumption was calculated from feed added to the feeder minus the feed left in the feeder at the end of the feeding phase. Pigs were weighed individually upon entry to the room on d 0 (start of the trial) and d 8, 15, 22, and 30. Pig body weights were recorded to the nearest 0.05

kg. ADG, ADFI, and GF were calculated from the weights of pigs and feeders and averaged by pen.

### *Biological Measurements*

Rectal temperatures were measured using a digital thermometer (iProven, Beaverton, OR) and surface temperatures were measured using a 220x160 HT-18 thermal imaging infrared camera (HTI, Dongguan City, Guangdong Province, China) at the hottest point between the shoulders on d 2, 4, 8, 11, 15, 18, 22, 25, and 30. Respiration rate was also taken on d 2, 4, 8, 11, 15, 18, 22, 25, and 30 by recording number of flank movements in 30 seconds on pigs lying laterally.

### *Systemic Cortisol and Cytokine Assays*

Blood samples were taken from pigs on d 2, 4, 8, 11, 15, 18, 22, and 25 by venipuncture into 10 mL vacuum tubes (BD Vacutainer Systems, Franklin Lakes, NJ) after temperature and respiration rate measurements had been collected. Blood was centrifuged at 900 x g for 20 minutes at 25°C to collect serum. HPA axis activation was determined by quantitatively measuring serum concentrations of cortisol (Saint Louis Zoological Park Endocrinology Laboratory, Saint Louis, MO). Systemic inflammation status was determined by quantitatively measuring serum concentrations of GM-CSF, IFN $\gamma$ , IL1 $\alpha$ , IL1Ra, IL1 $\beta$ , IL2, IL4, IL6, IL8, IL10, IL12, IL18, and TNF $\alpha$  cytokines (Eve Technologies Corporation, Calgary, AB Canada).

### *Bronchoalveolar lavage*

On d 15, 13 pigs were euthanized via captive bolt followed by exsanguination, 6 from the CON group and 7 from the HS group. On d 30, 12 pigs were euthanized, 6 from the CON group and 6 from the HS group. After being euthanized, the lungs were removed, and a balloon foley catheter was threaded through the trachea, into the primary bronchus, and advanced into the bronchus until it was met with resistance. The balloon was inflated with 10 CC of air and pulled back gently to ensure a seal and prevent backflow. A catheter syringe was used to inject 50 mL of ice cold 1x PBS (Fisher Scientific, Waltham, MA) buffer into the lung via the catheter. The lung was gently agitated, and the PBS was recovered in a 50 mL conical tube and frozen. If the lung lavage contained any blood, the sample was discarded, and the other lung was sampled.

Total protein of the bronchoalveolar lavage fluid (BALF) was determined using a Pierce™ BCA protein assay kit (Thermo Scientific™ Waltham, MA). Each sample was normalized to 1000 µg/mL total protein, and was sent to Eve Technologies Corporation (Calgary, AB Canada) for quantitative cytokine analysis.

### *Intestinal Histology and Mucosa Sampling*

After removing the lungs, intestinal sampling was performed. Approximately 5 to 7 cm of ileum sections were excised 20 cm proximal to the ileocecal junction, flushed with phosphate buffered saline (1x PBS; Fisher Scientific, Waltham, MA), and fixed in 10% formalin (The Lab Depot, Dawsonville, GA). Another 5 to 7 cm section was cut, and the section was opened to expose the mucosa. The mucosa was scraped using a microscope slide and placed into a cryotube and flash frozen.

Approximately 5 to 7 cm of jejunum sections were cut 20 cm from the pancreas, flushed with 1x PBS (Fisher Scientific, Waltham, MA), and fixed in 10% formalin (The Lab Depot, Dawsonville, GA). Another 5 to 7 cm section was cut, and the section was opened to expose the mucosa. The mucosa was scraped into a cryotube and flash frozen.

The formalin fixed intestinal samples were embedded in paraffin, and 2 sections were cut and mounted on slides (NCSU College of Veterinary Medicine Diagnostic Testing Lab, Raleigh, NC). They were then stained with hematoxylin and eosin (H&E). Slides were imaged on a Laxco SeBa digital microscope at 10x magnification. Villi height and crypt depth were determined using ImageJ using 10 well oriented villi per pig.

Mucosa scrapings were suspended in Fisher Scientific 1x PBS (81 Wyman Street, Waltham, MA), sonicated, and centrifuged to remove cellular debris. Total protein of the mucosa was determined using Pierce™ BCA Colormetric Assay (Thermo Scientific™, Waltham, MA). Each sample was normalized to 1500 µg/mL total protein using 1x PBS for quantitative cytokine analysis (Eve Technologies Corporation, Calgary, AB Canada).

### *Behavioral Analysis*

Video recording cameras (Axis Communications, Lund, Sweden) were placed on each side of the room so that 8 focal barrows in 2 pens from each treatment were recorded for observation of drinking and feeding behaviors using Viso™ video recording software and Observer annotation software (Noldus Information Technology Inc., Leesburg, VA). Behaviors were recorded for 2-hour time blocks from 0800 to 1000, 1200 to 1400, and 1800 to 2000 on d 3, 6, 10, 13, 17, 20 and 28. Recording sessions were started on an automatic schedule as to avoid human interference with normal pig behaviors. Number of times seen at the waterer and at the

feeder, as well as duration of sitting, ventral lying, lateral lying, and standing/walking were recorded and analyzed between treatments.

### *Statistical Analysis*

Data was analyzed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). Pen was used as the experimental unit for performance and respiration rate. Pig was used as an experimental unit for rectal temperature, surface temperature, behavior analysis, cytokine analysis, and cortisol analysis. Rectal temperature, serum measurements, and performance parameters were analyzed with repeated measures by day. Behaviors were analyzed with repeated measures with the variable time nested within day. Main effects and interactions were considered statistically significant at  $P \leq 0.05$  and tendencies were considered when  $0.05 \leq P \leq 0.10$ . Least squared means were reported with the SEM.

### **Results and Discussion**

The temperature settings established in the experimental design were not maintained due to unseasonably hot outdoor temperatures exceeding the capacity of the cooling systems in the barn. Figures 3.19 and 3.20 show actual temperatures during the study. From d 1 to d 12 of the project, the CON room temperature averaged  $23.87^{\circ}\text{C} \pm 2.47^{\circ}\text{C}$  (min  $18.85^{\circ}\text{C}$ ; max  $30.35^{\circ}\text{C}$ ) with an average relative humidity of  $73.27\% \pm 16.18\%$ . The HS room temperature was set to mimic a constant heat-stressed environment and averaged  $28.71^{\circ}\text{C} \pm 1.25^{\circ}\text{C}$  (min  $24.89^{\circ}\text{C}$ ; max  $32.13^{\circ}\text{C}$ ) with an average relative humidity of  $59.51\% \pm 11.57\%$ . From d 13 to d 17, the CON room temperature averaged  $26.75^{\circ}\text{C} \pm 2.71^{\circ}\text{C}$  (min  $20.85^{\circ}\text{C}$ ; max  $32.35^{\circ}\text{C}$ ) with an average relative humidity of  $72.77\% \pm 10.11\%$ . The HS room temperature averaged  $27.77^{\circ}\text{C} \pm 2.32^{\circ}\text{C}$

(min 22.14°C; max 32.375°C) with an average relative humidity of 68.22% ± 9.58% creating a constant heat stressed environment for HS and a diurnal heat stressed environment for CON. From d 18 to d 21 of the project both rooms maintained thermoneutral conditions. The CON room temperature averaged 24.55°C ± 1.55°C (min of 22.1°C; max 28.35°C) with an average relative humidity of 69.26% ± 10.32%. The HS room temperature was 24.16°C ± 2.00°C (min 22.14°C; max 28.39°C) with an average relative humidity of 70.46% ± 11.22%. From d 22 through the conclusion of the trial on d 30, the CON room temperature was 28.08°C ± 3.10°C (min 22.6°C; max 35.84°C) with an average relative humidity of 61.17% ± 12.25%. The HS room temperature averaged 27.81°C ± 2.93°C (min 21.39°C; max 35.37°C) with an average relative humidity of 61.90% ± 11.41%.

### *Performance*

A significant ( $P \leq 0.05$ ) treatment by time interaction was found for ADG (Figure 3.21). The mean ADG for the CON group was 0.92 kg/d for week 1, 0.99 kg/d for week 2, 1.13 kg/d for week 3, and 0.97 for week 4. The HS group was 1.00 kg/d for week 1, 0.90 kg/d for week 2, 1.00 kg/d for week 3, and 1.06 kg/d for week 4. The ADG for CON was greater than HS for week 3, during which the CON group was experiencing diurnal heat stress and the HS group was under constant heat stress. When a diurnal heat stress impacted both groups during week 4, there was a decline in ADG for CON but not HS. The HS group may have developed acclamatory mechanisms from the first exposure to heat stress (Horowitz, 2002) that reduced the energy cost of maintaining homeostasis and protected them from performance losses (Afsal et al., 2018). Pearce et al., (2014) reported changes in gastric inhibitory peptide, ghrelin, and cholecystokinin during heat stress, which may play a role in appetite attenuation and disparate metabolism.

Figure 3.22 shows the significant increase in ADFI between week 1 (1.68 kg/d) and week 2 (2.20 kg/d) and week 2 and weeks 3 (2.38 kg/d) and 4 (2.51 kg/d), regardless of treatment. Feed intake increased over time as the pigs grew, but intake stopped increasing between weeks 3 and 4. This could be due to high ambient temperature in both rooms, as CON pigs did gain less weight between week 3 and 4 (Figure 3.22). There was a tendency ( $P \leq 0.10$ ) for lower ADFI in the HS pigs ( $2.14 \text{ kg/d} \pm 0.04 \text{ kg/d}$ ) than CON ( $2.24 \text{ kg/d} \pm 0.04 \text{ kg/d}$ ) which also could have been due to the increased temperature for the CON pigs late in the trial.

Figure 3.23 depicts the significant interaction between treatment and week for (GF). In week 1, the GF ratio for HS (0.62) was significantly greater than CON (0.52). Both values were greater than any other week for both HS (week 2 - 0.43, week 3 - 0.44, week 4 - 0.41) and CON (week 2 - 0.44, week 3 - 0.46, week 4 - 0.39). There was a tendency ( $P \leq 0.10$ ) for ADFI to be lesser for the HS group ( $2.14 \text{ kg} \pm 0.01 \text{ kg}$ ) compared to the CON group ( $2.24 \text{ kg} \pm 0.04 \text{ kg}$ ). However, the ADG for both groups was the same for week 1 (Figure 3.21), which would explain the greater feed efficiency for HS pigs during that time (Figure 3.23).

### *Biological Measurements*

Rectal temperatures were significantly ( $P \leq 0.05$ ) different by day, regardless of treatment. Rectal temperatures dropped from  $40.06^\circ\text{C}$  on d 2 to  $39.58^\circ\text{C}$  on d 8. Temperature increased to  $39.78^\circ\text{C}$  on d 11,  $39.76^\circ\text{C}$  on d 15, and  $39.76^\circ\text{C}$  on d 18. There is another rectal temperature increase to  $40.10^\circ\text{C}$  on d 22 before it drops back down to  $39.82^\circ\text{C}$  on d 25 and  $39.86^\circ\text{C}$  on d 30 (Figure 3.24). The rectal temperature may have been slightly elevated at the start of the trial due to residual moving and mixing stress (Geers et al., 1997), and on d 22, both rooms exceed the heat stress threshold for environmental temperature. There was no significant

difference in rectal temperatures by treatment (CON  $39.68^{\circ}\text{C} \pm 0.05^{\circ}\text{C}$ ; HS  $39.81^{\circ}\text{C} \pm 0.05^{\circ}\text{C}$ ), which is likely due to both groups being exposed to the same environmental temperatures for part of the trial.

Figure 3.25 depicts a significant interaction of treatment by day for surface temperature of the pigs. The surface temperature for HS on d 2 ( $39.28^{\circ}\text{C}$ ), d 4 ( $40.12^{\circ}\text{C}$ ), d 8 ( $39.70^{\circ}\text{C}$ ), d 11 ( $39.07^{\circ}\text{C}$ ), and d 15 ( $39.11^{\circ}\text{C}$ ) was significantly greater than CON ( $37.40^{\circ}\text{C}$ ,  $37.81^{\circ}\text{C}$ ,  $37.38^{\circ}\text{C}$ ,  $37.34^{\circ}\text{C}$ , and  $37.92^{\circ}\text{C}$ , respectively). During the initial heat stress phase from d 1 through d 17, the HS group had the greater surface temperature compared to CON pigs, which is in agreement with previous research in swine (Morrison et al., 1967; Brown-Brandl et al., 2013; Soerensen and Pedersen, 2015; Afsal et al., 2018). Blood is shunted to the skin in animals approaching their UCT in order to exchange heat with the environment (Nakamura and Morrison, 2008). HS was significantly lower on d 18 ( $36.41^{\circ}\text{C}$ ) and d 22 ( $38.56^{\circ}\text{C}$ ) compared to CON ( $37.77^{\circ}\text{C}$  and  $39.34^{\circ}\text{C}$ , respectively) during which, both groups were in a thermoneutral phase. This could be an acclamatory effect from cardiovascular reserve increase in the HS group during chronic heat stress which would have allowed for more heat exchange between the skin and the environment (Horowitz, 2002). Upon return to heat stress (diurnal instead of constant) on d 22, the HS group skin surface temperature remained lower than the CON group, likely due to a decreased reaction threshold in the HS group improving the efficiency of heat dissipation upon repeat exposure (Horowitz, 2002). On d 25 and d 30, neither group experienced increased temperature, indicating that the diurnal heat stress does not elicit the same skin reaction as constant heat stress.

Figure 3.26 depicts a significant interaction between treatment and day for respiration rate. Respiration rate was greater for HS than CON on d 2 (42.69 breaths/30sec and 22.75 breaths/30sec), d 4 (45.38 breaths/30sec and 35.91 breaths/30sec), d 8 (39.21 breaths/30sec and

29.94 breaths/30sec), d 11 (38.19 breaths/30sec and 27.63 breaths/30sec), and d 15 (40.91 breaths/30sec and 31.94 breaths/30sec), but not different for d 18 (30.00 breaths/30sec and 32.23 breaths/30sec), d 22 (33.5 breaths/30sec and 31.19 breaths/30sec), d 25 (27.56 breaths/30sec and 29.13 breaths/30sec), and d 30 (29.06 breaths/30sec and 27.94 breaths/30sec). Respiration rate was greater in the HS group during the HS phase d 1 through d 17, but not different during the thermoneutral phase, which is consistent with previous research (Brown-Brandl et al., 2001; Huynh et al., 2005). The diurnal heat stress from d 22 through d 30 did not increase respiration rate from the rate recorded during the thermoneutral phase.

### *Cortisol*

Figure 3.27 depicts the significant interaction of treatment and day for serum cortisol concentrations. The concentration of cortisol in serum for HS ( $40.12 \text{ ng/mL} \pm 3.60 \text{ ng/mL}$ ) was greater than CON on d 2 ( $27.26 \text{ ng/mL} \pm 3.60 \text{ ng/mL}$ ). Increased cortisol has been observed in pigs housed under hyperthermic conditions (Marple et al., 1974). Cortisol concentration in CON was greater than HS on d 22 ( $48.85 \text{ ng/mL} \pm 4.56 \text{ ng/mL}$  and  $32.93 \text{ ng/mL} \pm 4.56 \text{ ng/mL}$ , respectively) and d 25 ( $41.95 \text{ ng/mL} \pm 4.56 \text{ ng/mL}$  and  $25.38 \text{ ng/mL} \pm 4.56 \text{ ng/mL}$ , respectively). CON and HS cortisol concentrations were not different on d 4 ( $30.73 \text{ ng/mL} \pm 3.60 \text{ ng/mL}$  and  $23.18 \text{ ng/mL} \pm 3.60 \text{ ng/mL}$ , respectively), d 8 ( $21.88 \text{ ng/mL} \pm 3.60 \text{ ng/mL}$  and  $27.53 \text{ ng/mL} \pm 3.60 \text{ ng/mL}$ , respectively), d 11 ( $26.67 \text{ ng/mL} \pm 3.60 \text{ ng/mL}$  and  $26.85 \text{ ng/mL} \pm 3.60 \text{ ng/mL}$ , respectively), d 15 ( $28.06 \text{ ng/mL} \pm 3.60 \text{ ng/mL}$  and  $22.14 \text{ ng/mL} \pm 3.60 \text{ ng/mL}$ , respectively), or d 18 ( $35.60 \text{ ng/mL} \pm 4.56 \text{ ng/mL}$  and  $26.57 \text{ ng/mL} \pm 4.56 \text{ ng/mL}$ , respectively). HS cortisol concentration dropped from d 2 to d 4 during constant heat stress, indicating possible endocrine acclimation (Sutherland et al., 2006; Afsal et al., 2018). CON cortisol concentration

increased on d 22 and d 25 and was greater than HS, which is another indicator of endocrine acclimation in the HS group. The cortisol response in HS pigs do not react as strongly to the second exposure of heat stress as CON does to their first exposure (Horowitz, 2002; Afsal et al., 2018). Also, CON dropped in ADG in week 4 aligns with times of high cortisol on d 22 and d 25. Cortisol leads to favoring energy mobilization over energy storage and growth (Afsal et al., 2018). This is supported by (Pearce et al., 2014), who reported an increase in circulating glucose in heat stressed pigs. The high cortisol concentrations may contribute to the drop in growth seen in the CON group at week 4 (Figure 3.21, Figure 3.27).

### *Systemic Cytokines*

Figure 3.28 shows that profiles for IFN $\gamma$ , IL1A, IL1 $\beta$ , and IL1Ra were significantly ( $P \leq 0.05$ ) different by day, regardless of treatment. IFN $\gamma$  concentration was highest at d 2 (856.63 pg/mL  $\pm$  152.92 pg/mL) and d 4 (806.04 pg/mL  $\pm$  152.92 pg/mL), dropped for d 8 (372.29 pg/mL  $\pm$  152.45 pg/mL) and d 11 (321.55 pg/mL  $\pm$  158.29 pg/mL), rose again on d 15 (612.29 pg/mL  $\pm$  160.73 pg/mL), then finally dropped for d 18 (205.17 pg/mL  $\pm$  205.17 pg/mL), d 22 (169.27 pg/mL  $\pm$  198.74 pg/mL), and d 25 (266.61 pg/mL  $\pm$  198.74 pg/mL). IFN $\gamma$  is inhibited by glucocorticoids (Wiegers and Reul, 1998; Sapolsky et al., 2000), of which, cortisol began to rise in both groups around d 18 (Figure 3.21). IL1A concentration was lesser on d 2 (33.85 pg/mL  $\pm$  24.31 pg/mL), d 4 (28.91 pg/mL  $\pm$  23.88 pg/mL), and d 8 (22.51 pg/mL  $\pm$  24.31 pg/mL) than any other day. It rose on d 11 (95.24 pg/mL  $\pm$  23.88 pg/mL), then again on d 15 (199.60 pg/mL  $\pm$  23.49 pg/mL), d 18 (231.66 pg/mL  $\pm$  29.71 pg/mL), d 22 (232.83 pg/mL  $\pm$  29.71 pg/mL), and d 25 (224.38 pg/mL  $\pm$  29.71 pg/mL). IL1 $\beta$  concentration starts high on d 2 (371.23 pg/mL  $\pm$  31.30 pg/mL), then dropped over d 4 (339.71 pg/mL  $\pm$  31.30 pg/mL), 8 (266.94 pg/mL  $\pm$  31.30

pg/mL), and 11 (192.01 pg/mL  $\pm$  31.30 pg/mL) until settling over d 15 (37.38 pg/mL  $\pm$  31.30 pg/mL), 18 (42.66 pg/mL  $\pm$  39.60 pg/mL), 22 (39.60 pg/mL  $\pm$  39.60 pg/mL), and 25 (41.33 pg/mL  $\pm$  39.60 pg/mL). IL1Ra concentration dropped over d 2 (480.51 pg/mL  $\pm$  51.53 pg/mL), d 4 (428.93 pg/mL  $\pm$  51.53 pg/mL), d 8 (347.34 pg/mL  $\pm$  51.53 pg/mL), and d 11 (283.16 pg/mL  $\pm$  51.53 pg/mL), and leveled out for d 15 (293.96 pg/mL  $\pm$  51.53 pg/mL), d 18 (291.21 pg/mL  $\pm$  65.18 pg/mL), d 22 (252.57 pg/mL  $\pm$  65.18 pg/mL), and d 25 (271.95 pg/mL  $\pm$  65.18 pg/mL) (Figure 3.29). IL1A and IL1 $\beta$  are both agonists within the same family of proinflammatory cytokines, and IL1Ra is a competitive IL1 receptor antagonist whose release is stimulated by IL1 $\beta$  as a self-regulatory measure (Arend, 2002). IL1 stimulates the HPA axis, which can result in suppression of its own production by glucocorticoids (Wiegers and Reul, 1998). The drop in systemic IL1 $\beta$  and IL1Ra corresponds with the drop in cortisol concentration (Figure 3.29, Figure 3.21).

