TEMPERATURE AND CORRELATED PHYSIOLOGIC RESPONSES AS INDICATORS OF WELL-BEING

BY

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THESIS

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ABSTRACT

It is well documented that young pigs are highly susceptible to stress, and body temperature in young pigs is altered in response to a variety of stressors. Exposure to pathogens, extreme fluctuations in ambient temperature and social stress may all result in dramatic changes in core body temperature. Neonatal piglets have a very limited thermogenic capacity, making them highly susceptible to chilling and disease. While improvements in piglet husbandry, nutrition, and management have benefited swine in production settings, another effective means of maintaining a high level of piglet well-being would be to limit the effects of stressors by being better able to identify the early signs of the stress response in piglets to various stressors. This has proven to be a difficult challenge for researchers because easily identifiable objective measures that signal the onset of a stress response have not been well identified. Deriving correlations between temperature and changes with other physiologic measures during the stress response would validate whether it is possible to use body temperature as an indicator of changes in well-being in response to various stressors in young pigs.

Chapter 1 examined the effects of feeding a gestation diet supplemented with a plant-extract, "capsicum", to sows to assess its modulatory effects on thermogenic and immune effects of their offspring. This was conducted across two experiments in which sows were fed either a gestation diet supplanted with capsicum for 2-wks prior to expected farrowing date (TRT-ST) or a standard gestation diet (CONT). In Experiment 2 an additional treatment was added feeding the treated diet for the entire gestation period (TRT-LT). In both experiments piglets were randomly assigned an age treatment (24 h or 72 h) and a challenge treatment (LPS or saline). The acute intraperitoneal injection of

LPS was used to assess if piglets from sows fed TRT diet could evoke a more robust febrile response shortly after birth. It has been well documented that neonatal piglets have a minimal capacity to thermoregulate, thus improving thermogenic capacity might reduce the number of animals that succumb to chilling. However, thermogenic ability was not vastly improved as rectal temperature in response to LPS was not greater in piglets from sows fed a TRT-diet compared to piglets from sows fed a CONT-diet. However, piglets from sows fed TRT-diet had different immune status. Plasma levels of cytokines were altered by TRT-diet. Piglets from sows fed TRT-ST diet and injected with LPS had lower plasma levels of interleukin-12 (IL-12), an inflammatory cytokine compared to piglets from sows fed CONT diet. However, feeding TRT-LT diet to sows did not alter piglet IL-12 levels. Interleukin-10 (IL-10), on the other hand, was altered in piglets whose dams were fed TRT-diet. Feeding a diet supplemented with capsicum to sows seems to have modulatory effects on the immune response of her piglets, but had little effect on their thermogenic ability.

Chapter 2 explored correlations between body core temperature and other physiologic responses as indicators of well-being by subjecting weaned pigs to various stressors. Pigs were mixed and acutely challenged with LPS in experiments 1 and 3. In experiment 2, pigs were also challenged repeatedly with LPS to simulate a chronic exposure to the immunogen and to assess methods of a chronic LPS challenge. In experiment 3, pigs were cold stressed and acutely challenged with LPS. Pigs were implanted with RFID thermosensors near the anterior jugular vein and in the flank fold, two locations that had previously been determined to be representative of pig core body temperature.

Acute LPS challenge resulted in increased body core temperature but with variable results across groups. In experiment 1, acute challenge with LPS resulted in greater (P <0.05) pig rectal and jugular temperatures within 45 min post-injection compared to saline-injected pigs. However, flank fold temperature was not altered by LPS. In experiment 3, regardless of ambient temperature, rectal temperature was greater (P <0.01) in LPS-injected pigs at 4 h post-injection compared to saline-injected pigs. Pigs also had increased rectal temperatures (P < 0.05) at various times following mixing in experiments 2 and 3.

In experiment 2, pig rectal and jugular temperatures were greater (P < 0.05) at 2 h post-injection among those treated chronically with LPS + peanut oil compared to pigs treated with peanut oil only. There was no difference in rectal or jugular temperatures of pigs treated with LPS + saline compared to pigs treated with saline only.

Pig body temperature decreased in response to cold. In experiment 3, pigs housed in cold ambient temperature (COLD 50 F) during both mixing and LPS challenge had lower rectal temperature (P <0.01) than did pigs kept at a thermoneutral ambient temperature (TNT). Regardless of whether pigs were subjected to cold stress, pigs injected with LPS (71.52 ng/mL ± 11.04) had greater (P < 0.05) plasma cortisol levels than did saline-treated pigs (52.00 ng/mL ± 10.86).

Concurrently, pig behavior was changed in response to acute LPS challenge and cold stress. Pigs injected with LPS spent less time drinking, eating, and fighting (P < 0.05) than did saline-injected pigs. Pigs kept in a COLD spent greater (P < 0.05) time sitting in contact with other pigs compared to pigs in TRT chamber, regardless of LPS treatment.