Figure 3.29 depicts cytokine differences by treatment. There was a tendency ( $P \leq 0.10$ ) for IL1 $\beta$  concentration to be greater for HS pigs compared to (190.42 pg/mL  $\pm$  17.32 pg/mL) CON pigs (143.42 pg/mL  $\pm$  17.32 pg/mL). IL12 concentration was significantly greater in HS (922.21 pg/mL  $\pm$  28.57 pg/mL) than CON (828.5 pg/mL  $\pm$  41.24 pg/mL). IL1 cytokines can stimulate glucocorticoid production via HPA axis activation, bolstering the stress response (Kovács et al., 2005). Mice show an increase in IL1 cytokine concentration during heat stress, but the opposite is observed in humans (Sapolsky et al., 2000). Both IL1 $\beta$  and IL12 are suppressed by glucocorticoids and catecholamines (Elenkov et al., 1999; Sapolsky et al., 2000; Kovács et al., 2005), which are activated during the heat stress response (Afsal et al., 2018).

### *Intestine Cytokines*

Figure 3.30 depicts cytokines measured within the mucosa of the ileum or jejunum by treatment. There was a tendency for ( $P \leq 0.1$ ) differences in IL6 concentration in the ileum. There was a numeric difference in IL1A concentration in the jejunum ( $P = 0.1014$ ) and a significant ( $P \leq 0.05$ ) difference in IL12 concentration in the jejunum. Ileum IL6 concentration was greater in HS ( $50.40 \text{ pg/mL} \pm 7.05 \text{ pg/mL}$ ) than CON ( $30.88 \text{ pg/mL} \pm 7.28 \text{ pg/mL}$ ). Jejunum IL1A concentration and IL12 concentration was greater in CON ( $13.54 \text{ pg/mL} \pm 1.84 \text{ pg/mL}$  and  $18.13 \text{ pg/mL} \pm 1.68 \text{ pg/mL}$ ) than HS ( $8.97 \text{ pg/mL} \pm 1.93 \text{ pg/mL}$  and  $11.84 \text{ pg/mL} \pm 1.76 \text{ pg/mL}$ ). The ileum is equipped with more immune specialized mucosa-associated lymphoid tissues (MALT) and Peyer's patches than the jejunum (Brandtzaeg et al., 1999). IL12 stimulates IL1 in circulation, but is inhibited by it, in turn (Hopkins et al., 2018). IL6 and IL1A are both normal responders to mucosal immune challenge, but both are also involved in pathogenic autoinflammatory reactions (Bamias and Cominelli, 2016). However, in mice, IL6 inhibits cell death in the intestine during pro-apoptotic stimulus (Jin et al. 2010). Locally, IL1 is stimulated during stress (Elenkov and Chrousos, 2002), but it is inhibited by cortisol systemically (Elenkov et al., 1999). IL8 concentration in the ileum tended to be greater on d 30 ( $7401.00 \text{ pg/mL} \pm 763.02 \text{ pg/mL}$ ) than d 15 ( $5334.97 \text{ pg/mL} \pm 729.30 \text{ pg/mL}$ ). IL8 concentration in the jejunum was significantly greater on d 30 ( $5006.35 \text{ pg/mL} \pm 586.77 \text{ pg/mL}$ ) than d 15 ( $2894.55 \text{ pg/mL} \pm 560.84 \text{ pg/mL}$ ). IL12 concentration was significantly greater in the jejunum, on d 30 ( $17.88 \text{ pg/mL} \pm 1.76 \text{ pg/mL}$ ) than d 15 ( $12.08 \text{ pg/mL} \pm 1.68 \text{ pg/mL}$ ). On d 15 the HS group was under heat stress conditions and the CON group was not. However, on d 30, both groups were experiencing diurnal heat stress which may have contributed to the differences observed.

### *Lung Cytokines*

Figure 3.31 depicts cytokine differences in BALF by treatment. GMCSF had a numerically ( $P = 0.0151$ ) greater concentration in HS ( $6.92 \text{ pg/mL} \pm 0.73 \text{ pg/mL}$ ) group than CON ( $5.27 \text{ pg/mL} \pm 0.61 \text{ pg/mL}$ ). IL4 and IL12 concentrations were significantly greater in HS ( $7.66 \text{ pg/mL} \pm 1.02 \text{ pg/mL}$  and  $34.83 \text{ pg/mL} \pm 2.57 \text{ pg/mL}$ ) than CON ( $4.17 \text{ pg/mL} \pm 0.85 \text{ pg/mL}$  and  $24.48 \text{ pg/mL} \pm 2.57 \text{ pg/mL}$ ). GMCSF is involved in normal alveolar macrophage function, but an elevation in GMCSF as a result of hyperoxia has been recorded in mice (Baleeiro et al., 2006). Respiration rates increase during heat stress that may result in hyperventilating the alveoli (Robertshaw, 2006). Elevated GMCSF has been shown to stimulate the production of IL4 (Wang et al., 2009).

There was a significant ( $P \leq 0.05$ ) difference of IL1Ra and IL4 concentration and a tendency ( $P \leq 0.10$ ) for differences of IL18 concentration by day in BALF, regardless of treatment. IL1Ra and IL18 concentrations were greater on d 15 ( $69.51 \text{ pg/mL} \pm 9.36 \text{ pg/mL}$  and  $102.52 \text{ pg/mL} \pm 21.50 \text{ pg/mL}$ ) than on d 30 ( $41.12 \text{ pg/mL} \pm 9.36 \text{ pg/mL}$  and  $50.82 \text{ pg/mL} \pm 21.50 \text{ pg/mL}$ ). IL4 concentration was greater on d 30 ( $7.52 \text{ pg/mL} \pm 0.96 \text{ pg/mL}$ ) than d 15 ( $4.31 \text{ pg/mL} \pm 0.91 \text{ pg/mL}$ ). On d 15 the HS group was under heat stress conditions and the CON group was not. However, at d 30, both groups were heat stressed.

### *Histology*

There were no significant differences in villi height or crypt depth histological measurements for the ileum and jejunum during heat stress (Table 3.1). This may be partially explained by the protective effect of IL6 in the ileum (Jin et al. 2010).

### *Behavior Duration*

There was a significant ( $P \leq 0.05$ ) difference in total minutes at the feeder between HS and CON based on time of day and day (Table 3.2). The diurnal fluctuation in temperature for both rooms likely influenced the time of day that pigs ate (Feddes et al., 1989), especially for the HS pigs. In addition, the normal circadian rhythm of cortisol drives morning feeding behavior (Boumans et al., 2017). This may be seen in CON pigs that spent more time at the feeder in the morning than HS pigs until d 20, when both groups were heat stressed and CON cortisol was elevated (Figure 3.27). The total amount of time CON pigs spent at the feeder dropped after d 13, which may correspond with their elevated cortisol (Figure 3.27) and drop in ADG during week 3 (Figure 3.21) (Boumans et al., 2017). Overall, pigs may be adjusting their nutrient intake to regulate energy and heat generation to mitigate heat stress (Rhoads et al., 2013), and the strong interaction between treatment, day, and time was likely due to the unintended heat stress that impacted both groups.

There was a significant effect of time by day for duration at drinker, regardless of treatment. On d 3, pigs drank for  $0.44 \text{ min} \pm 0.26 \text{ min}$  in the morning,  $0.99 \text{ min} \pm 0.26 \text{ min}$  in the afternoon, and  $0.31 \text{ min} \pm 0.26 \text{ min}$  in the evening. On d 6, pigs drank for  $0.64 \text{ min} \pm 0.26 \text{ min}$  in the morning,  $0.76 \text{ min} \pm 0.26 \text{ min}$  in the afternoon, and  $0.63 \text{ min} \pm 0.26 \text{ min}$  in the evening. On d 10, pigs drank for  $0.88 \text{ min} \pm 0.26 \text{ min}$  in the morning,  $1.08 \text{ min} \pm 0.26 \text{ min}$  in the afternoon, and  $1.31 \text{ min} \pm 0.26 \text{ min}$  in the evening. On d 13, pigs drank for  $0.97 \text{ min} \pm 0.26 \text{ min}$  in the morning,  $2.24 \text{ min} \pm 0.26 \text{ min}$  in the afternoon, and  $0.66 \text{ min} \pm 0.26 \text{ min}$  in the evening. On d 20, pigs drank for  $1.08 \text{ min} \pm 0.26 \text{ min}$  in the morning,  $0.51 \text{ min} \pm 0.26 \text{ min}$  in the afternoon, and  $0.52 \text{ min} \pm 0.26 \text{ min}$  in the evening. On d 29, pigs drank for  $0.27 \text{ min} \pm 0.26 \text{ min}$  in the morning,  $1.12 \text{ min} \pm 0.26 \text{ min}$  in the afternoon, and  $0.64 \text{ min} \pm 0.26 \text{ min}$  in the evening. A majority of drinking

behaviors are preprandial (Bigelow and Houpt, 1988), so this interaction was likely driven by feeding behaviors.

Table 3.3 shows a significant effect of time by treatment by day in duration of lying in both ventral and lateral postures. Laying laterally maximizes contact with the floor to improve the efficiency of conductive heat exchange (Nienaber and Hahn, 2004; Collier and Gebremedhin, 2015), and heat stressed pigs spend more time lying down than pigs in thermoneutral environments (Banhazi et al., 2008). Other stressors, such as shipping, cause a change in lying behaviors (Hicks et al., 1998), so it may be more than just heat exchange driving the shift.

There was a significant effect of time for duration of sitting by day, regardless of treatment. On d 3, pigs sat for  $3.01 \text{ min} \pm 0.56 \text{ min}$  in the morning,  $1.74 \text{ min} \pm 0.56 \text{ min}$  in the afternoon, and  $0.57 \text{ min} \pm 0.56 \text{ min}$  in the evening. On d 6, pigs sat for  $1.42 \text{ min} \pm 0.56 \text{ min}$  in the morning,  $1.26 \text{ min} \pm 0.56 \text{ min}$  in the afternoon, and  $1.10 \text{ min} \pm 0.56 \text{ min}$  in the evening. On d 10, pigs sat for  $2.37 \text{ min} \pm 0.56 \text{ min}$  in the morning,  $1.67 \text{ min} \pm 0.56 \text{ min}$  in the afternoon, and  $1.43 \text{ min} \pm 0.56 \text{ min}$  in the evening. On d 13, pigs sat for  $0.48 \text{ min} \pm 0.56 \text{ min}$  in the morning,  $2.14 \text{ min} \pm 0.56 \text{ min}$  in the afternoon, and  $0.38 \text{ min} \pm 0.56 \text{ min}$  in the evening. On d 20, pigs sat for  $2.80 \text{ min} \pm 0.56 \text{ min}$  in the morning,  $0.40 \text{ min} \pm 0.56 \text{ min}$  in the afternoon, and  $1.38 \text{ min} \pm 0.56 \text{ min}$  in the evening. On d 29, pigs sat for  $0.90 \text{ min} \pm 0.61 \text{ min}$  in the morning,  $1.90 \text{ min} \pm 0.56 \text{ min}$  in the afternoon, and  $1.06 \text{ min} \pm 0.56 \text{ min}$  in the evening.

There was also a significant difference in sitting behavior for treatment by time. In the morning, CON sat  $2.71 \text{ min} \pm 0.35 \text{ min}$  and HS sat  $0.95 \text{ min} \pm 0.32 \text{ min}$ . In the afternoon, CON sat  $1.74 \text{ min} \pm 0.32 \text{ min}$  and HS sat  $1.30 \text{ min} \pm 0.32 \text{ min}$  (Figure 3.32). In the evening, CON sat  $0.94 \text{ min} \pm 0.32 \text{ min}$  and HS sat  $1.03 \text{ min} \pm 0.32 \text{ min}$ . Sitting was often observed as a transition between two behaviors and may be indicative of general activity.

### *Behavior Frequency*

There was a significant effect of time by day for frequency at feeder, regardless of treatment. On d 3, pigs visited the feeder  $13.06 \pm 2.44$  times in the morning,  $15.75 \pm 2.44$  in the afternoon, and  $5.69 \pm 2.44$  in the evening. On d 6, pigs visited the feeder  $11.00 \pm 2.44$  times in the morning,  $13.44 \pm 2.44$  in the afternoon, and  $18.56 \pm 2.45$  in the evening. On d 10, pigs visited the feeder  $10.56 \pm 2.44$  times in the morning,  $25.63 \pm 2.44$  in the afternoon, and  $10.44 \pm 2.44$  in the evening. On d 13, pigs visited the feeder  $5.63 \pm 2.44$  times in the morning,  $9.00 \pm 2.44$  in the afternoon, and  $2.78 \pm 2.52$  in the evening. On d 20, pigs visited the feeder  $8.94 \pm 2.44$  times in the morning,  $3.19 \pm 2.44$  in the afternoon, and  $19.88 \pm 2.44$  in the evening. On d 29, pigs visited the feeder  $6.31 \pm 2.67$  times in the morning,  $4.00 \pm 2.44$  in the afternoon, and  $9.25 \pm 2.44$  in the evening.

There was a significant difference of frequency at the feeder for treatment by time. CON pigs visited the feeder  $9.80 \pm 1.51$  times in the morning and HS visited the feeder  $8.70 \pm 1.37$  times. In the afternoon, CON visited the feeder  $10.21 \pm 1.41$  times and HS visited the feeder  $13.46 \pm 1.41$  times. In the evening, CON visited the feeder  $7.80 \pm 1.42$  times and HS visited the feeder  $14.40 \pm 1.41$  times (Figure 3.33). Both duration and frequency at the feeder was lowest in the morning (Table 3.1, Figure 3.15). The HS pigs likely shifted their feeding frequency to the cooler parts of the day, as seen by Feddes et al., 1989.

There was a significant effect of time by day for frequency at drinker, regardless of treatment. On d 3, pigs visited the drinker  $4.19 \pm 1.10$  times in the morning,  $7.31 \pm 1.10$  in the afternoon, and  $1.88 \pm 1.10$  in the evening. On d 6, pigs visited the drinker  $3.44 \pm 1.10$  times in the morning,  $6.25 \pm 1.10$  in the afternoon, and  $4.38 \pm 1.10$  in the evening. On d10, pigs visited the drinker  $5.81 \pm 1.10$  times in the morning,  $9.25 \pm 1.10$  in the afternoon, and  $6.63 \pm 1.10$  in the

evening. On d 13, pigs visited the drinker  $3.56 \pm 1.10$  times in the morning,  $9.13 \pm 1.10$  in the afternoon, and  $3.38 \pm 1.10$  in the evening. On d 20, pigs visited the drinker  $4.88 \pm 1.10$  times in the morning,  $2.56 \pm 1.10$  in the afternoon, and  $4.38 \pm 1.10$  in the evening. On d 29, pigs visited the drinker  $1.90 \pm 1.10$  times in the morning,  $3.81 \pm 1.10$  in the afternoon, and  $3.31 \pm 1.10$  in the evening. As with drinking duration, this pattern was likely driven by the shift in meal intake (Bigelow and Houpt, 1988). Drinking frequency was not significantly impacted by treatment.

There was a significant effect of time by day for frequency lying ventrally, regardless of treatment. On d 3, pigs laid down  $16.75 \pm 1.85$  times in the morning,  $15.63 \pm 1.85$  in the afternoon, and  $7.25 \pm 1.85$  in the evening. On d 6, pigs laid down  $16.69 \pm 1.85$  times in the morning,  $16.63 \pm 1.85$  in the afternoon, and  $14.06 \pm 1.85$  in the evening. On d 10, pigs laid down  $18.38 \pm 1.85$  times in the morning,  $21.38 \pm 1.85$  in the afternoon, and  $18.44 \pm 1.85$  in the evening. On d 13, pigs laid down  $10.56 \pm 1.85$  times in the morning,  $17.00 \pm 1.85$  in the afternoon, and  $13.19 \pm 1.85$  in the evening. On d 20, pigs laid down  $10.88 \pm 1.85$  times in the morning,  $7.31 \pm 1.85$  in the afternoon, and  $10.44 \pm 1.85$  in the evening. On d 29, pigs laid down  $9.85 \pm 2.22$  times in the morning,  $14.13 \pm 1.85$  in the afternoon, and  $10.75 \pm 1.85$  in the evening. Pigs lie down more often when they were warm (Nienaber and Hahn, 2004) and are more likely to be settling down to sleep in the evening videos.

Figure 3.34 shows a significant effect of treatment by day in frequency of both ventral and lateral lying postures. CON pigs laid down ventrally  $10.95 \pm 1.51$  times on d 3,  $13.13 \pm 1.51$  on d6,  $13.00 \pm 1.51$  on d 10,  $12.71 \pm 1.51$  on d 13,  $9.04 \pm 1.51$  on d 20, and  $11.44 \pm 1.76$  on d 29. HS pigs laid down ventrally  $15.46 \pm 1.51$  on d 2,  $18.46 \pm 1.51$  on d 6,  $25.79 \pm 1.51$  on d 10,  $14.46 \pm 1.51$  on d 13,  $10.04 \pm 1.51$  on d 20, and  $11.71 \pm 1.48$  on d 29. CON pigs laid down laterally  $6.08 \pm 1.01$  times on d 3,  $7.71 \pm 1.01$  on d 6,  $6.71 \pm 1.01$  on d 10,  $7.08 \pm 1.01$  on d 13,

4.79 ± 1.01 on d 20, and 6.70 ± 1.14 on d 29. HS pigs laid down laterally 9.79 ± 1.01 on d 2, 10.88 ± 1.01 on d 6, 15.50 ± 1.01 on d 10, 9.58 ± 1.01 on d 13, 5.83 ± 1.01 on d 20, and 6.79 ± 0.95 on d 29. HS pigs laid down in both postures more frequently than CON pigs during the initial heat stress phase, which has been observed in heat stress in previous research (Nienaber and Hahn, 2004; Banhazi et al., 2008). HS lying behaviors in both postures was similar to CON for the remainder of the trial.

Table 3.3 shows a significant effect of treatment by day by time for frequency of sitting. Sitting was often observed when pigs were using the drinkers or changing postures.

## **Implications**

Due to unforeseen circumstances and a loss of climate control, there were additional layers of complexity to the data interpretation. However, adaptation responses in the HS group may have been observed in ADG. ADG for CON pigs decreased when exposed to heat stress for the first time between week 3 and week 4, but ADG for HS pigs increased, as it was the group's second exposure to heat stress. The overreaction of HS surface temperature on d 18 and d 22, following the secession of heat stress, may be an indicator of an adaptive cardiovascular response readjusting to thermoneutral conditions (Figure 3.25). Most convincing is the systemic cortisol increase in the CON group following d 18 that is not present in the HS group (Figure 3.27). Cortisol is one of the primary drivers of the stress response (Sapolsky et al., 2000), and its lack of reaction to repeat stress in HS pigs may prevent some of the other physiologic heat stress reactions that often lead to pathogenesis (Afsal et al., 2018).

The cytokine response, especially IL1 $\beta$  and IL6 concentrations, in both the bloodstream and organs indicate an immune response that could be impacting energy use and growth

(Johnson, 1997). A combination of cytokines and cortisol are the likely drivers of eating behavioral changes (Table 3.1, Figure 3.29, Figure 3.27) and drinking changes by proxy.

Overall, heat stress reduces performance, impacts cortisol, increases circulating, ileum, and lung cytokines, and decreases jejunum cytokines. These physiologic changes occur simultaneously with changes in the frequency and duration of posture, eating, and drinking behaviors, indicating a potential relationship between them. A deeper understanding of the molecular drivers of heat stress coupled with behavioral reactions could help in the generation of more effective mitigation strategies in the future.

## **Conclusion**

Behavioral observation can be subjective and difficult to interpret. It is worthwhile to layer observable behaviors with concrete, objective biological parameters to understand what the behavior changes mean. Overall, this research addresses some of the potential physiologic responses induced by chronic heat stress in terms of systemic inflammatory response that occurs in tandem with eating, drinking, and postural changes. While varied research has been done in the field, there is still more to do to understand and link behavior and the systemic cytokine and cortisol reactions. Furthermore, additional physiologic parameters need to be assessed in order to solidify the relationship between behavioral changes and internal pathogenic reactions in order to determine a non-invasive method for assessing stress state. This link could lead to the development of a functional, non-invasive predictor of stress so that mitigation strategies can be applied earlier and more effectively.

## Literature Cited

- Afsal, A., V. Sejian, M. Bagath, G. Krishnan, C. Devaraj, and R. Bhatta. 2018. Heat Stress and Livestock Adaptation: Neuro-endocrine Regulation. *International Journal of Veterinary and Animal Medicine*. 1. doi:10.31021/ijvam.20181108. Available from: <https://www.boffinaccess.com/journals/international-journal-veterinary-animal-medicine/index.php>
- Arend, W. P. 2002. The balance between IL-1 and IL-1Ra in disease. *Cytokine and Growth Factor Reviews*. 13:323–340. doi:10.1016/S1359-6101(02)00020-5.
- Baleeiro, C. E. O., P. J. Christensen, S. B. Morris, M. P. Mendez, S. E. Wilcoxon, and R. Paine. 2006. GM-CSF and the impaired pulmonary innate immune response following hyperoxic stress. *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 291:L1246–L1255. doi:10.1152/ajplung.00016.2006. Available from: <https://www.physiology.org/doi/10.1152/ajplung.00016.2006>
- Bamias, G., and F. Cominelli. 2016. Cytokines and intestinal inflammation. *Current Opinion in Gastroenterology*. 32:437–442. doi:10.1097/MOG.0000000000000315. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/27673380>
- Banhazi, T. M., A. J. Aarnink, H. T. Thuy, S. Pedersen, J. Hartung, H. Payne, B. Mullan, and D. Berckmans. 2008. Review of Issues Related to Heat Stress in Intensively Housed Pigs. In: *Livestock Environment VIII*, 31 August - 4 September 2008, Iguassu Falls, Brazil. American Society of Agricultural and Biological Engineers, St. Joseph, MI. Available from: <http://elibrary.asabe.org/abstract.asp?JID=1&AID=25578&CID=lenv2009&T=1>
- Bigelow, J. A., and T. R. Houpt. 1988. Feeding and drinking patterns in young pigs. *Physiology and Behavior*. 43:99–109. doi:10.1016/0031-9384(88)90104-7. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/3413258>
- Boumans, I. J. M. M., I. J. M. de Boer, G. J. Hofstede, S. E. la Fleur, and E. A. M. Bokkers. 2017. The importance of hormonal circadian rhythms in daily feeding patterns: An illustration with simulated pigs. *Hormones and Behavior*. 93:82–93. doi:10.1016/j.yhbeh.2017.05.003.
- Brandtzaeg, P., E. S. Baekkevold, I. N. Farstad, F. L. Jahnsen, F. E. Johansen, E. M. Nilsen, and T. Yamanaka. 1999. Regional specialization in the mucosal immune system: What happens in the microcompartments? *Immunology Today*. 20:141–151. doi:10.1016/S0167-5699(98)01413-3.
- Brown-Brandl, T. M., R. A. Eigenberg, John A. Nienaber, Stephen D. Kachman, J. A. Nienaber, and S. D. Kachman. 2001. Thermoregulatory profile of a newer genetic line of pigs. Available from: <https://digitalcommons.unl.edu/usdaarsfacpubhttps://digitalcommons.unl.edu/usdaarsfacpub/178www.elsevier.com/locate/livprodsci>

- Brown-Brandl, T. M., R. A. Eigenberg, and J. L. Purswell. 2013. Using thermal imaging as a method of investigating thermal thresholds in finishing pigs. *Biosystems Engineering*. 114:327–333. doi:10.1016/j.biosystemseng.2012.11.015.
- Collier, R. J., and K. G. Gebremedhin. 2015. Thermal Biology of Domestic Animals. *Annu. Rev. Anim. Biosci.* 3:513–545. doi:10.1146/annurev-animal-022114-110659. Available from: [www.annualreviews.org](http://www.annualreviews.org)
- Elenkov, I. J., and G. P. Chrousos. 2002. Stress Hormones, Proinflammatory and Antiinflammatory Cytokines, and Autoimmunity.
- Elenkov, I. J., E. L. Webster, D. J. Topry, and G. P. Chrousos. 1999. Stress, Corticotropin-Releasing Hormone, Glucocorticoids, and the Immune/Inflammatory Response: Acute and Chronic Effects. *Annals of the New York Academy of Sciences*. 876:1–13. doi:10.1111/j.1749-6632.1999.tb07618.x. Available from: <http://doi.wiley.com/10.1111/j.1749-6632.1999.tb07618.x>
- Feddes, J. J. R., B. A. Young, and J. A. DeShazer. 1989. Influence of temperature and light on feeding behaviour of pigs. *Applied Animal Behaviour Science*. 23:215–222. doi:10.1016/0168-1591(89)90112-3.
- Geers, R., R. Puers, and V. Goedseels. 1997. Electronic identification and monitoring of pigs during housing and transport. *Computers and Electronics in Agriculture*. 17:205–215. doi:10.1016/S0168-1699(96)01305-1.
- Hicks, T. A., J. J. McGlone, C. S. Whisnant, H. G. Kattesh, and R. L. Norman. 1998. Behavioral, Endocrine, Immune, and Performance Measures for Pigs Exposed to Acute Stress. *Journal of Animal Science*. 76:474–483. doi:10.2527/1998.762474x.
- Hopkins, B., Y. Pan, M. Tucker, and Z. (Jacky) Huang. 2018. A Model-Based Investigation of Cytokine Dynamics in Immunotherapies. *Processes*. 7:12. doi:10.3390/pr7010012. Available from: <http://www.mdpi.com/2227-9717/7/1/12>
- Horowitz, M. 2002. From molecular and cellular to integrative heat defense during exposure to chronic heat. *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology*. 131:475–83. doi:10.1016/s1095-6433(01)00500-1. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11867273>
- 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. doi:10.1016/j.applanim.2004.10.020.
- Jin, X., T. A. Zimmers, Z. Zhang, R. H. Pierce, and L. G. Koniaris. 2010. Interleukin-6 is an important in vivo inhibitor of intestinal epithelial cell death in mice. doi:10.1136/gut.2008.151175. Available from: <http://gut.bmj.com/>

- Kovács, K. J., I. H. Miklós, and B. Bali. 2005. Chapter 6.1 Psychological and physiological stressors. In: *Techniques in the Behavioral and Neural Sciences*. Vol. 15. Academic Press. p. 775–792.
- Marple, D. N., D. J. Jones, C. W. Alliston, and J. C. Forrest. 1974. Physiological and endocrinological changes in response to terminal heat stress in swine. *Journal of animal science*. 39:79–82. doi:10.2527/jas1974.39179x.
- Morrison, S. R., T. E. Bond, and H. Jr. H. Hubert. 1967. Skin and Lung Moisture Loss from Swine. *Transactions of the ASAE*. 10:0691–0692. doi:10.13031/2013.39762. Available from: <http://elibrary.asabe.org/abstract.asp??JID=3&AID=39762&CID=t1967&v=10&i=5&T=1>
- Nakamura, K., and S. F. Morrison. 2008. A thermosensory pathway that controls body temperature. *Nature Neuroscience*. 11:62–71. doi:10.1038/nn2027.
- Nienaber, J. A., and G. L. Hahn. 2004. *Engineering and Management Practices to Ameliorate Livestock Heat Stress*.
- Pearce, S. C., M. v. Sanz-Fernandez, J. H. Hollis, L. H. Baumgard, and N. K. Gabler. 2014. Short-term exposure to heat stress attenuates appetite and intestinal integrity in growing pigs1. *Journal of Animal Science*. 92:5444–5454. doi:10.2527/jas.2014-8407.
- Rhoads, R. P., L. H. Baumgard, J. K. Suagee, and S. R. Sanders. 2013. Nutritional Interventions to Alleviate the Negative Consequences of Heat Stress. *Advances in Nutrition*. 4:267–276. doi:10.3945/an.112.003376.
- Robertshaw, D. 2006. Mechanisms for the control of respiratory evaporative heat loss in panting animals. *Journal of Applied Physiology*. 101:664–668. doi:10.1152/jappphysiol.01380.2005. Available from: <https://www.physiology.org/doi/10.1152/jappphysiol.01380.2005>
- Sapolsky, R. M., L. M. Romero, and A. U. Munck. 2000. How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions\*. Available from: <https://academic.oup.com/edrv/article-abstract/21/1/55/2423840>
- Soerensen, D. D., and L. J. Pedersen. 2015. Infrared skin temperature measurements for monitoring health in pigs: A review. *Acta Veterinaria Scandinavica*. 57. doi:10.1186/s13028-015-0094-2.
- Sutherland, M. A., S. R. Niekamp, S. L. Rodriguez-Zas, and J. L. Salak-Johnson. 2006. Impacts of chronic stress and social status on various physiological and performance measures in pigs of different breeds 1. Available from: <https://academic.oup.com/jas/article-abstract/84/3/588/4778470>

Wang, Xinglong, J. Li, P. Jiang, Y. Li, B. Zeshan, J. Cao, and Xianwei Wang. 2009. GM-CSF fused with GP3 and GP5 of porcine reproductive and respiratory syndrome virus increased the immune responses and protective efficacy against virulent PRRSV challenge. *Virus Research*. 143:24–32. doi:10.1016/j.virusres.2009.02.017.

Wiegers, G. J., and J. M. H. M. Reul. 1998. Induction of cytokine receptors by glucocorticoids: Functional and pathological significance. *Trends in Pharmacological Sciences*. 19:317–321. doi:10.1016/S0165-6147(98)01229-2.

**Table 3.1:** The least squared mean and SEM of villi height and crypt depth of the ileum and jejunum for pigs subjected (HS) or not subjected to heat stress (CON). No significant differences.

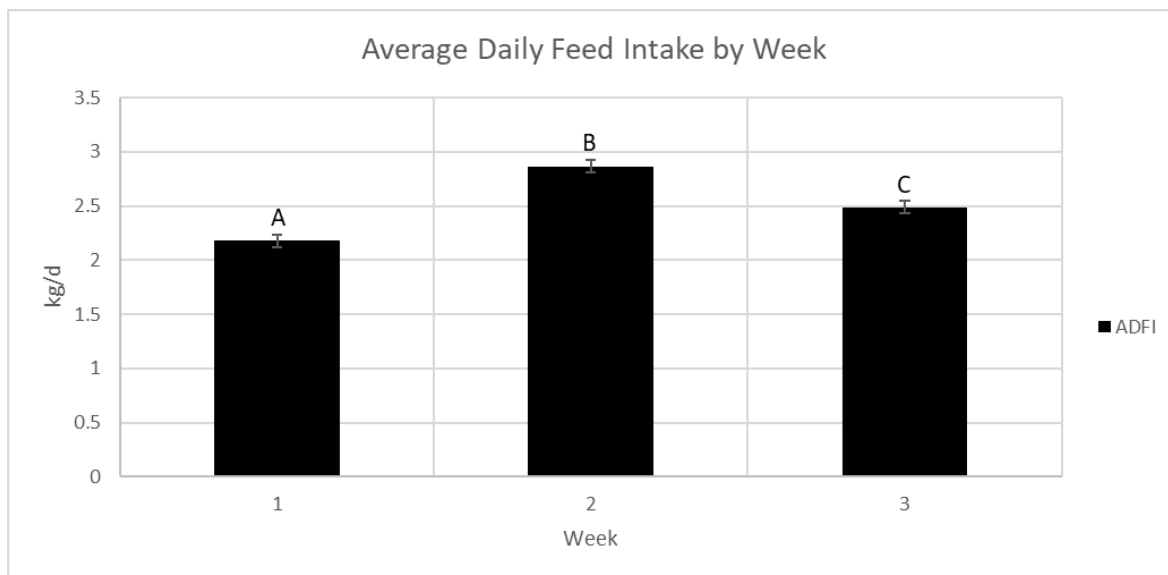
Intestine Histology - Treatment					
		Villi Height	SEM	Crypt Depth	SEM
Ileum	CON	410.63	22.72	246.05	15.79
	HS	382.62	23.77	272.22	16.52
Jejunum	CON	535.00	38.86	284.76	15.43
	HS	529.22	40.66	272.91	16.15

**Table 3.2:** The least squared mean and SEM of feeder duration by treatment (heat stress - HS and control - CON), day, and time (morning, afternoon, and evening) in minutes. P = 0.0032.

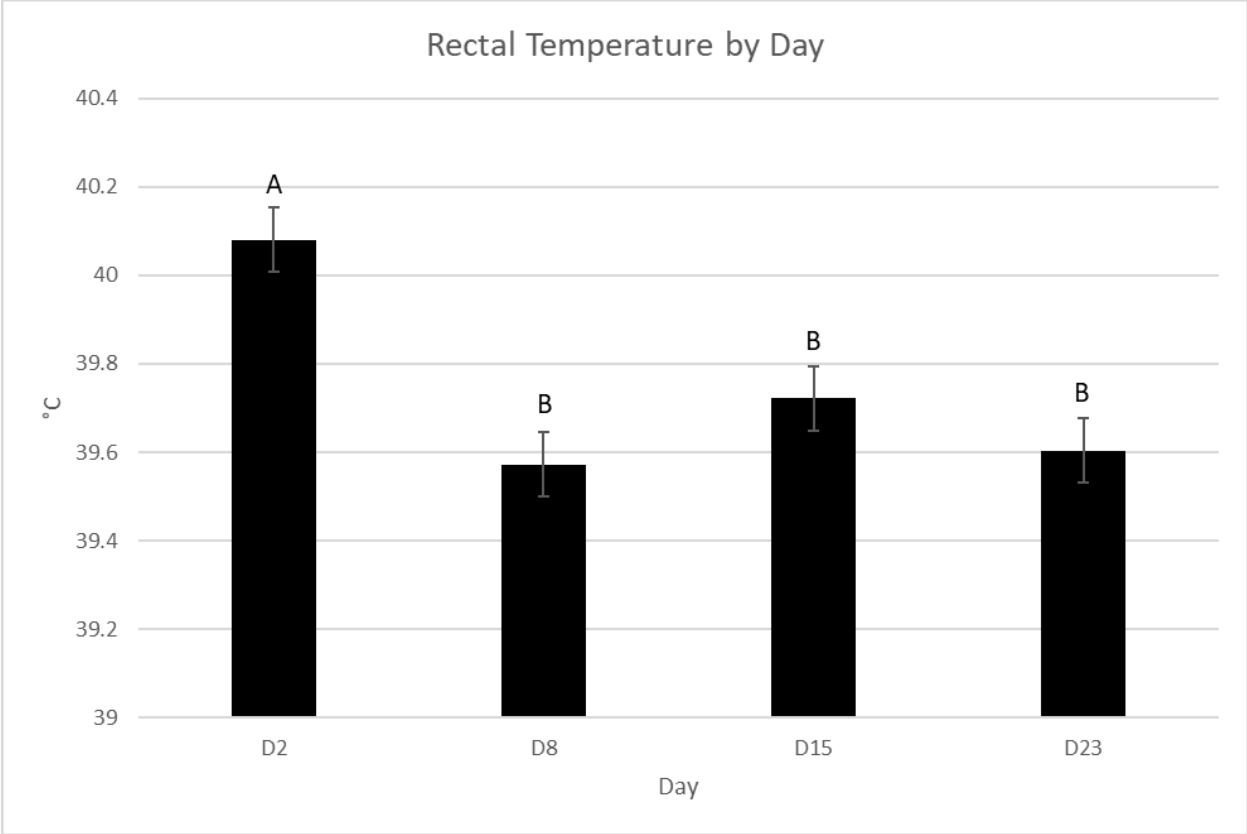
<b>Duration at the Feeder in Minutes</b>							
	<b>CON</b>			<b>HS</b>			
	<b>Morning</b>	<b>Afternoon</b>	<b>Evening</b>	<b>Morning</b>	<b>Afternoon</b>	<b>Evening</b>	<b>SEM</b>
<b>D3</b>	<b>14.58</b>	<b>10.44</b>	<b>1.19</b>	<b>8.45</b>	<b>16.39</b>	<b>9.43</b>	2.90
<b>D6</b>	<b>22.70</b>	<b>9.72</b>	<b>8.78</b>	<b>8.97</b>	<b>13.62</b>	<b>19.57</b>	2.90
<b>D10</b>	<b>20.53</b>	<b>24.30</b>	<b>13.90</b>	<b>13.63</b>	<b>18.23</b>	<b>18.31</b>	2.90
<b>D13</b>	<b>20.62</b>	<b>25.32</b>	<b>6.10</b>	<b>3.50</b>	<b>14.61</b>	<b>7.39</b>	2.90
<b>D20</b>	<b>10.52</b>	<b>5.17</b>	<b>15.67</b>	<b>13.50</b>	<b>13.63</b>	<b>10.53</b>	2.90
<b>D29</b>	<b>15.04</b>	<b>12.40</b>	<b>10.37</b>	<b>9.12</b>	<b>3.83</b>	<b>15.94</b>	4.74

**Table 3.3:** The least squared mean and SEM of lying duration by treatment (heat stress - HS and control - CON), day, and time (morning, afternoon, and evening) in minutes. Lying Ventrally P = 0.0251; Lying Laterally P = 0.0001.