Social status also played a role in the response pigs had to LPS and cold stress. In experiment 3, intermediate (INT) piglets injected with LPS had greater ($P < 0.05$) rectal temperature compared to either dominant (DOM) or submissive (SUB) pig. The DOM pigs spent greater ($P < 0.05$) time drinking than did other pigs. Surprisingly, when challenged with LPS and kept in cold temperature, SUB pigs spent greater ($P < 0.05$) time eating than did either DOM or SUB piglets.

Monitoring changes in body temperature can be an easy and effective physiologic indicator of exposure to stress, but it must be understood that a change in body temperature might not indicate an impingement on pig well-being. To effectively assess piglet well-being, other parameters should be incorporated into the assessment of the animal.

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LIST OF ABBREVIATIONS

NK – Natural Killer

IL – Interleukin

CRF – Corticotropin Releasing Factor

HPA – Hypothalamic-Pituitary-Adrenal

ACTH – Adrenocorticotropin Hormone

PVN – Paraventricular Nucleus

APC – Antigen Presenting Cell

LPS – Lipopolysaccharide

TNF – Tumor Necrosis Factor

INF – Interferon

NE – Norepinephrine

REVIEW OF LITERATURE

Introduction

Through advances in nutrition, technology, and management practices, the swine industry has made strides on improving animal production. But piglets are still routinely exposed to stressors that may affect their well-being, including various standard husbandry practices such as teeth resection, tail docking, identification, just to name a few (Marchant-Forde, 2009). Also, alterations to the microenvironment, or individual space, such as changes in ambient temperature and/or changes to the social dynamics of a group can also result in undue stress. Currently, there is no universally accepted way to determine an animal's level of well-being. There are some who believe that the feelings of the animals should be considered when evaluating well-being (Duncan, 1996), but it is beyond the ability of science to realistically evaluate feelings. Still others are convinced that performance measures such as growth rate, mortality rate or reproductive efficiency are the most appropriate method of measuring the level of pig well-being (Curtis, 2007). While these measures are indicative of severe impingements on well-being, they fall short in recognizing the point at which the animal cannot maintain a satisfactory level of wellness. Instead, more easily quantifiable measures that will provide real-time changes should be used to develop a system of monitoring well-being.

Our knowledge of stress systems, primarily the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system is quite extensive. Levels of glucocorticoids and catecholamines, the end product of activation of these two systems, have been shown to increase shortly after the onset (Germann, 2002) of a variety of

stressors that will be discussed later. The state of the immune system may also be a sensitive indicator to stress as different stressors have been shown to affect the condition of the immune system to various degrees. The nature of the stressor, including the intensity and duration under which stress is experienced, dictates whether aspects of the immune system are enhanced or inhibited. Other studies point to behavior as a sensitive indicator of changes in well-being (Skinner, 2008) as behavioral changes to stress often outlast other physiologic measures.

Finally, it has been well documented that body core temperature fluctuates in response to infection, changes in ambient temperature, and social stress. The physiologic systems that regulate all of these measures are interconnected. For example, proinflammatory cytokines play a role in initiating the febrile response as well as the initiation of sickness behaviors through a release of corticotropin releasing factor (CRF) at the hypothalamus. This also results in increases in cortisol, the primary glucocorticoid in pigs that has a modulatory effect on immune activity, and along with the catecholamine norepinephrine, upregulates thermogenesis throughout the body. Incorporating all of these measures in a multi-faceted approach would represent the most complete explanation of the stress response, potentially providing a detailed blueprint for quantitatively evaluating changes in pig well-being.

Stress Physiology

Defining Stress

Stress can be defined as the biological response that occurs when an individual perceives a threat to its homeostasis. Adverse conditions tend to disrupt this homeostasis,

resulting in a variety of biological responses from the animal. These responses may include neuroendocrine, thermogenic, immune, or behavioral changes, and they occur out of an effort to return to a homeostatic state. While some stimuli may be vital to animals, excessive or chronic exposure to a stressor can be extremely detrimental to the health and well-being of an animal; however, there is some evidence that stress hormones may mediate brain regions associated with cognitive learning and memory (Akirav and Richter-Levin, 2005). When an animal's physiologic and behavioral efforts to return to homeostasis are not successful an animal may fall into a state of distress.

Common physiologic changes to acute stress include an increase in cardiac output and respiration rate as well as a redirecting of blood flow to the brain, heart, and muscle (Chrousos and Gold, 1992). These changes are regulated by sympathetic nervous activity and other stress systems including the hypothalamic-pituitary-adrenal (HPA) axis as a means of preparing the body to respond to a particular stressor. For example, Andersson et al. (1964) showed that sympathetic activation at the level of the hypothalamus results in release of norepinephrine, which has been shown to increase blood pressure. As stated previously, the sympathetic nervous system and the HPA axis are two physiologic systems that play an important role in the response to stress, but because catecholamines are often difficult to measure, HPA activity is perhaps a better way to measure levels of stress hormones.

Role of the HPA Axis

It has been well documented that stress activates regions of the brain including the hypothalamus and anterior pituitary and subsequently the adrenal cortex, collectively known as the HPA axis. The major role of the HPA axis is maintenance of homeostasis

following stress (Abel, 2005). Sutanto and de Kloet, 1994, imply that the HPA axis is "the pivot for the animal's ability to adaptation and coping with stress" (Sutanto and de Kloet, 1994). The hypothalamus contains parvocellular neurons that are responsible for secreting the 41-amino acid peptide, corticotropin releasing hormone (CRH) and vasopressin (Grinevich, 2001), with CRH being the primary modulator of the stress response in pigs (Abel, 2005). These neurons are located primarily in the paraventricular nucleus (PVN) of the hypothalamus (Vale et al, 1981). CRH is secreted in response to stressors and travels through portal capillaries to the anterior pituitary where it synergizes the production and release of proopiomelanocortin or its products including adrenocorticotropin releasing hormone (ACTH) from corticotrope cells (Jones and Gillman, 1988; Antoni, 1989). ACTH subsequently causes release of glucocorticoids from the adrenal cortex (Whitnall, 1993) where it encounters its receptor, melanocortin receptor type 2 (Abel, 2005). The HPA activation is not as immediate as the sympathetic response of epinephrine and norepinephrine release; however, cortisol, the primary glucocorticoid in pigs (Minton, 1994), is primarily responsible for mobilizing energy sources, but has regulatory effects on the immune system and in general aids in returning the animal to homeostasis.

Glucocorticoids, both cortisol and corticosterone, have a regulatory effect on several physiologic processes. Cortisol, in pigs, is responsible for mobilizing energy stores in times of higher energy demand when available free glucose levels are not sufficient (Germann, 2002). Generally speaking, glucocorticoids also serve to modulate immune function. In particular, corticosteroids are necessary for regulating an overshooting of the inflammatory response to some immune challenges. This is apparent

in adrenalectomized rats (Kapcala, 1995) and CRF knockout mice (Karalis, 1997) which cannot curb the lethal inflammatory effects of the immune response to endotoxin. In a laboratory setting, glucocorticoids inhibit the production of several cytokines in activated T lymphocytes by increasing production of I-kB. Nuclear factor kappa B (NF-kB) translocation is inhibited when bound to I-kB resulting in decreased NF-kB translocation to the nucleus and subsequently decreased cytokine production (Auphan, 1995). Exposing murine macrophages to dexamethasone (DEX), a synthetic glucocorticoid, results in decreased macrophage production of the proinflammatory cytokine, interleukin-12 (IL-12) (Dekruyff, 1998). A related study showed that exposing human monocytes to DEX reduces the capacity of monocytes to produce IL-12 (Blotta, 1997). Furthermore, Ramirez et al. (1996) showed that, in vitro, CD4+ T lymphocyte production of inflammatory factors such as interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) were also decreased in the presence of DEX. Conversely, in the same study, mRNA levels of the anti-inflammatory cytokines IL-4, IL-10, and IL-13 were all increased. The role of cytokines and the immune system in the stress response will be discussed in greater detail shortly.

The HPA axis also regulates its own action by negatively feeding back on itself. Glucocorticoid release provides a means of self-regulation by acting directly on the hypothalamus to decrease CRH release and also both directly and indirectly acting on the anterior pituitary to control release of ACTH (Keller-Wood and Dallman, 1987; Dallman, 1987). This negative feedback consequently controls the secretion of glucocorticoids from the adrenals, and at rest, basal levels of cortisol are enough to maintain this.

Role of the Immune System in the Stress Response

Any immune challenge that threatens an animal's homeostasis can be considered a stressor (Elenkov and Chrousos, 1999). Evidence to support this includes data that suggest the immune system can activate the HPA axis (Kapcala, 1995), send signals to the brain to initiate the febrile response (Banks, 1991) and dictate the onset of behavioral changes associated with sickness. In short, the immune response is not isolated and instead involves communication with other physiologic systems to coordinate the appropriate response to effectively combat infectious stressors.

First, some detail about the organization of the porcine immune system is necessary. The immune response in pigs consists of two equally important, but not exclusively independent "arms". The first "arm" is referred to as being innate in nature due to its method of antigen recognition. Specific patterns on the surfaces of pathogens called pathogen associated molecular patterns (PAMPs) are recognized by antigen-presenting cells (APCs) like monocytes/macrophages, dendritic cells and other phagocytic cells. Examples of PAMPs include flagella or lipopolysaccharide (LPS), the lipid cell membrane constituent of gram negative bacteria (Janeway, 2005; Fearon and Locksley, 1996). Macrophages are generally considered to be the most important APC in the recognition process of LPS. The macrophage possesses a receptor for LPS, CD14, which associates with a particular Toll-like receptor, TLR-4 to accomplish recognition of LPS (Hoshino, 1999). It is this recognition that induces the release of a cascade of cytokines and chemokines such as IL-8 (Schall, 1994) IL-1β, IL-10 and IL-12 which recruit other cell types such as neutrophils and play a role in the differentiation of T lymphocytes.