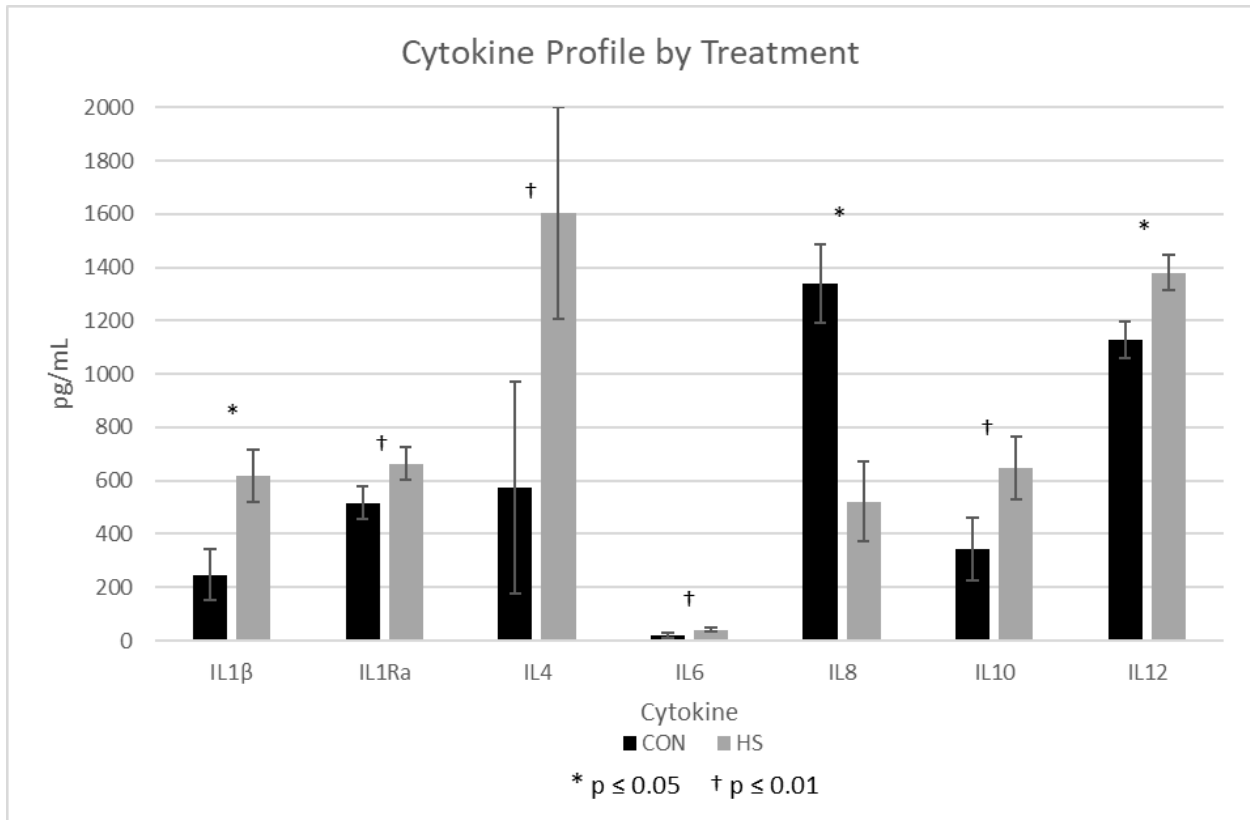
Duration Lying Down by Posture in Minutes								
		CON			HS			SEM
		Morning	Afternoon	Evening	Morning	Afternoon	Evening	
Ventral	D3	36.77	29.27	5.80	39.49	33.78	22.50	5.51
	D6	44.88	29.54	22.90	32.33	33.81	31.04	5.51
	D10	46.73	34.98	38.38	36.95	47.20	45.67	5.51
	D13	38.90	30.89	34.26	28.06	34.29	35.06	5.51
	D20	30.63	28.78	36.50	57.33	27.87	29.88	5.51
	D29	47.96	43.69	39.97	41.74	45.55	45.60	5.20
Lateral	D3	42.03	67.76	111.83	62.11	50.17	80.83	8.09
	D6	32.45	67.41	80.89	70.83	58.22	53.36	8.09
	D10	29.53	36.62	62.32	57.68	33.42	39.61	8.09
	D13	28.94	44.63	75.49	66.55	58.03	69.39	8.09
	D20	69.05	83.66	57.25	29.39	77.56	69.58	8.09
	D29	42.40	53.68	62.01	46.93	49.85	40.96	13.21



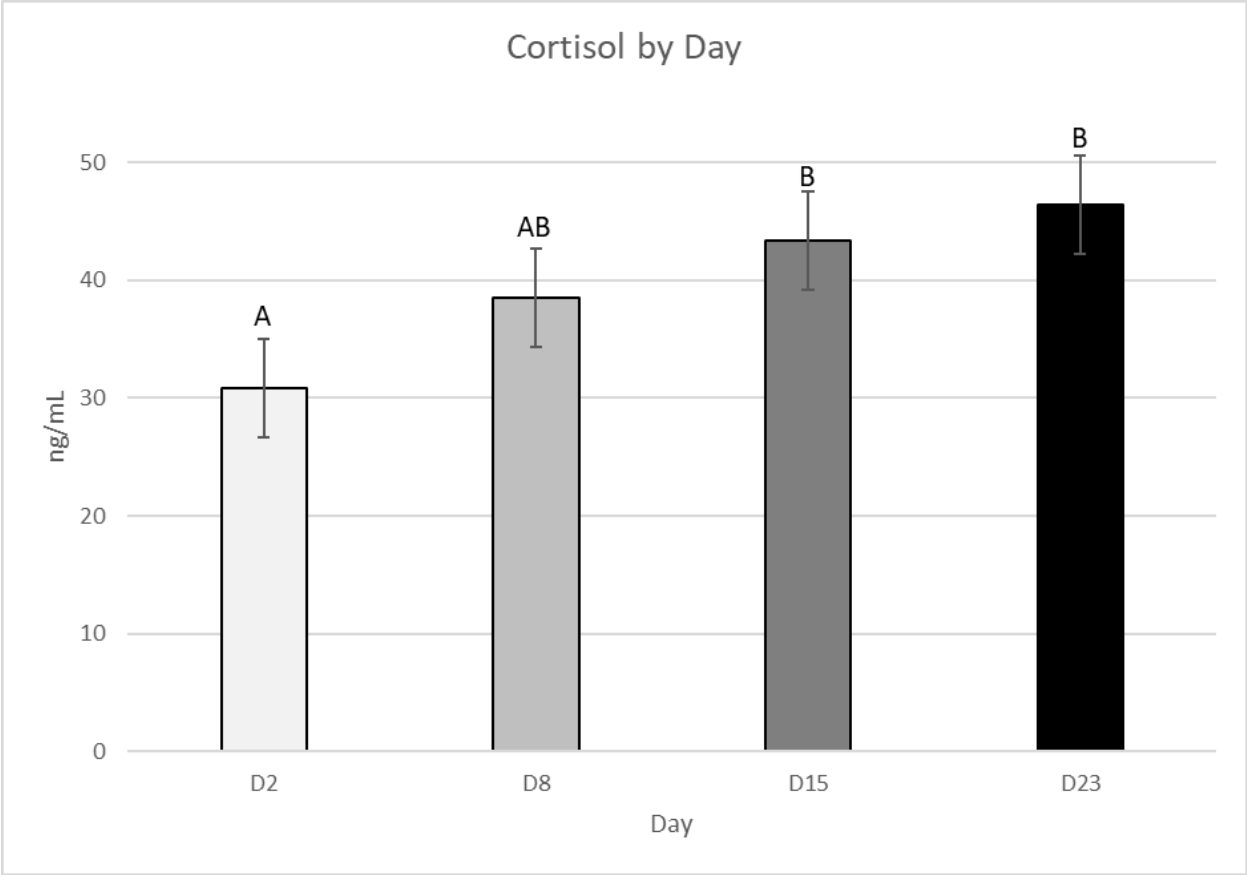
**Figure 2.5:** The least squared mean ( $\pm$ SEM) Average Daily Feed Intake (ADFI) in kg/day calculated from weekly feed weights, regardless of treatment. Effect of week -  $P < 0.0001$ . Differences of  $P \leq 0.05$  in the least square mean between weeks are marked with different superscripts.



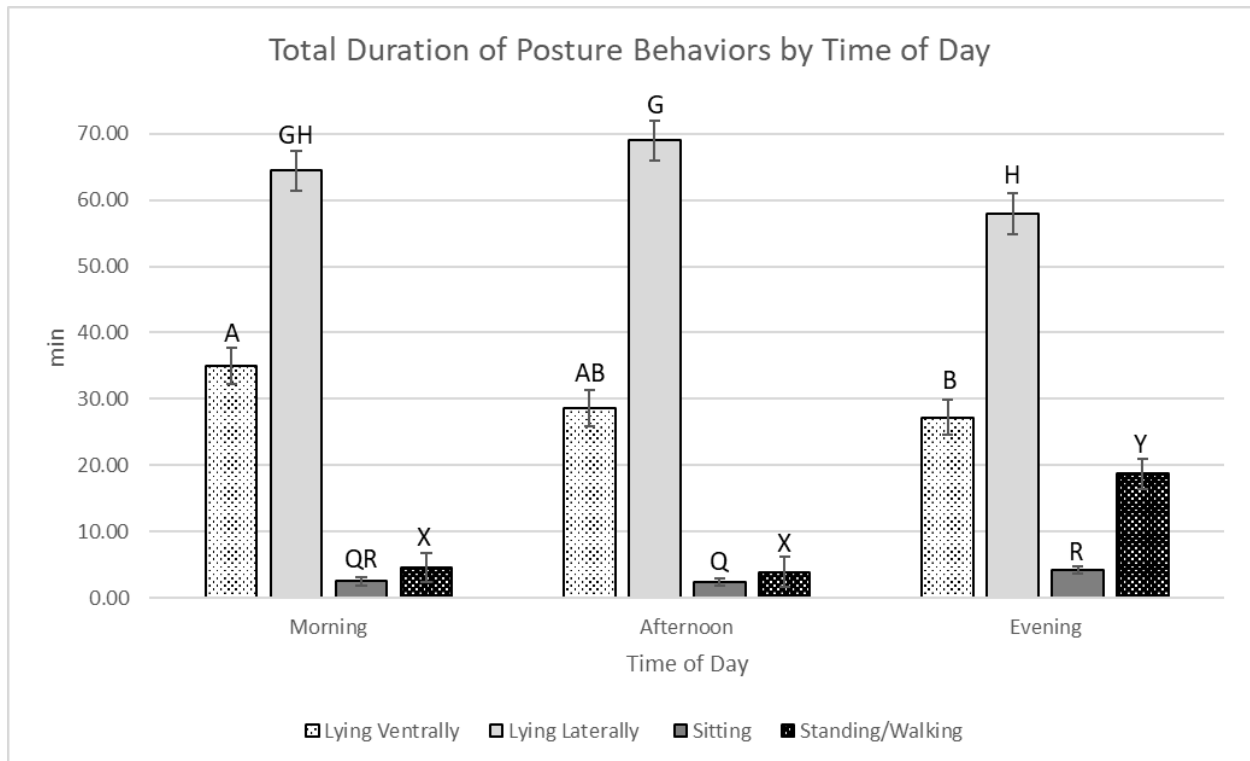
**Figure 2.6:** The least squared mean ( $\pm$ SEM) of rectal temperature by day, regardless of treatment. Effect of day -  $P < 0.0001$ . Differences of  $P \leq 0.05$  in the least square mean between weeks are marked with different superscripts.



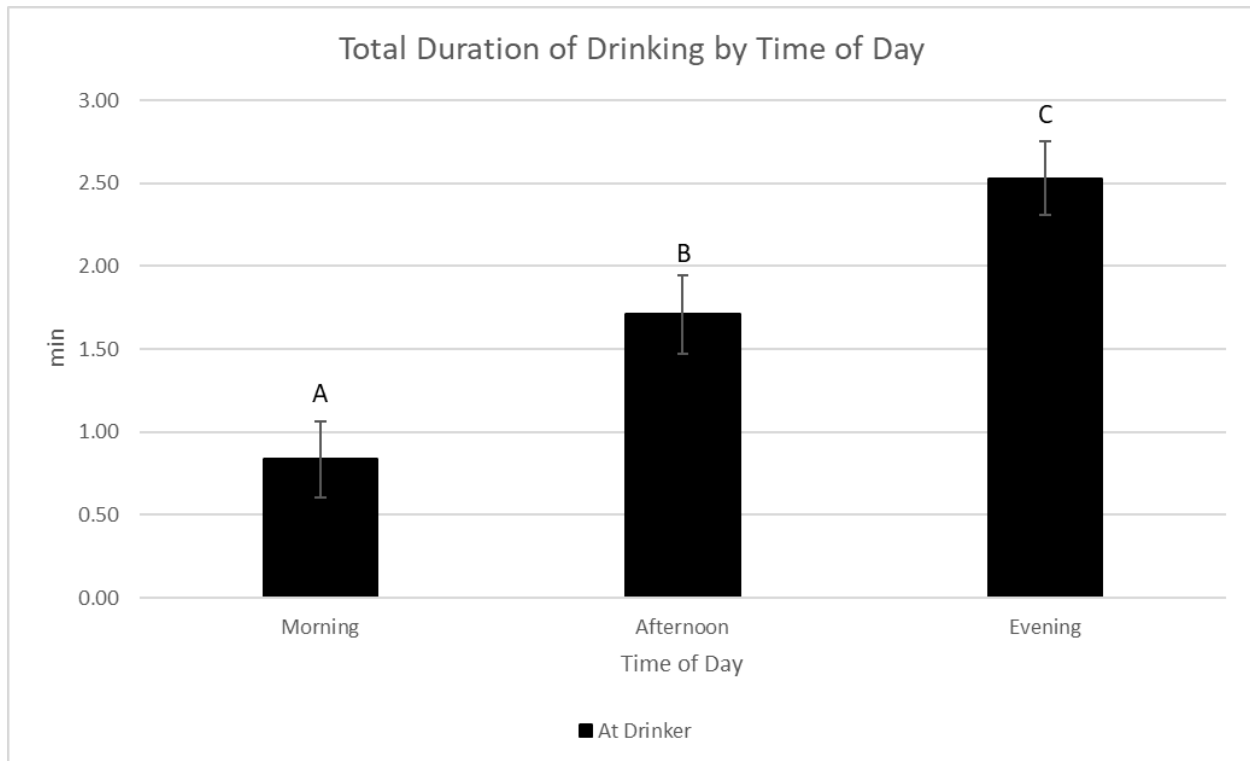
**Figure 2.7:** The least squared mean ( $\pm$ SEM) of cytokine concentration in pg/mL by treatment (control – CON and heat stress – HS). Effect of treatment - IL1 $\beta$  P = 0.009; IL1Ra P = 0.0920; IL4 P = 0.0719; IL6 P = 0.0914; IL8 P = 0.0003; IL10 P = 0.0714; IL12 P = 0.0107. Differences of  $P \leq 0.05$  in the least square mean between treatments are indicated by “\*” and by “†” for  $P \leq 0.01$ .



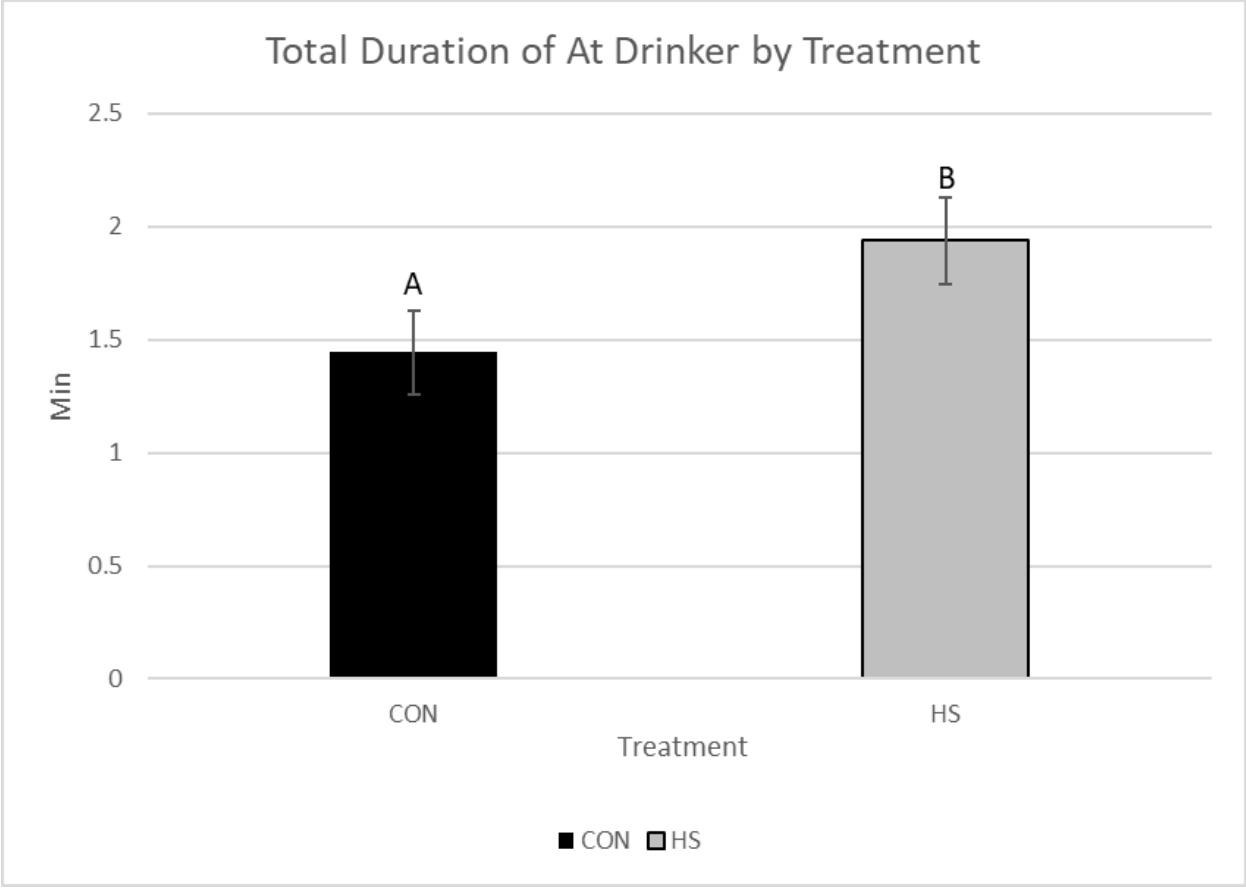
**Figure 2.8:** The least squared mean ( $\pm$ SEM) of serum cortisol concentration in ng/mL by day, regardless of treatment. Effect of day -  $P = 0.0564$ . Differences of  $P \leq 0.05$  in the least square mean between days are marked with different superscripts.