The second arm of the immune system has an adaptive mechanism that allows it to remember pathogens in which it comes in contact. From an evolutionary standpoint, it is newer, found in higher order organisms and is comprised of a cell mediated aspect and a humoral aspect, each associated with a particular T helper subclass (Fearon and Locksley, 1996; Mosmann and Sad, 1996). The adaptive arm of the immune system is classified as such because naïve lymphocytes differentiate into the T helper 1 or 2 subclasses depending on the particular pathogen. The Th1 subclass functions primarily against intracellular pathogens, whereas those naïve T lymphocytes that differentiate into a Th2 subclass are effective against extracellular pathogens including bacteria and parasites. Because of this ability to differentiate, the adaptive immune system is much more flexible in its activity than the innate immune system and has long lasting immune protection due to memory (Parham, 2009).

The innate and adaptive immune systems are not exclusively independent of each other. In fact, signaling mechanisms from one system can affect function and activity of the other. Cytokines and chemokines are important factors that allow "communication" between the two immune systems (Mosmann, 1996). Those cytokines that drive a cell-mediated, Th1, response are often labeled as proinflammatory and those that drive the humoral, Th2, response are considered to be anti-inflammatory though this is not completely black and white. The proinflammatory cytokines that are released during the acute immune response include tumor necrosis factor alpha (TNF- α), interferon-γ (INF-γ) (Carroll, 2002), interleukins-1β, -2, -6, -8, and -12 (Janeway, 2005). Interleukin-12 is one of the first proinflammatory cytokines secreted from APCs such as macrophages or dendritic cells at sites of infection (Ashkar, 2000; Scheicher, 1995). A heterodimer, IL-

12 consists of a p35 and p40 subunit and along with other members of the IL-12 family, including free p40 and IL-23, IL-12 functions to promote and maintain a Th1 response. It has been shown that both natural killer (NK) cells and T lymphocytes express the two receptors, β1 and β2, required for IL-12 activity. Data supports that IL-12 has the ability to induce proliferation of NK cells and differentiated T lymphocytes with a functional IL-12 receptor molecule (Valiente, 1992). IL-12 also elicits the release of INF-γ from lymphocytes, further promoting a Th1 response.

Polymorphonuclear neutrophilic leukocytes, or simply neutrophils, are recruited heavily in the acute immune response. In fact, neutrophils are one of the "first waves of cells that cross the blood vessel wall to enter inflammatory sites" (Janeway, 2005). Neutrophils play an integral role in the host defense against bacterial infections. For example, mice injected with LPS had a 7.5 fold increase in neutrophils by 0.5 h within the lung vasculature (Hirano, 1996). Neutrophil recruitment is by chemotactic cytokines, specifically IL-8, which are given off by macrophages during antigen recognition (Bochenska-Marciniak, 2003). Because macrophages are affected during stress, this phenomenon of neutrophil recruitment is further supported by increases in the ratio of neutrophils to lymphocytes in the periphery shortly after the onset of many stressors (Lay, 2008; Puppe, 1997).

The Th2 differentiation from naïve T lymphocytes is also driven by cytokines, albeit a different group of them. Both IL-4 and IL-10 have been suggested to down-regulate the production of inflammatory cytokines as well as shift the differentiation of naïve T-cells to a Th2 phenotype. The effects of IL-10 are synergistic with glucocorticoid effects in that they inhibit IL-2 production and subsequently the

proliferation of CD4+ T cells (Brunetti, 2002). In a study by de Waal Malefyt et al. (1993) it was suggested that inhibition of CD4+ T cell proliferation was in fact due to a reduction in IL-2.

The response of the porcine immune system to infection is significant not only because of what it does within the context of the immune response, but also the effect it has at the level of the brain. Chrousos (1995) showed that the acute phase immune response, that is the initial antigen recognition and subsequent response by the immune system, activates the HPA axis. Increases in inflammatory cytokines were followed by increases in cortisol levels. Exogenous interleukin-1, in particular, has been shown to activate neurons in the hypothalamus to secrete CRH (Berkenbosch, 1987). Increases in hypothalamic CRH following immune challenge further strengthen this point.

It is well documented that HPA activation in turn has effects on the immune system. Glucocorticoids inhibit some immune functions including lymphocyte proliferation, migration and cytotoxicity (Elenkov, 1999). As previously stated, glucocorticoids also have been shown to block NF-κB transcription. Fitzgerald et al. (2007) reported that the NF-κB transcription pathway drives production of IL-8 in epithelial cells, thus cortisol blockage of NF- κB pathways may reduce the IL-8 directed migratory capacity of neutrophils to local inflammatory sites. Glucocorticoids also drive human CD8+ T cell proliferation towards a phenotype with high IL-10 production (Richards, 2000). IL-10 has a general inhibitory effect on inflammatory cytokine production, so by activating the HPA, the innate immune response indirectly activates cortisol release, driving a shift towards humoral defense and an increase in anti-inflammatory cytokine levels (Ramirez, 1996).

Body Temperature and Connection to Stress

Pigs are homeothermic, that is they are able to maintain their body core temperature regardless of changes in ambient temperature. Decreases in ambient temperature have been shown to rapidly decrease skin temperature, but not body core temperature in several species including rats (Bratincsak and Palkovits, 2006), cats (Foster and Ferguson, 1952), and dogs (Hellstrom and Hammel, 1967). An increase in body temperature is also important during the febrile response to infection as it creates an unfavorable environment for many pathogens while enhancing immune function. Animals are able to regulate their body core temperature through various physiologic, endocrine, and behavioral mechanisms.

While it is well documented that thermoreceptors are present in both the body core and central nervous system, it is cutaneous thermoreceptors that initiate thermoreception. Findings from recent studies suggest that specific cation channels in sensory neurons, the transient receptor potential (TRP) family, mediate temperature sensation across a broad range of temperatures. In particular, TRPM8 has been shown to dominate sensation to cold environmental stimuli as TRPM8-deficient mice exhibit a reduced ability to avoid cold temperatures (Bautista, 2007; Colburn, 2007; Dhaka, 2007). TRPM8 channels can be activated by other means such as application of menthol and icillin (McKemy, 2002; Peier, 2002). Tajino et al. (2007) showed that application of menthol to the trunk of mice induces warm seeking behavior as well as shivering induced-thermogenesis, increased oxygen consumption, and vasoconstriction in the tail. As reviewed by Morrison (2009), cold-defensive mechanisms are initiated in central nervous system structures, such as the preoptic area of the hypothalamus.

Thermogenesis - important in maintaining body core temperature - is modulated by the central nervous system in response to cold environments, a fall in body core temperature, or pyrogenic cytokines (Morrison, 2009). Sympathetic activity of the hypothalamus is responsible for behavioral changes including shivering (Marsden, 1970; Bülbring, 1976). Shivering is a result of bursts of activity in α-motoneurons innervating skeletal muscle causing rhythmic contractions. While it is not completely understood, several areas of the hypothalamus have been shown to be responsible for these cold evoked bursts of α-motoneuron activity including the lateral parabrachial nucleus (Nakamura and Morrison, 2008a), the preoptic area (Zhang, 1995), and the rostral ventromedial medulla (Tanaka, 2006).

As previously stated, the hypothalamus has been shown to mediate glucocorticoid and catecholamine release. Norepinephrine has been implicated as having thermogenic properties, including mobilizing fuels for increased metabolism (Young, 1977), specifically acting on brown adipose tissue as in young animals (Smith, 1964), and modulating blood flow to certain tissues (Gale, 1973). Norepinephrine release is controlled at least in part by the hypothalamus (Acuna, 2009). This again implicates the hypothalamus as an important brain region as it pertains to thermogenesis. Moreover, Madden and Morrison (2009) showed that blocking sympathetic neurons decreased both brown adipose tissue thermogenesis and induction of fever mechanisms in response to cold temperatures.

The importance of the hypothalamus during feverish states has become clearer over the past two decades. Fever is important not only to create an unfavorable environment for infectious agents but also to enhance some immune functions. For

example, human neutrophils have increased nitric oxide production activity at elevated temperatures (Rosenspire, 2002). As discussed earlier, cytokines are messengers of the immune system and have pyrogenic properties including IL-1β, TNF-α, and IL-6 (Kluger, 1991). Poveda et al. (1999) showed that IL-1β, TNF-α, and IL-6 are in greater concentrations during chronic bacterial infection. The role of TNF-α in the febrile response is somewhat controversial (Stefferl, 1996) though its role may be through IL-1 activity. Interleukin-1 can effectively circumvent the blood brain barrier (Banks, 1991), and the receptor for IL-1 has more recently been found to be expressed in the human hypothalamus (Hammond, 1999). Administration of human monocyte derived IL-1 to mice resulted in elevated glucocorticoid levels, further evidence that IL-1 can activate the brain at the hypothalamic-pituitary level (Besedovsky, 1986). IL-6 activity has also been better characterized in the brain in recent years (Beurel, 2009). While only weakly pyrogenic when injected peripherally, IL-6 is important in the febrile response as shown by a lack of fever in IL-6 knockout mice (Kozak, 1998; Nilsberth 2009). Thus, the mechanism of inducing fever during infection is through the release of several inflammatory cytokines that act either directly or indirectly on the hypothalamus.

A final point to consider is that pig core body temperature naturally fluctuates throughout the day in a circadian pattern. This circadian rhythm has been well studied, including a recent experiment by Hanneman et al. (2005) that showed that over a period of 5-9 days, temperature fluctuated around a mean by as much as 0.7 and 1.6 °C.

Behavioral Responses to Stress

Animals respond to stress through many physiologic changes, but often the least costly way they respond to stressful stimuli is through behavioral changes. As Banks

(1982) discussed, behavior is the most difficult aspect of an animal's phenotype to measure in a quantitative, replicable manner. However, just like measuring changes of some physiologic parameters to stress can glean some idea of an animal's well-being so too can observable changes in an animal's behavior offer information about their state of well-being.