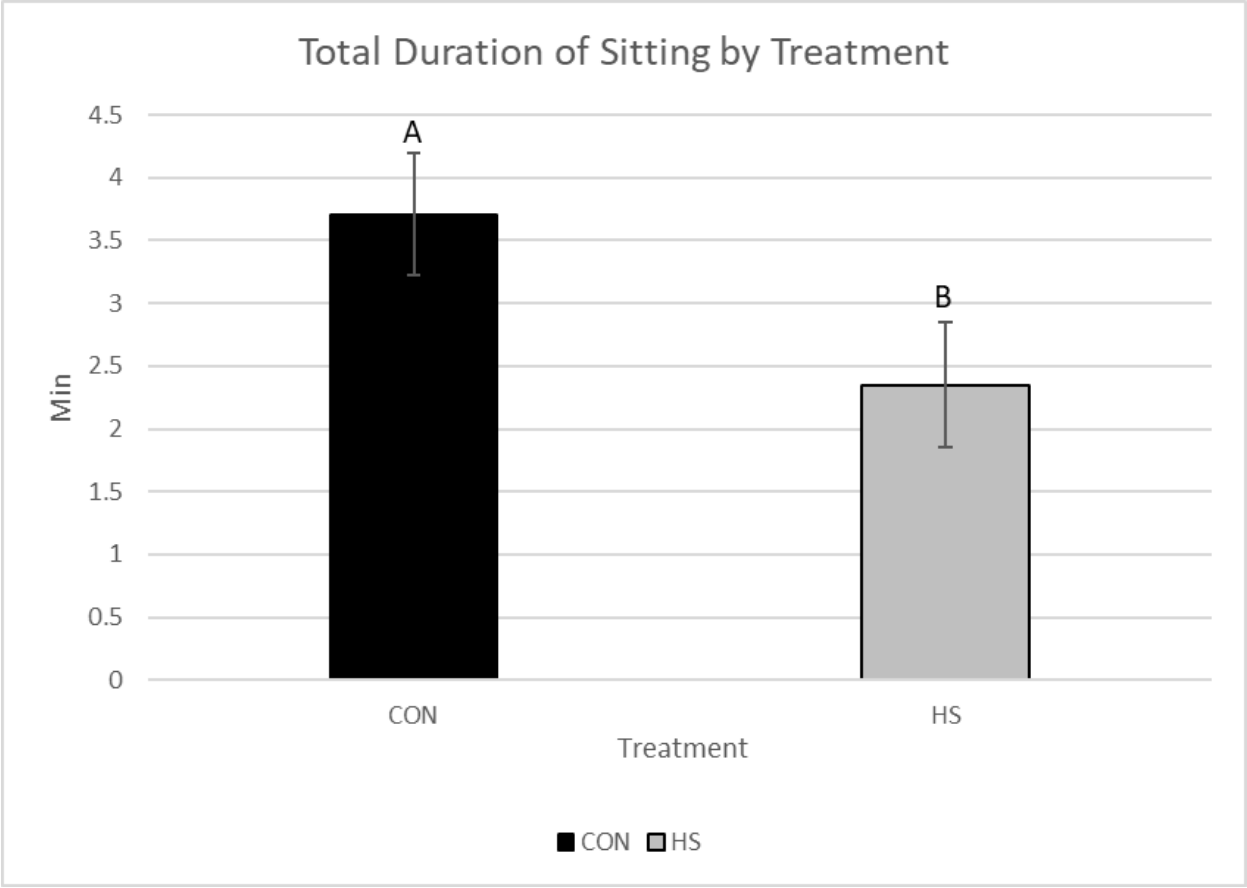
**Figure 2.9:** The least squared mean ( $\pm$ SEM) of behavior duration by time of day (morning, afternoon, and evening) in minutes, regardless of treatment. Effect of time – Lying ventrally  $P = 0.1002$ ; Lying Laterally  $P = 0.0398$ ; Sitting  $P = 0.0636$ ; Standing/Walking  $P < 0.0001$ . Differences of  $P \leq 0.05$  in the least square mean between times are marked with different superscripts. Lying Ventrally – ABC; Lying Laterally GH; Sitting – QR; Standing/Walking – XY.



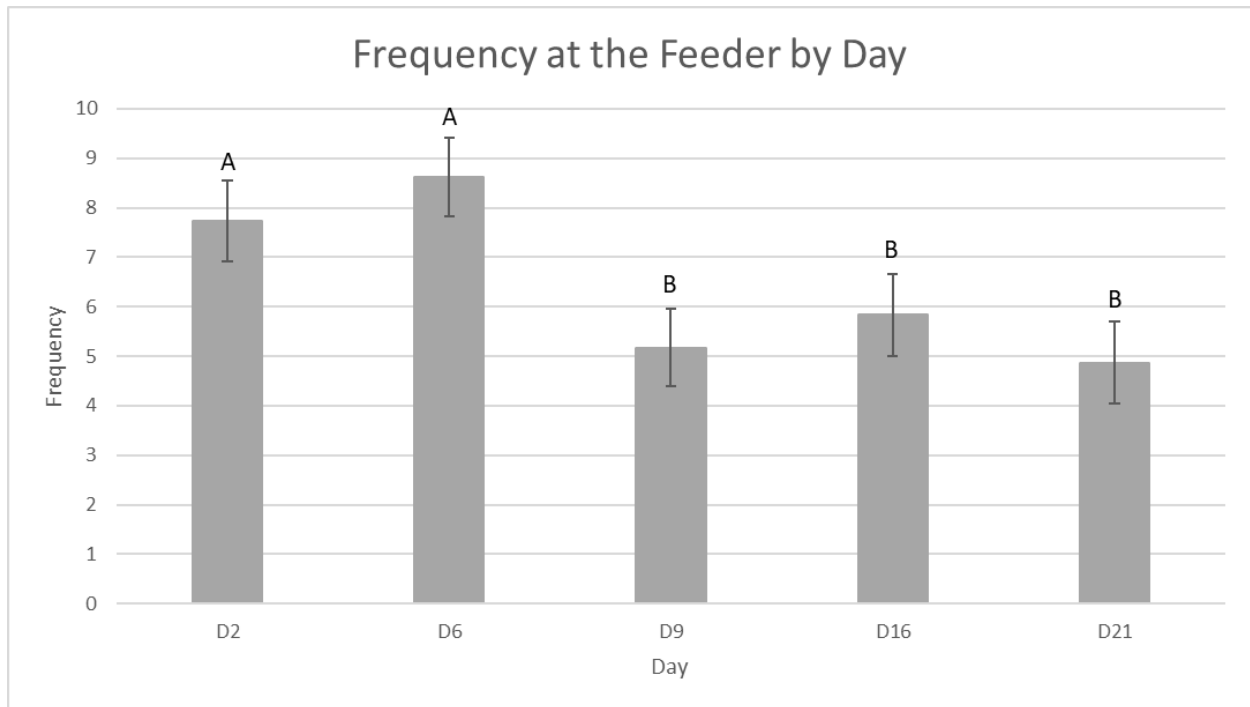
**Figure 2.10:** The least squared mean ( $\pm$ SEM) of drinking duration by time of day (morning, afternoon, and evening) in minutes, regardless of treatment. Effect of time –  $P < 0.0001$ . Differences of  $P \leq 0.05$  in the least square mean between times are marked with different superscripts.



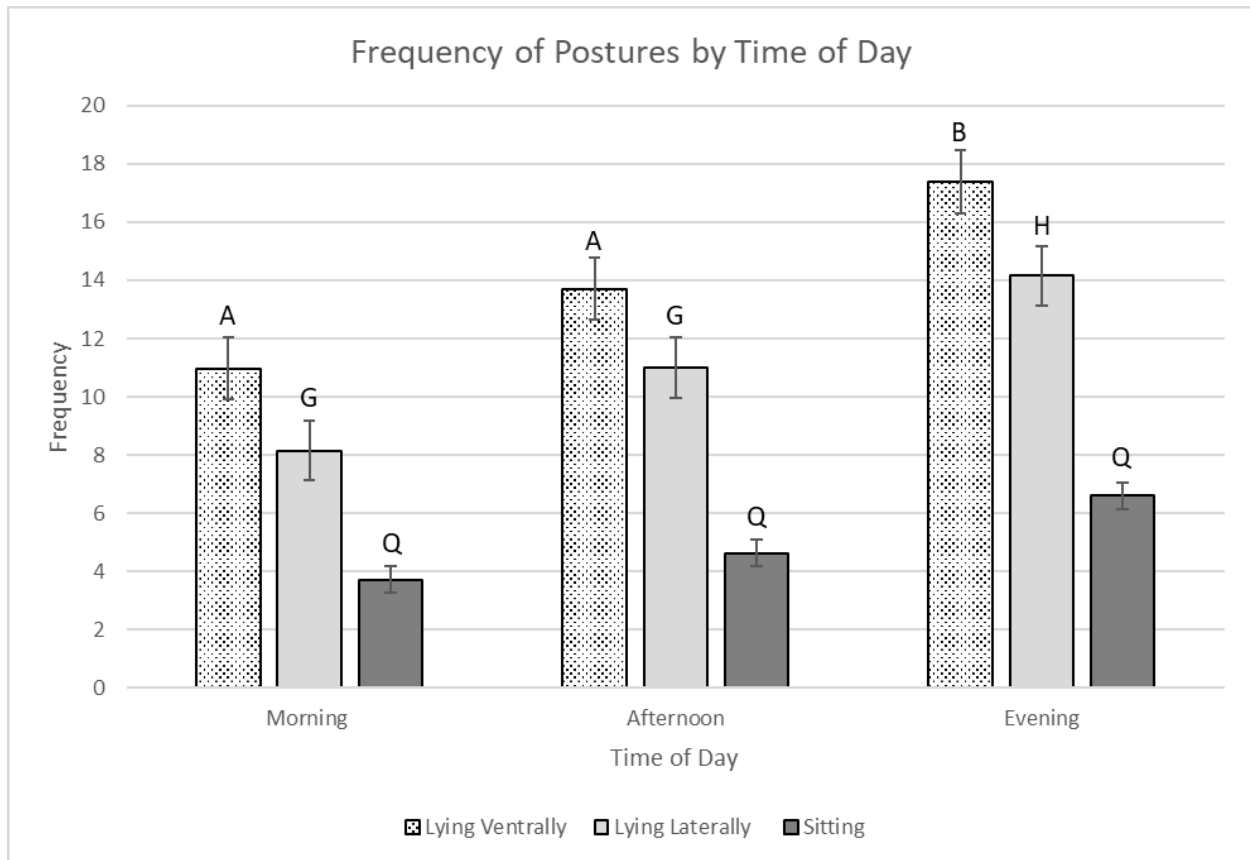
**Figure 2.11:** The least squared mean ( $\pm$ SEM) of duration at the drinker by treatment (control – CON and heat stress - HS) in minutes. Effect of treatment –  $P = 0.0404$ . Differences of  $P \leq 0.05$  in the least square mean between treatments are marked with different superscripts.



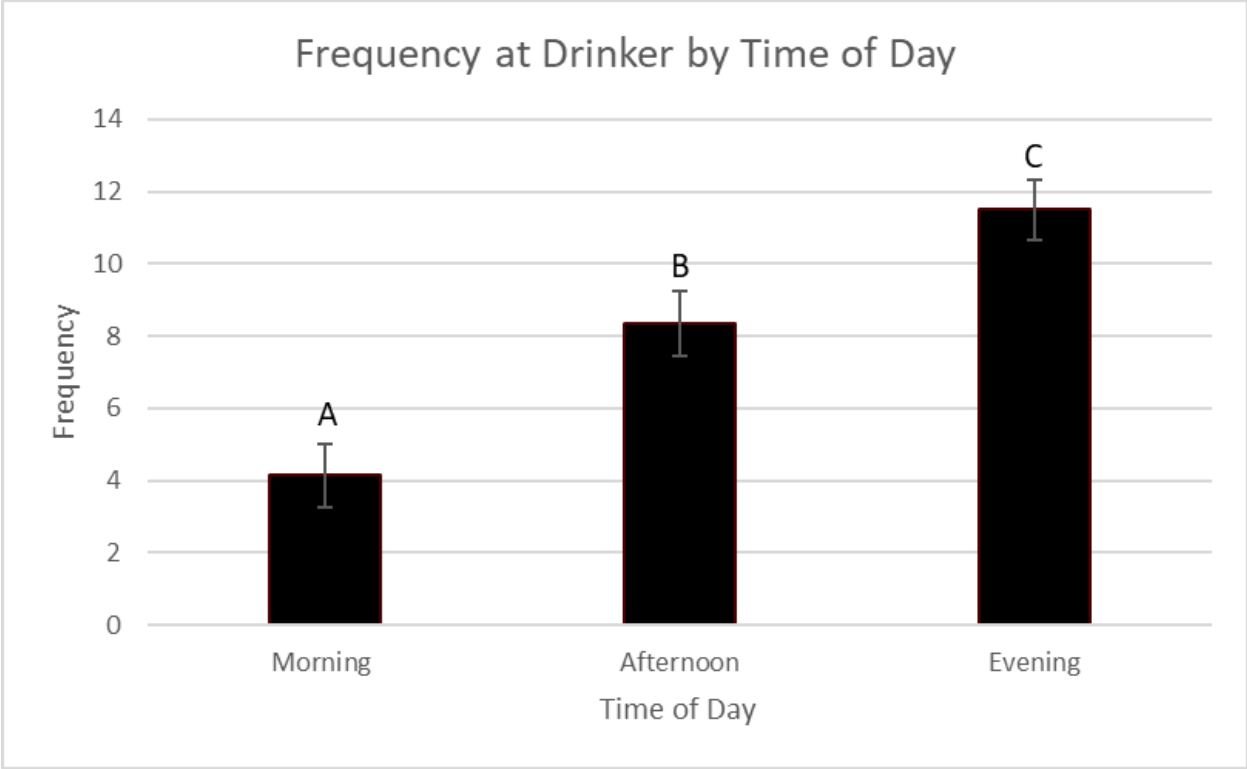
**Figure 2.12:** The least squared mean ( $\pm$ SEM) of sitting duration by treatment (control – CON and heat stress – HS) in minutes. Effect of treatment –  $P = 0.0547$ . Differences of  $P \leq 0.05$  in the least square mean between treatments are marked with different superscripts.



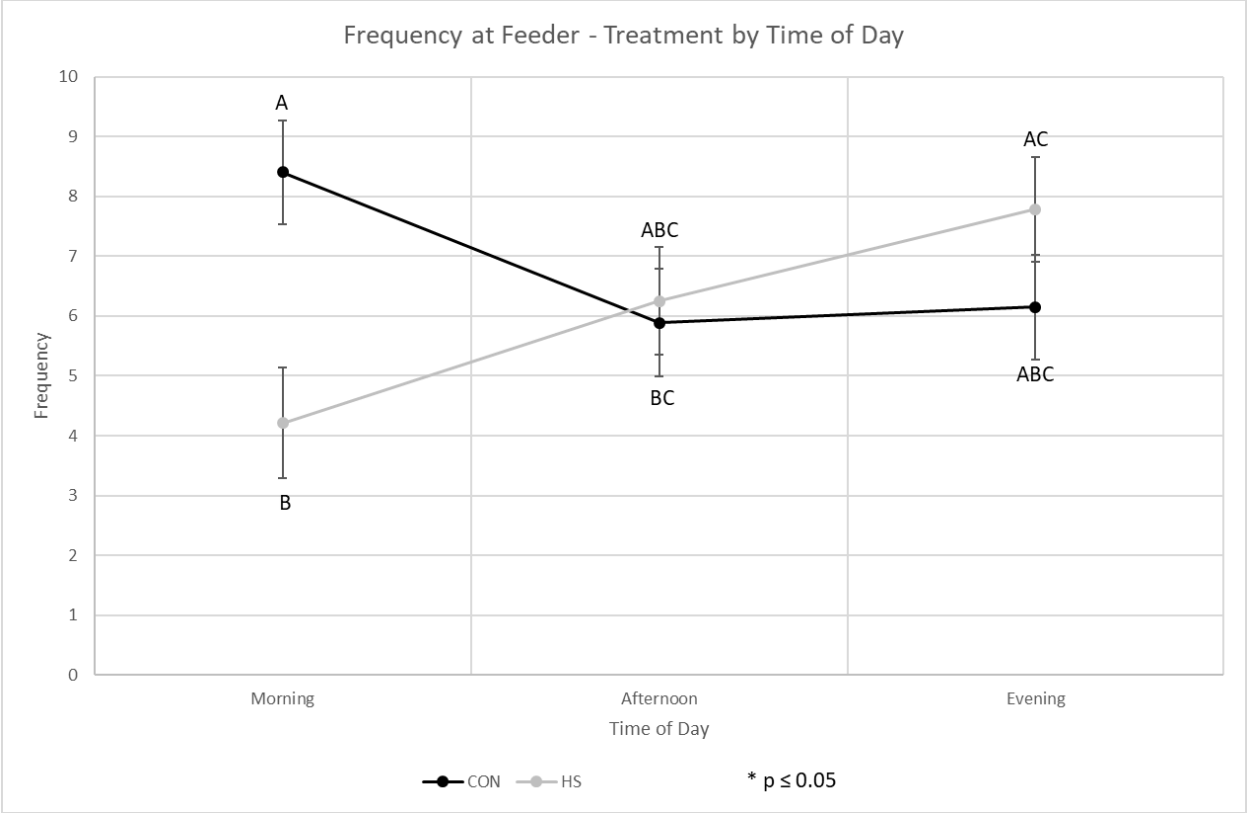
**Figure 2.13:** The least squared mean ( $\pm$ SEM) of frequency at the feeder by day, regardless of treatment. Effect of day –  $P = 0.0030$ . Differences of  $P \leq 0.05$  in the least square mean between days are marked with different superscripts.



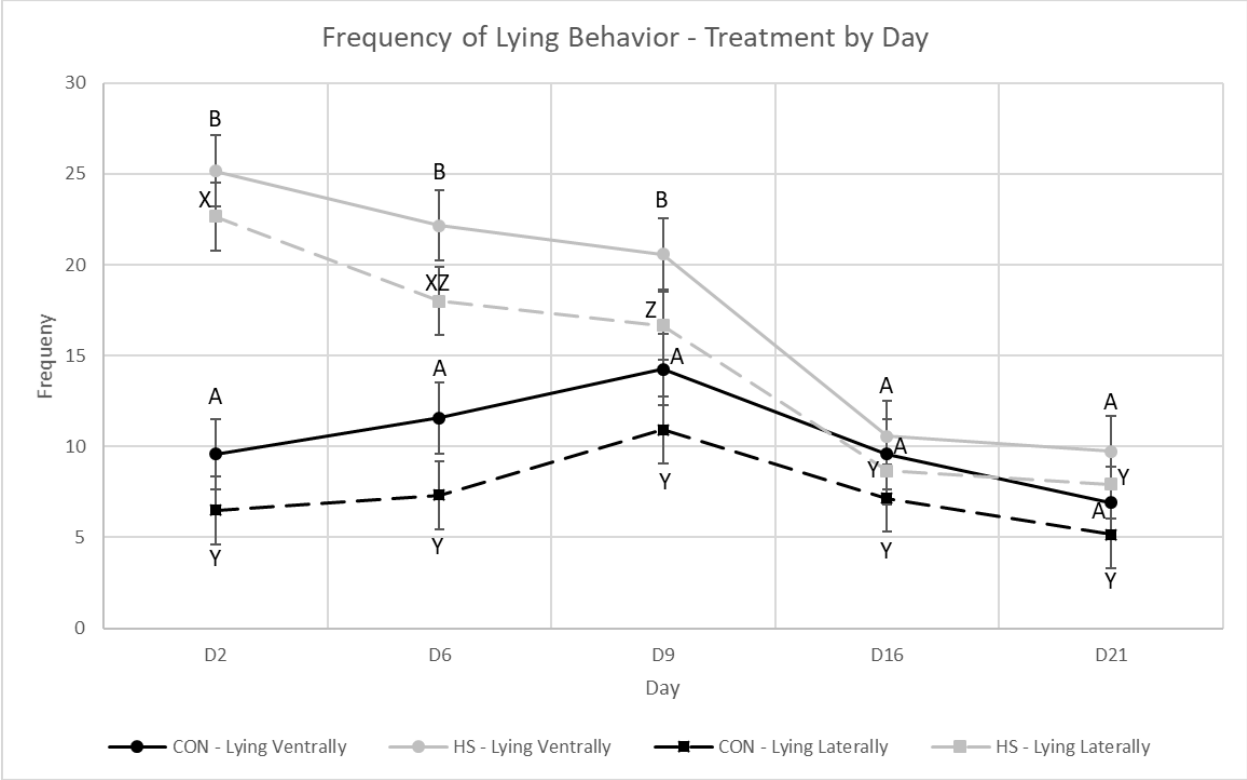
**Figure 2.14:** The least squared mean ( $\pm$ SEM) of behavior frequency by time of day (morning, afternoon, and evening), regardless of treatment. Effect of time – Lying ventrally  $P = 0.0002$ ; Lying Laterally  $P = 0.0003$  Sitting  $P < 0.0001$ . Differences of  $P \leq 0.05$  in the least square mean between times are marked with different superscripts. Lying Ventrally – AB; Lying Laterally GH; Sitting – QR.