Pigs will alter their behavior in response to a variety of stressors. Their behavioral responses to infection, or sickness behavior, have been particularly well characterized. Sickness behavior is a collective term referring to non-specific behavioral changes such as increased sleep, pain, depression, lethargy, and suppression of appetite (Dantzer, 2001). These behavioral changes may at times be a better indicator of infectious stress than fever. Sickness behaviors such as lethargy, fatigue, and anorexia tend to outlast fever in rats (Bennett, 1998). Some of the same proinflammatory cytokines that play a role in activating the febrile response have also been implicated in the induction of sickness behaviors. Tazi et al. (1988) showed that administration of IL-1 to rats could elicit a taste aversion to a saccharin solution, evidence suggesting the onset of sickness behaviors. Furthermore, rats injected with high doses of IL-10 showed little signs of inactivity or sickness induced depression as late as 6 hours after intraperitoneal injection of LPS (250ug/kg), indicating that IL-10 may modulate proinflammatory cytokine production centrally (Bluthe et al, 1999). On the contrary, other studies have produced conflicting results. Konsman et al. (2008) showed that infusion of IL-1 receptor agonist into the lateral brain ventricle of rats attenuated sickness behaviors as well as c-fos expression in the amygdala as late as 4 hours after peripheral administration of bacterial lipopolysaccharide.

Sickness behaviors are also commonly observed in response to stimuli such as dramatic changes in ambient temperature. Depending on increases or decreases in ambient temperature, animals may alter their position closer or further away from each other to either help conserve or dispel excess body height. Shivering and panting are more specific examples of behaviors associated with thermoregulation.

Effects of Stressors and Other Factors Contributing to the Stress Response

The response to stress depends on multiple factors including the type of stressor, duration and intensity, as well as social status of the organism. The behavior and physiologic responses to stress are also dictated by the nature of the stressor, including the intensity and duration that the stress is experienced. For example, psychological stressors such as uncertainty or conflict with conspecifics have the ability to activate the HPA axis through cognitive recognition (de Kloet, 1991) whereas infectious challenges activate stress responses via immune mechanisms. The intensity of the stressor dictates the level of physiologic and behavioral change as seen in dose responsive experiments. For example, rats injected with LPS (50 μg/kg) have greater abdominal temperature and exhibit a greater decrease in wheel running behavior compared to rats injected with 10 μg/kg LPS (Skinner, 2008). Simultaneous exposure to multiple stressors can also produce more potent effects. Van Gucht et al. (2003) showed that exposing pigs to PRRS and LPS resulted in enhanced respiratory disease compared to inoculating pigs with just PRRS or LPS. One factor in particular that does not always receive enough attention is the role of dominance in species that establish social hierarchies as pigs do.

Role of Dominance in the Stress Response

Pigs are social animals, and as such, they establish a social hierarchy with their pen mates in the form of a social hierarchy. There is evidence to show that dominance status affects the physiologic response of pigs to different stressors. Aspects of the immune system, for example, may be enhanced or diminished by social stress. Those pigs classified as dominant had greater total white blood cell counts when infected with PRRS virus than did submissive pigs infected with PRRS. On the other hand, natural killer cytotoxicity was greater for subordinate pigs classified as PRRS (+) than it was for dominant, PRRS (+) pigs (Sutherland, 2007). However, exposure to acute cold enhanced NK cytotoxicity among dominant and intermediate pigs (Hicks, 1998) compared with submissive pigs.

The immune system is not the only physiologic system that seems to be affected by social status of the animal. There is also evidence to show that more subordinate pigs had an enhanced HPA activity at rest as well as following aggressive dyadic confrontations (Fernandez, 1993). This is also true in a three pig hierarchy, where plasma and salivary cortisol levels were elevated in intermediate pigs shortly after conflict with an unfamiliar dominant pig (Koopmans, 2005). So, there is solid evidence to support that social status can directly affect the degree of the physiologic response of pigs to stress.

Management Stressors

Management practices such as crowding, mixing of unfamiliar animals, transportation, weaning, tail docking, etc. can all invoke a stress response in domestic farm animals (Minton, 1994). Nyberg et al. (1988) showed that transporting 12-week-old

pigs for 5 hours increased plasma cortisol concentrations but decreased glucocorticoid receptor concentration immediately after transport. Though not statistically different, 4-week-old piglets had increased plasma cortisol (50.4 ng/mL) in response to 4 hours of shipping stress compared to control piglets (43.2 ng/ml) (Hicks, 1998). Weaning has been shown to affect piglet physiology. Lay et al. (2008) showed that regardless of exposure to prenatal stress, within 2 hours after weaning cortisol levels had spiked, an indication that a stress response had occurred due to weaning.

Changes in Ambient Temperature

It has been well documented that ambient temperature can affect piglets by eliciting physiologic responses. When 7-week-old pigs were challenged with PRRS virus and heat stressed, regardless of PRRS status, rectal temperature was greater in pigs housed at 32 °C compared to those housed at 24 °C (Sutherland, 2007). Unlike typical heat stress situations, decreases in environmental temperature can be detrimental to young pigs. A study by Hicks et al. (1998) showed several physiologic responses of 4-week-old pigs in response to 4 hours of a cold stressor. Cold stressed pigs spent more time feeding, 18.0 min/h, compared to control pigs, 12.0 min/h, though this did not result in higher feed intake. Cold stressed pigs were also more active as they spent more time standing, 19.0 min/h, compared to control pigs, 10.4 min/h. In a different study, dominance affected immune status of pigs that were cold stressed. Cold stressed, dominant and intermediate pigs exhibited greater NK cytotoxicity than submissive pigs. Neonatal piglets challenged acutely with cold stress also display changes in physiologic measures, such that piglets at 24 h of age exposed to 18 °C had significantly lower rectal temperature than did piglets at 34 °C. Also, those same piglets at 24 h of age exposed to

both cold stress and challenged with LPS exhibited drops in body temperature consistent with hypothermia. This was compared to piglets of the same age, that when challenged with LPS and kept at a thermoneutral temperature that did not exhibit a drop in body temperature (Carroll, 2001). Cold stress also increases HPA activity. Neonatal pigs housed at 18 °C had elevated serum ACTH compared to neonates kept at 34 °C (Carroll, 2001).

Immune function is also altered by exposure to cold. In unpublished data by Salak-Johnson and Niekamp, pigs exposed to cold stress for 4 days had elevated NK cytotoxicity at an effector: target ratio of 50:1 compared to control pigs (Hicks, 1998).

Type of Pathogenic Challenge Dictates Response

Antigen or pathogen recognition and the corresponding response in pigs are dependent on the particular pathogen. Cytokine levels, for example, are influenced by different pathogenic challenges. Following mixing, pigs that had been vaccinated with pseudorabies had elevated IFNγ levels compared to control pigs (de Groot, 2001). In pigs vaccinated against parasitic *Taenia solium*, IFNγ as well as IL-2 were elevated (Diaz, 2003). However, IL-10 was not elevated after vaccination with pseudorabies or *Taenia solium* indicating that the acute phase inflammatory response was activated. The time at which these measures are taken may be a reason for this. Plasma levels of IL-10 was increased at 7 days after PRRS challenge whereas IFNγ was not different between pigs challenged with PRRS and control pigs (Sutherland, 2007). Pigs challenged with *Toxoplasma gondii* had elevated TNFα, IFNγ, and IL-4 (Dawson, 2005).

A common challenge to simulate a disease state is injection with LPS. Lipopolysaccharide has been shown to activate all of the discussed physiologic responses

that correlate with stress. In those mice injected interperitoneal with LPS, nighttime locomotor activity was decreased compared to saline injected mice. The responses to LPS, however, are dose dependent. Injecting mice with LPS at concentrations of 1.0 and 3.0 mg/kg, elicited an increase in body temperature whereas lower doses, between 0 and 0.1, did not (Kozak, 1994). Cytokine expression is also altered during LPS challenge. Interleukin-6 mRNA expression is increased in peripheral tissues and in the hypothalamus following LPS challenge in neonatal pigs (Klir, 1997). Webel et al. (1997) showed that doses as low as 5 µg/kg LPS caused similar increases in blood levels of IL-6 as well as TNF-α. Knockdown of IL-12 pathways reduces IL-12 production and mRNA expression in macrophages and dendritic cells during infectious challenge with LPS (Utsugi, 2009). Lipopolysaccharide injection also affects the HPA axis and induces sickness behavior. Injection of 0.5 µg/kg LPS in pigs was effective in elevating cortisol levels at 2 hours post-injection (Webel, 1997). Kinoshita et al. (2008) reported decreased locomotor activity in adult and middle-aged rats injected with 200 µg/kg LPS.

Stress and Impact on Piglet Well-being

The nature of a stressor will dictate whether a particular animal will have a stress response and to what degree that response will be. Changes in physiology (neuroendocrine, immune, and behavior) in response to management practices, infectious agents, and extreme changes in thermal environment all have the potential to impinge on a pig's level of well-being. For example, it has already been discussed that stress hormones affect the production of Th1 and Th2 cytokines, important for determining the appropriate immune response (Elenkov, 1999). Thus, exposure to a particular stressor

may shift an animal's immune system towards one type of immunity leaving the animal susceptible to some pathogens.

Weaning and Mixing are Deleterious to Piglet Well-Being

Weaning has several negative effects on piglet well-being including nutritional, social, and environmental changes that often elicit an acute stress response. It has been shown that at the time of weaning piglets are more susceptible to infection (Bailey, 1992). There is also evidence that viral and bacterial infections increase shortly after weaning as is indicated by increased inflammatory cytokines around that time (Wattrang, 1998). In 1978, Lecce and King showed that weaning of piglets makes them more susceptible to the effects of rotavirus: increased death rate (6%), decreased weight gain, and damage to the intestinal villa. Behavioral cues, such as increased vocalization during weaning, indicate that piglet well-being may be compromised at this time (Weary, 1997).