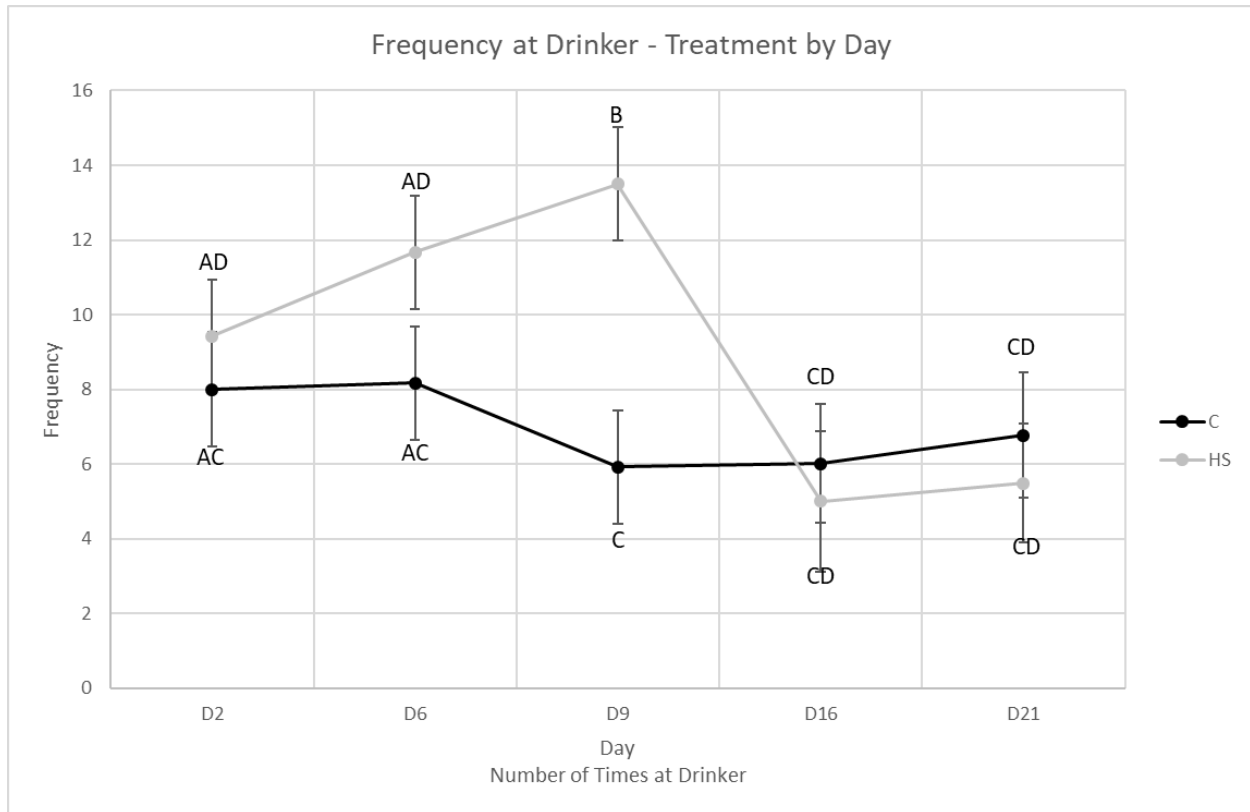
**Figure 2.15:** The least squared mean ( $\pm$ SEM) of frequency at the drinker by time of day (morning, afternoon, and evening), regardless of treatment. Effect of time -  $P < 0.0001$ . Differences of  $P \leq 0.05$  in the least square mean between times are marked with different superscripts.



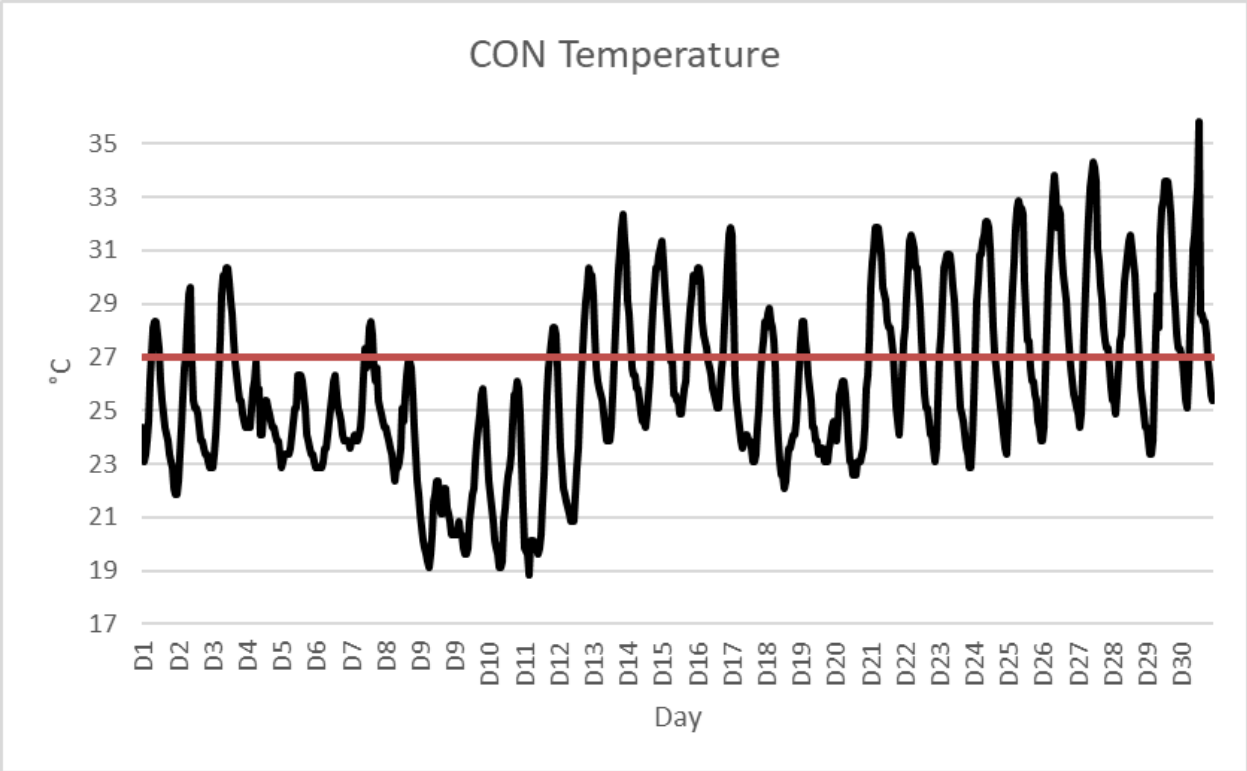
**Figure 2.16:** The least squared mean ( $\pm$ SEM) of frequency at the feeder by treatment (control – CON and heat stress – HS) and time of day (morning, afternoon, and evening). Effect of treatment\*time  $P = 0.0029$ . Differences of  $P \leq 0.05$  in the least square mean between treatment by time are marked with different superscripts.



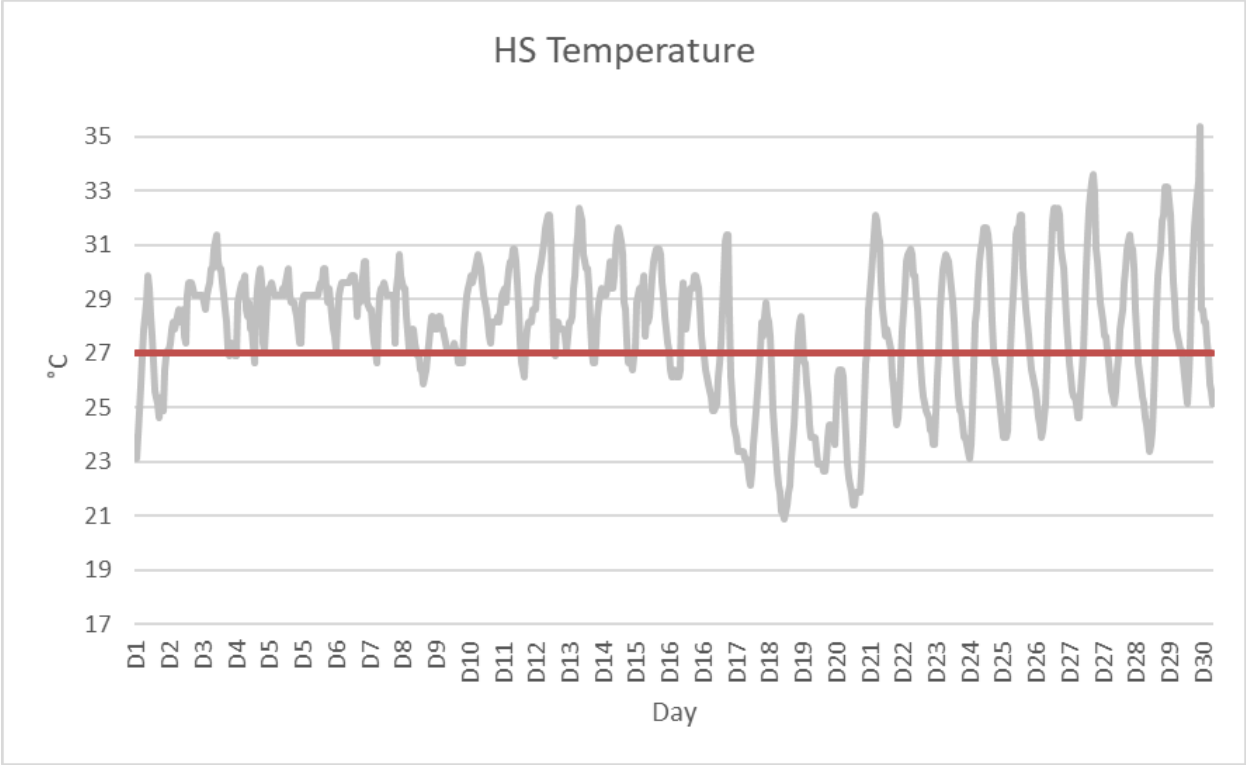
**Figure 2.17:** The least squared mean ( $\pm$ SEM) of ventral and lateral lying frequency by treatment (control – CON and heat stress – HS) and day. Effect of treatment\*day - Lying Ventrally  $P = 0.0006$ ; Lying Laterally  $P = 0.0018$ . Differences of  $P \leq 0.05$  in the least square mean between treatments and days are marked with different superscripts; Lying Laterally – ABC; Lying Ventrally - XYZ.



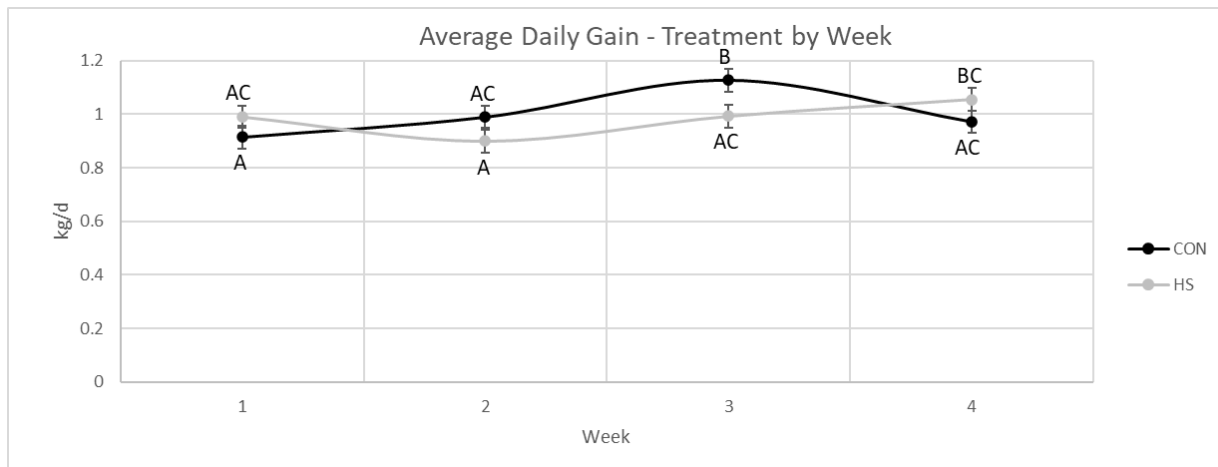
**Figure 2.18:** The least squared mean ( $\pm$ SEM) of frequency at the drinker by treatment (control – CON and heat stress – HS) and day. Effect of treatment\*day -  $P = 0.0361$ . Differences of  $P \leq 0.05$  in the least square mean between treatments and days are marked with different superscripts.



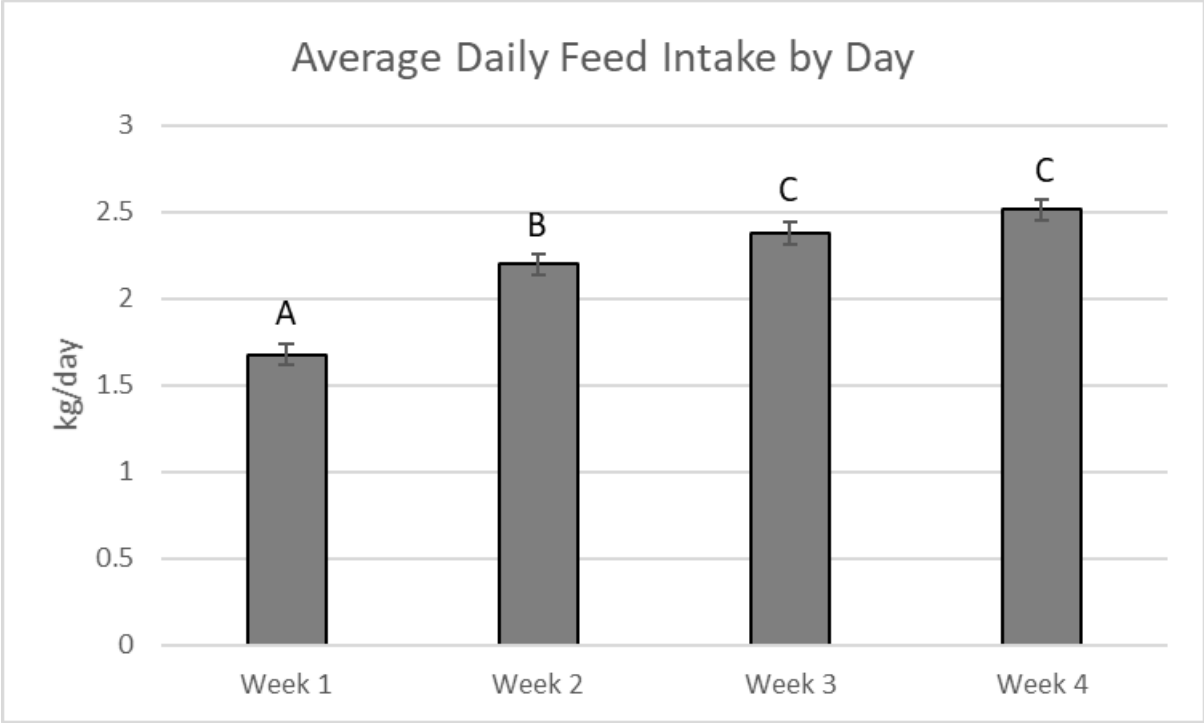
**Figure 3.19:** Hourly temperatures for the control (CON) room for the duration of the trial. Heat stress threshold marked at 27°C.



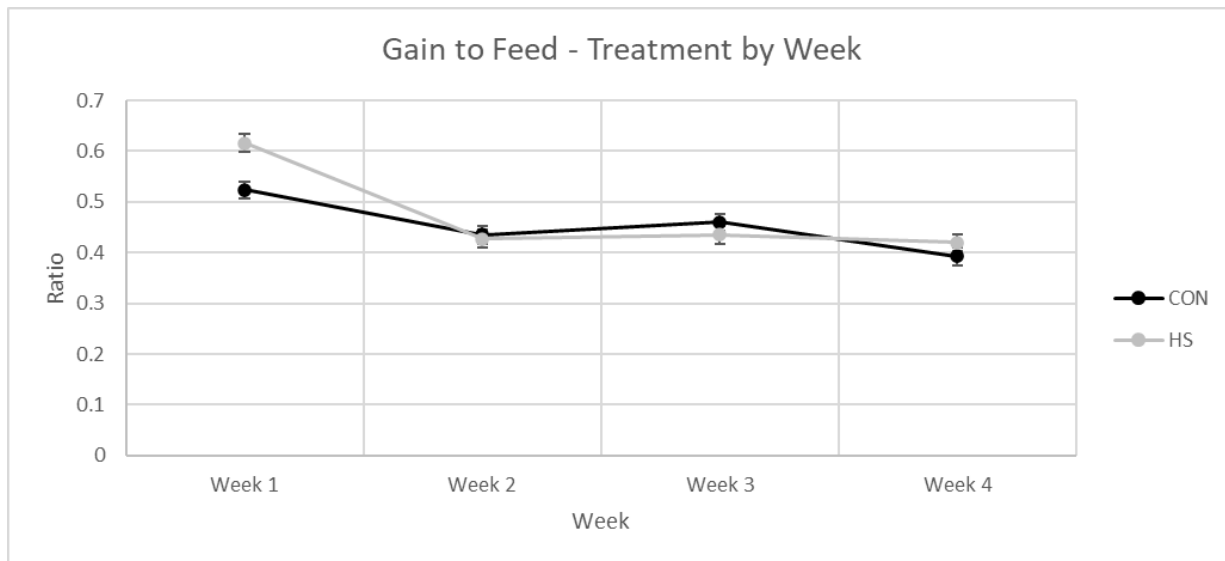
**Figure 3.20:** Hourly temperatures for the heat stress (HS) room for the duration of the trial. Heat stress threshold marked at 27°C.



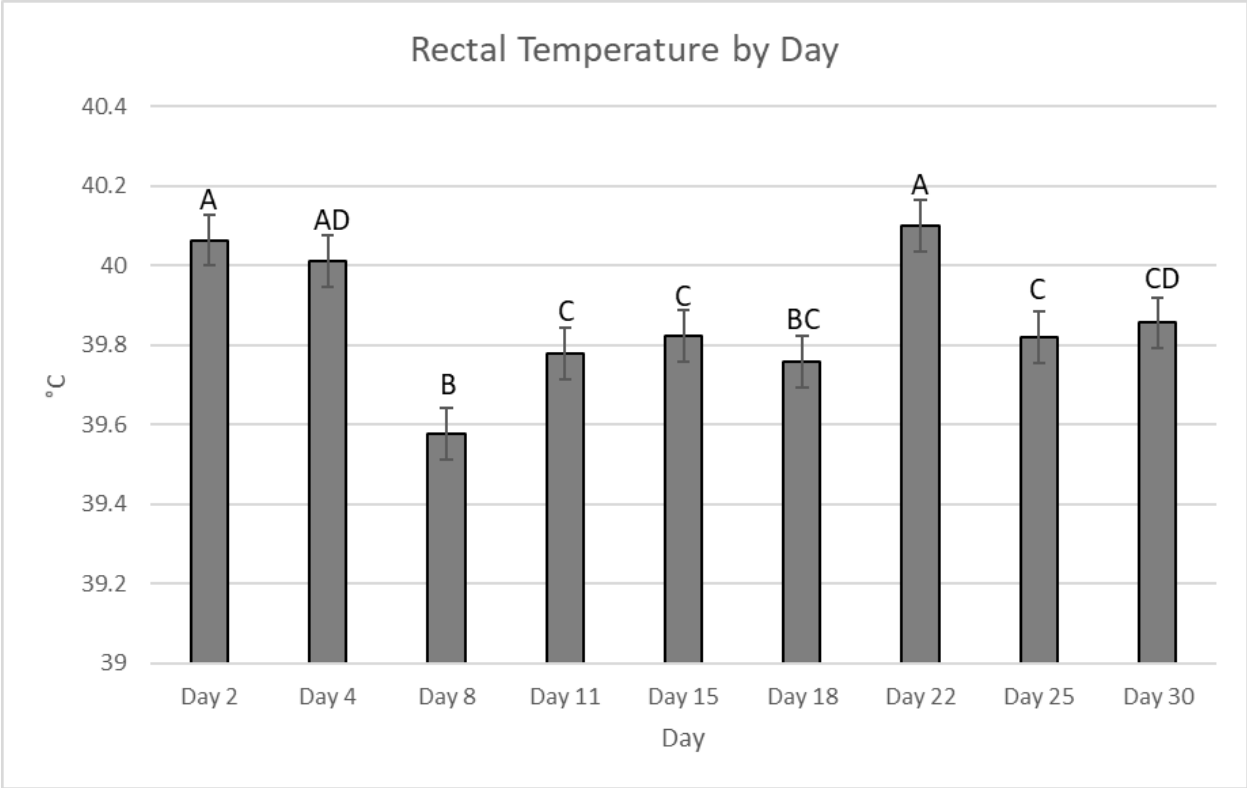
**Figure 3.21:** The least squared mean ( $\pm$ SEM) of average daily gain (ADG) calculated from weekly feed weights for week by treatment (control – CON and heat stress – HS) in kg/day. Effect of week\*treatment  $P = 0.0201$ . Differences of  $P \leq 0.05$  in the least square mean between weeks are marked with different superscripts.



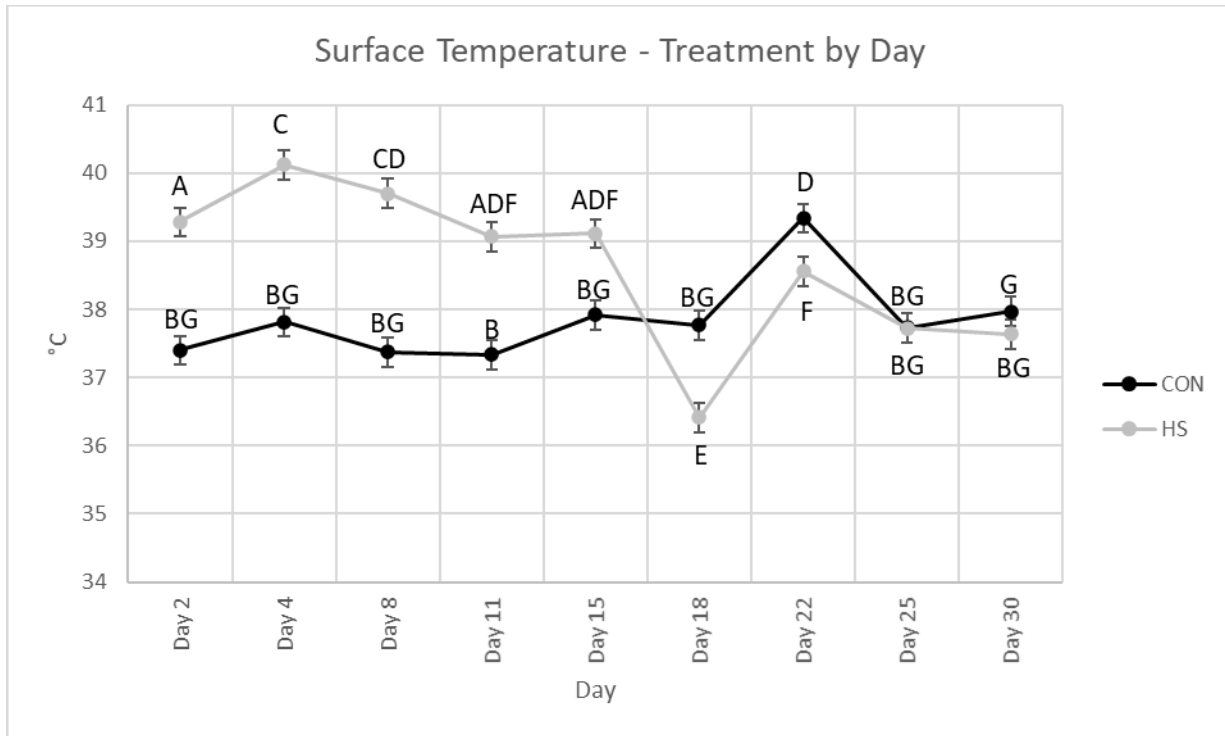
**Figure 3.22:** The least squared mean ( $\pm$ SEM) for average daily feed intake (ADFI) in kg/day calculated from weekly feed weights by week, regardless of treatment. Effect of week - ADFI  $P < 0.0001$ . Differences of  $P \leq 0.05$  in the least square mean between weeks are marked with different superscripts.



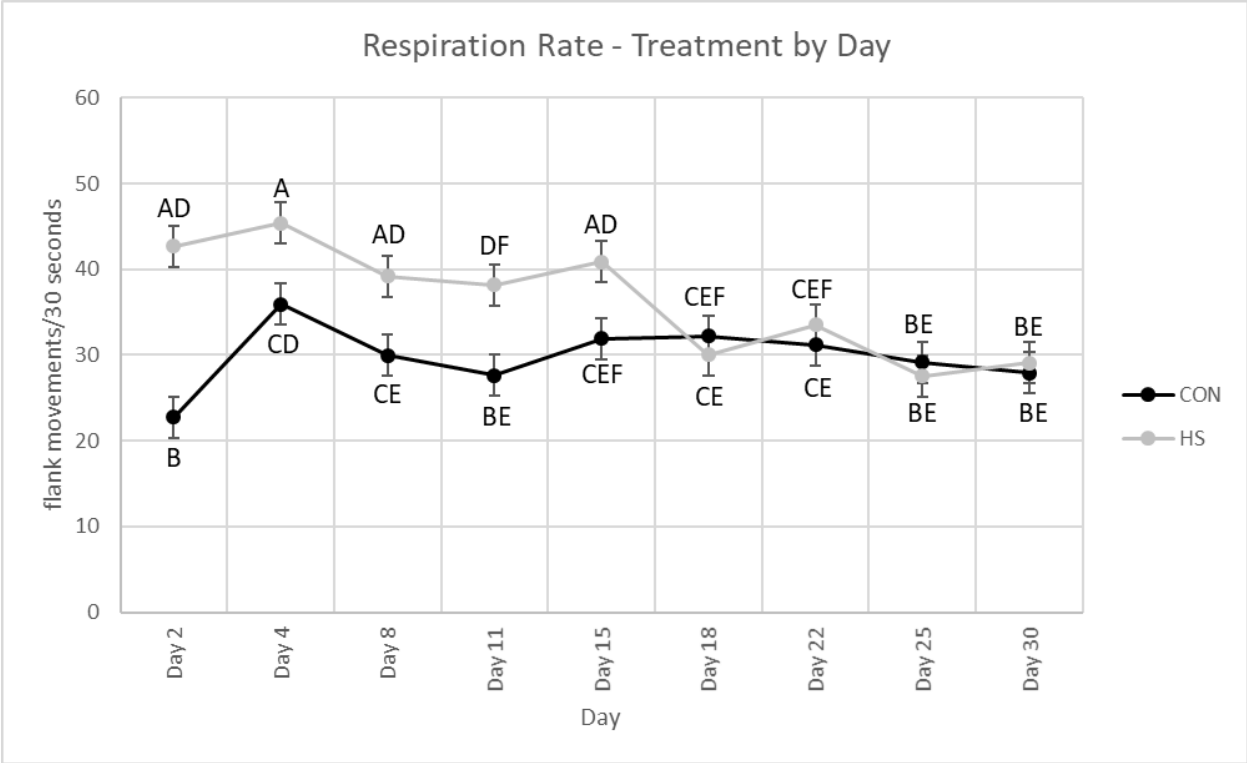
**Figure 3.23:** The least squared mean ( $\pm$ SEM) for the gain to feed ratio (GF) calculated from weekly feed and pig weights for week by treatment (control – CON and heat stress – HS). Effect of week\*treatment –  $P = 0.0059$ . Differences of  $P \leq 0.05$  in the least square mean between weeks are marked with different superscripts.



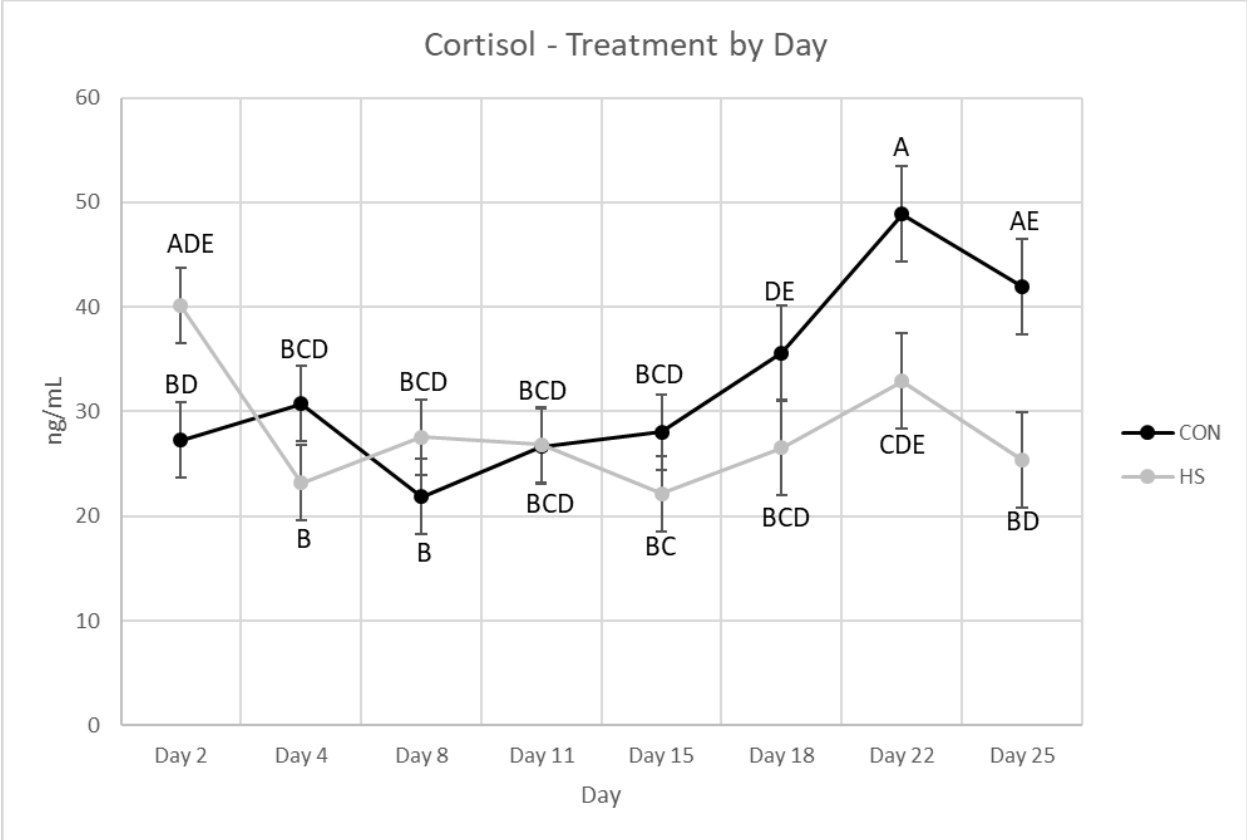
**Figure 3.24:** The least squared mean ( $\pm$ SEM) rectal temperature by day, regardless of treatment. Effect of day -  $P < 0.0001$ . Differences of  $P \leq 0.05$  in the least square mean between days are marked with different superscripts.



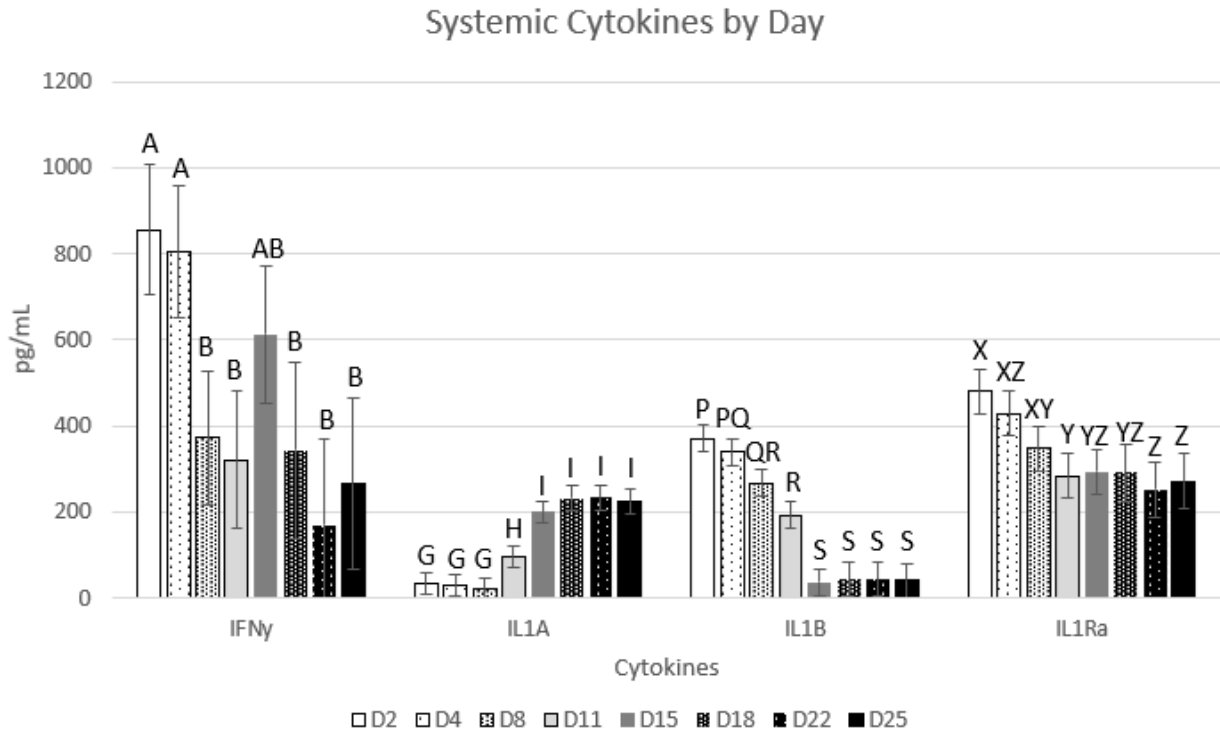
**Figure 3.25:** The least squared mean ( $\pm$ SEM) surface temperature by treatment (control – CON and heat stress – HS) and day. Effect of treatment\*day -  $P < 0.0001$ . Differences of  $P \leq 0.05$  in the least square mean for treatment by day are marked with different superscripts.



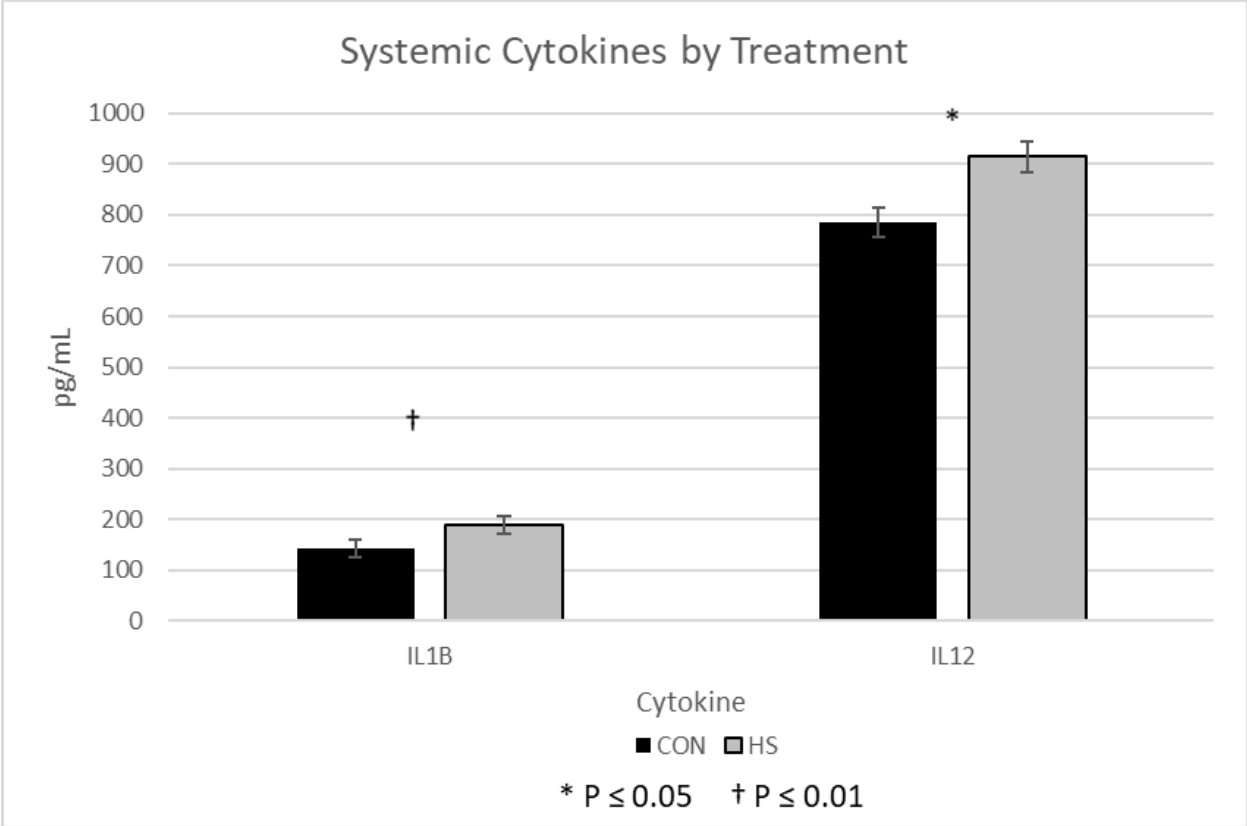
**Figure 3.26:** The least squared mean ( $\pm$ SEM) respiration rate by treatment (control – CON and heat stress – HS) and day measured as flank movements per 30 seconds. Effect of treatment\*day -  $P = 0.0001$ . Differences of  $P \leq 0.05$  in the least square mean for treatment by day are marked with different superscripts.



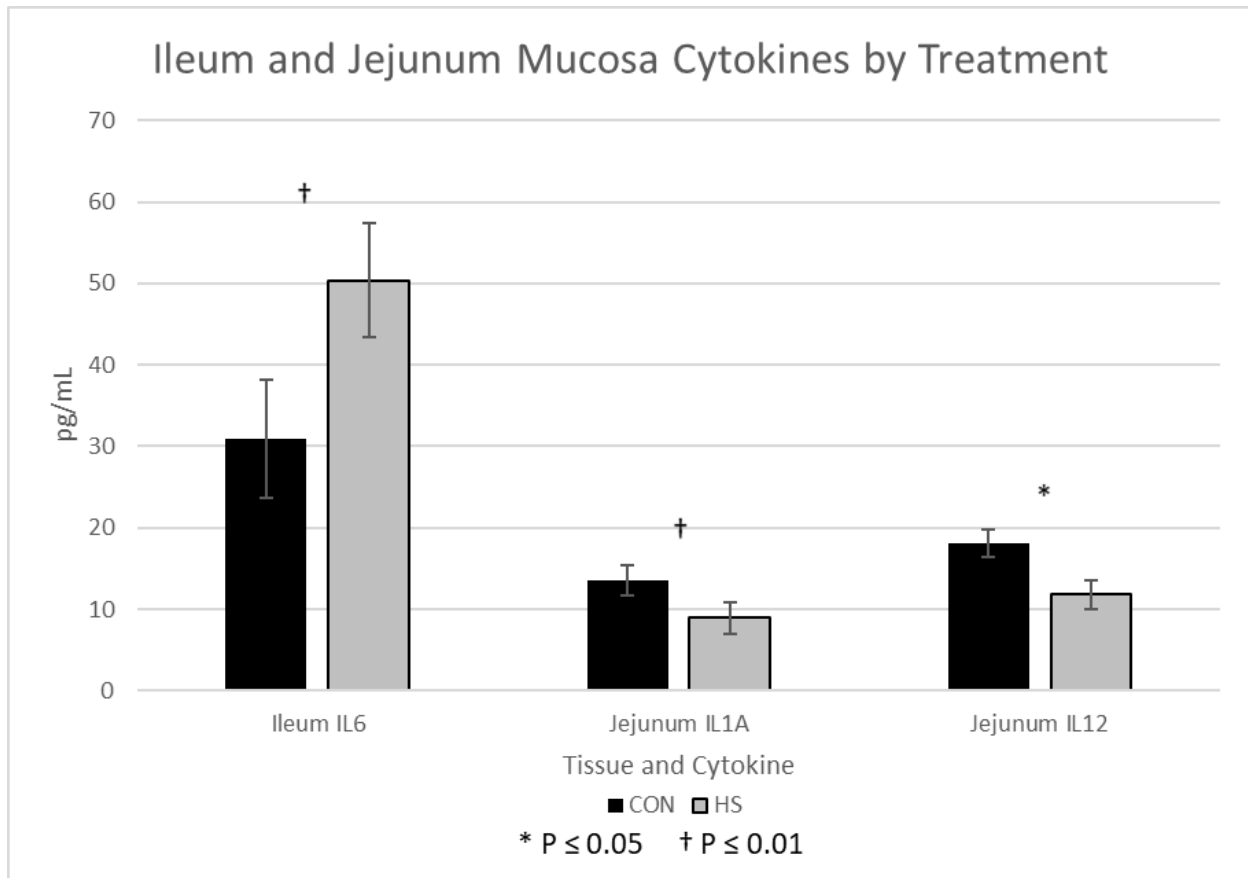
**Figure 3.27:** The least squared mean ( $\pm$ SEM) of cortisol concentration in ng/mL by treatment (control – CON and heat stress – HS) and day. Effect of treatment\*day -  $P = 0.0022$ . Differences of  $P \leq 0.05$  in the least square mean for treatment by day are marked with different superscripts.



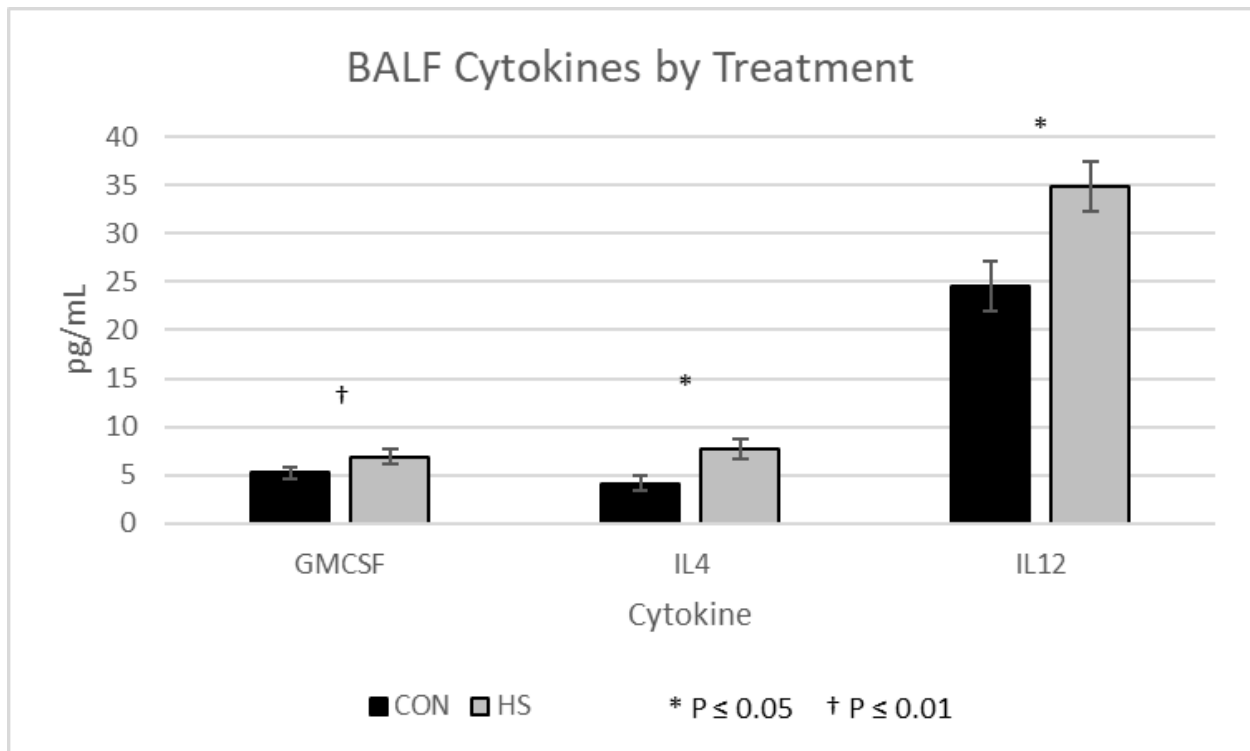
**Figure 3.28:** The least squared mean ( $\pm$ SEM) of cytokine concentration in pg/mL by day, regardless of treatment. Effect of day - P = IFN $\gamma$  P = 0.0240; IL1A P < 0.0001; IL1 $\beta$  < 0.0001; IL1Ra P = 0.0302. Differences of P  $\leq$  0.05 in the least square mean between day are marked with different superscripts. IFN $\gamma$  – AB; IL1A - GHI; IL1 $\beta$  – PQRS; IL1Ra - XYZ.



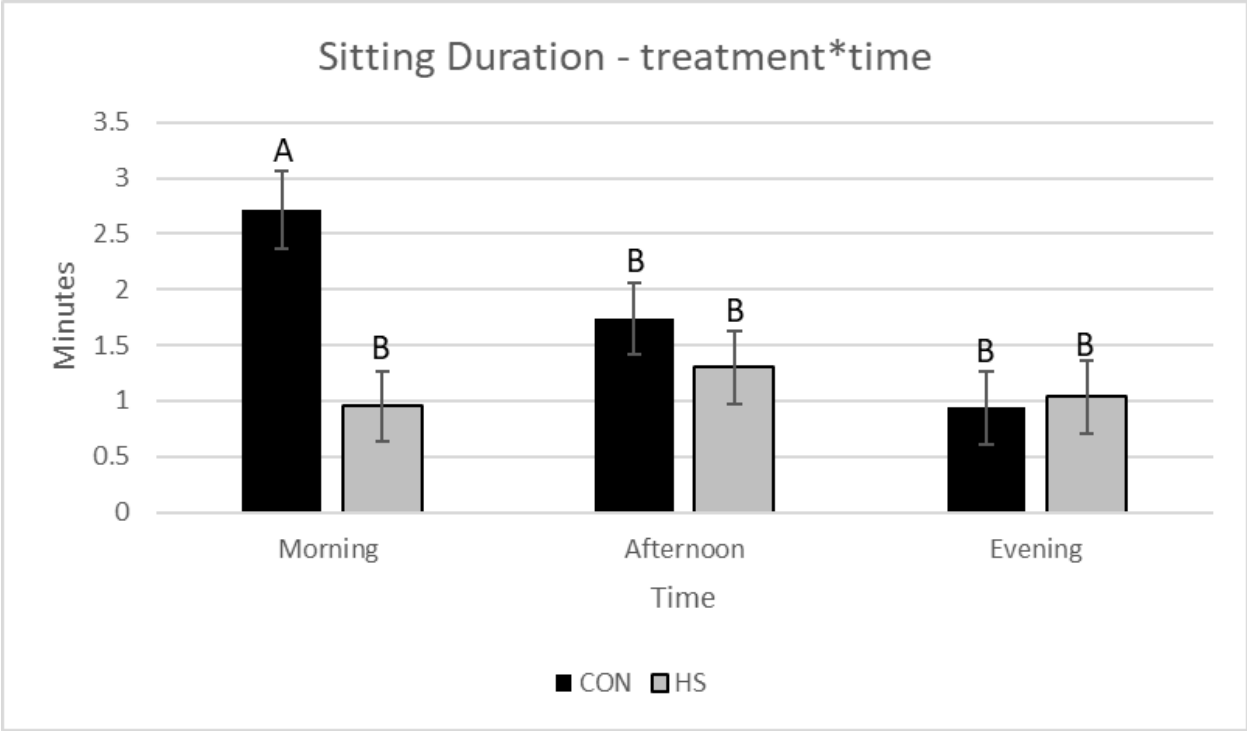
**Figure 3.29:** The mean least squared ( $\pm$ SEM) of systemic cytokine concentration in pg/mL by treatment (control – CON and heat stress – HS). Effect of treatment – IL1 $\beta$  P = 0.0582; IL12 P = 0.0024. Differences of P  $\leq$  0.05 in the least square mean between treatments are indicated by “\*” and by “†” for P  $\leq$  0.01.



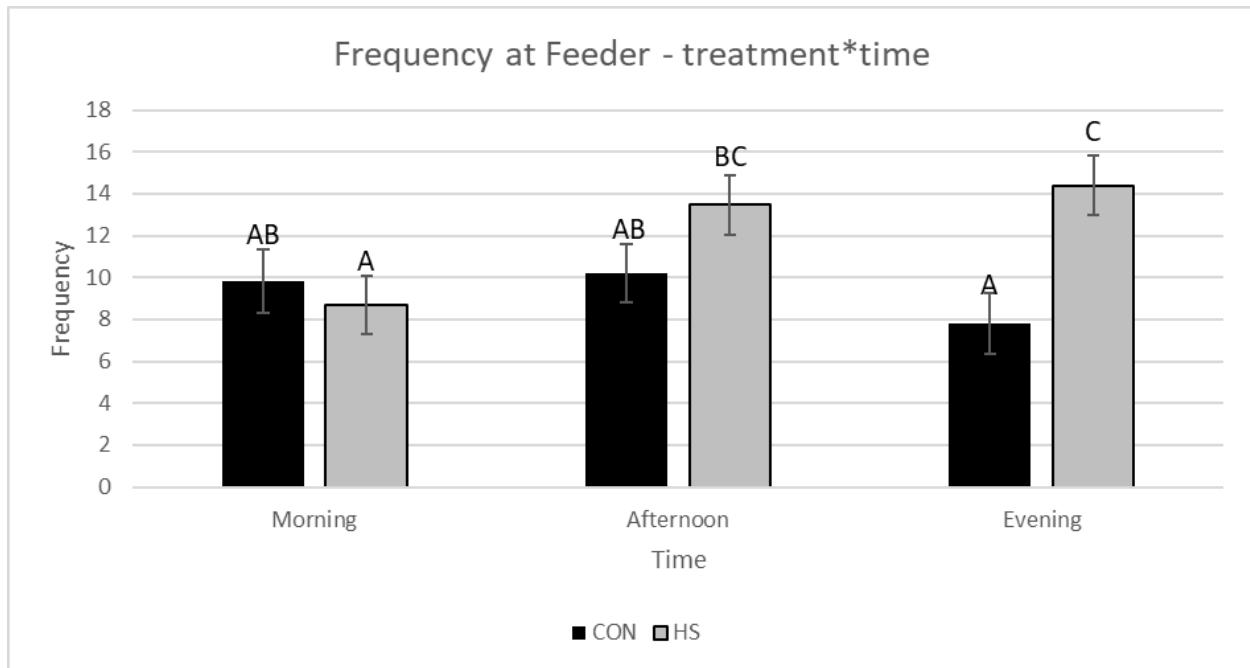
**Figure 3.30:** The least squared mean ( $\pm$ SEM) of organ cytokine concentration in pg/mL by treatment (control – CON and heat stress – HS). Effect of treatment – Ileum IL6  $P = 0.0765$ , Jejunum IL1A  $P = 0.1014$ ; Jejunum IL12  $P = 0.0172$ . Differences of  $P \leq 0.05$  in the least square mean between treatments are indicated by “\*” and by “†” for  $P \leq 0.01$ .



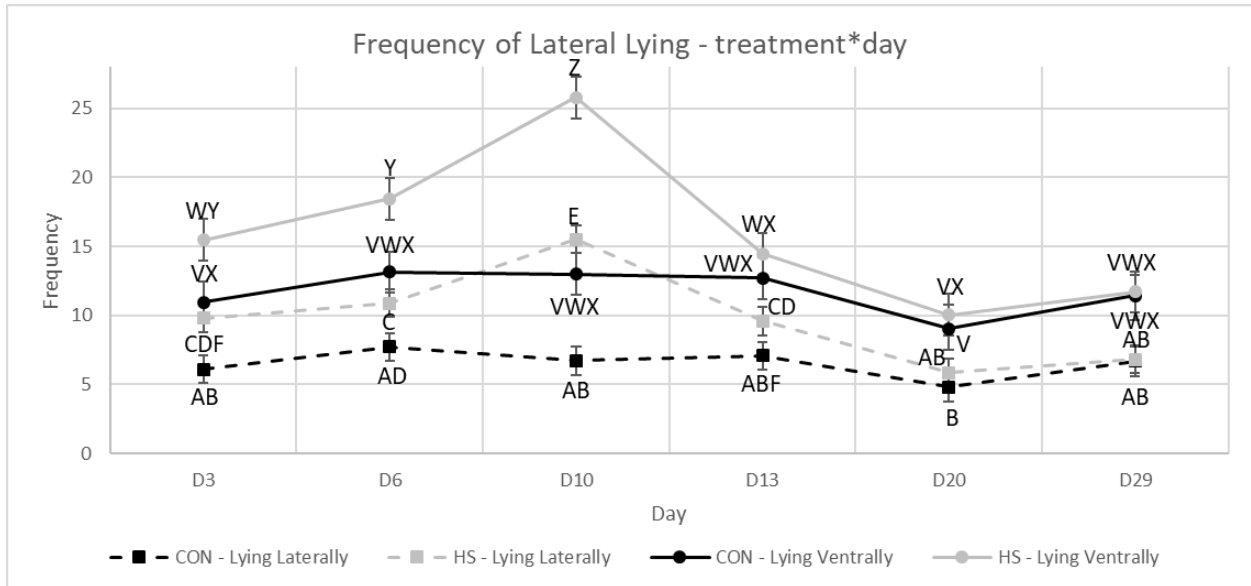
**Figure 3.31:** The least squared mean ( $\pm$ SEM) of bronchioalveolar lavage fluid (BALF) lung cytokine concentration by treatment (control – CON and heat stress – HS). Effect of treatment – GMCSF  $P = 0.1051$ , IL4  $P = 0.0152$ ; IL12  $P = 0.0101$ . Differences of  $P \leq 0.05$  in the least square mean between treatments are indicated by “\*” and by “†” for  $P \leq 0.01$ .



**Figure 3.32:** The least squared mean ( $\pm$ SEM) of sitting duration by treatment (control – CON and heat stress – HS) in minutes. Effect of treatment –  $P = 0.0166$ . Differences of  $P \leq 0.05$  in the least square mean between day are marked with different superscripts.



**Figure 3.33:** The least squared mean ( $\pm$ SEM) of frequency at the feeder for treatment by time (treatment = control – CON and heat stress – HS; time = morning, afternoon, and evening). Effect of treatment by time –  $P = 0.0288$ . Differences of  $P \leq 0.05$  in the least square mean for treatment by time are marked with different superscripts.



**Figure 3.34:** The least squared mean ( $\pm$ SEM) of ventral and lateral lying frequency by treatment (control – CON and heat stress – HS) and day. Effect of treatment\*day - Lying Ventrally  $P = 0.0005$ ; Lying Laterally  $P = 0.0006$ . Differences of  $P \leq 0.05$  in the least square mean for treatment by day are marked with different superscripts; Lying Laterally – ABCDE; Lying Ventrally - VWXYZ.

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## Appendix A

<b>Eve Technologies Cytokine Assay Sensitivity</b>	
<b>Cytokine</b>	<b>Sensitivity (pg/mL)</b>
GM-CSF	20
IFNg	42
IL-1 alpha	5
IL-1 beta	42
IL-1ra	10
IL-2	8
IL-4	20
IL-6	9
IL-8	5
IL-10	9
IL-12	5
IL-18	12
TNFa	6

## Appendix B

<b>St. Louis Zoo Cortisol Assay Sensitivity</b>	
<b>Parameter</b>	<b>Concentration (pg/mL)</b>
Sensitivity	27.6
Limit of Detection	45.4

## Appendix C

<b>Thermal Camera Sensitivity</b>	
Resolution	220x160
Thermal Sensitivity	0.07°C
Measuring Accuracy	±2°/ ±2%
Temperature Range	-20°~300°C