At the time of weaning young pigs are often mixed with other unfamiliar pigs, leading to agonistic interactions that result in social stress. This increase in social stress could be the reason that piglet immune function and piglet performance is impaired at weaning (Jones, 2001). More specifically, mixing piglets has been shown to reduce feed intake and subsequently body weight gain compared to piglets remaining with littermates (McGlone and Curtis, 1985). However, socially dominant pigs have increased lymphocyte proliferation following mixing compared to socially subordinate (Tuchscherer, 1998).

Cold Environment Detrimental to Young Pigs

Neonatal piglets, in particular, are highly susceptible to chilling as indicated by high death loss within the first 3 days of farrowing (Bauman, 1966; Curtis, 1974). Le

Dividich (1981) compared newly born piglets housed at 18-20 °C and piglets housed at 30-32 °C, a thermoneutral temperature for pigs of that age, for 3 days. By day 3 of life, 5 of the cold stressed piglets had died whereas all of the piglets housed at the warmer temperature survived. A high mortality rate due to cold stress is especially high for low birth weight piglets (Stanton, 1977). Thus, despite being a homeothermic species, neonatal piglets struggle to thermoregulate in very cold ambient temperatures.

Cold ambient temperatures can also affect the well-being of weanling age piglets. Frank et al (2003) showed that 4 days of exposure to 15.6 °C increased feed intake of 24 day old piglets by 24% compared to piglets housed at a thermoneutral temperature. However, increased feed intake translated into poor gain-to-feed ratio. This would indicate that an increased amount of energy is being expended in order to maintain a homeothermic state. Data to support this theory was gathered by Noblet and Le Dividich (1981) who showed that carbohydrate utilization was greater in neonatal pigs housed in a cold-environment. Another indicator that piglets have a greater energy demand during periods of cold stress is that they exhibit greater basal levels of serum ACTH and cortisol, presumably a response to mobilize energy stores (Frank, 2003).

In a study by Klir et al. (1997), it was shown that piglets housed at 21 °C did not mount a detectable febrile response after challenge with LPS. This lack of a febrile response is exacerbated in neonatal piglets as reported by Carroll et al. (2001). Acutely cold stressing neonatal piglets results in a period of hypothermia when they are injected with LPS i.p. It has also been shown that piglets housed at colder ambient temperature during weaning have increased fecal shedding of *Escherichia coli* (Jones, 2001), thus increasing exposure to bacteria and resulting in a higher prevalence of illness.

Disease State Decreases Well-Being

Production losses due to disease are reported to cost the U.S. swine industry in excess of $1.5 billion dollars yearly, including losses due to mortality and poor efficiency (Rothschild, 2002). PRRS, for example, has been shown to decrease ADG (Sutherland, 2007). Coffey and Cromwell (1995) showed that during the nursery phase, pigs raised in a controlled environment compared to be raised on a commercial farm. It was hypothesized that the pigs raised in a controlled environment had a higher level of well-being due to fewer exposures to pathogens and therefore did not have to divert resources away from growth to mount frequent immune responses.

As previously stated, endotoxin challenge with LPS has been used often in research settings to simulate a bacterial insult. Sickness behaviors such as reduced feed intake, inactivity and fever are common responses to an LPS challenge (Johnson, 1994; Wright, 2000). It has been suggested that animals, including pigs, exhibit these sickness behaviors as a means to conserve energy, thereby redirecting it for the clearance of the pathogen. Carroll et al. (2001) showed that piglets challenged with a cold environment, 18 °C, are able to maintain their body temperature; however, challenging those same piglets with LPS resulted in piglets that could not mount a fever and instead suffered from hypothermia. This would indicate that the immune system requires a great deal of resources, often at the expense of other physiologic systems.

Summary

Establishing a system to monitor piglet well-being is of primary concern. Stressors such as mixing, cold, and infection can dramatically affect the overall well-being of young piglets, primarily at stressful periods including farrowing (Carroll, 2001) and weaning. The acute phase immune response to infection such as LPS is characterized by increases in several cytokines including IL-12 (Utsugi, 2009) as well as an increase in body core temperature and cortisol (Carroll, 2001). Behavioral changes are apparent in response to fluctuations in temperature as due to pathogens. Also, functional aspects of the immune system such as neutrophil chemotaxis or lymphocyte proliferation may be affected by alterations in ambient temperature, exposure to social stress (Tuchscherer, 1998), or infectious challenge. It is important to establish methods to improve piglet well-being. Thus, in Chapter 1, we examine whether feeding a plant-extract (capsicum) to sows during pregnancy can be an effective means to improve thermogenic capacity of neonatal piglets. However, the results from this study, along with previous data further indicate the importance of establishing an effective means to monitor pig well-being. In Chapter 2, we examined the effectiveness of monitoring body temperature following weaning, LPS challenge, and cold challenge, as a means of measuring well-being and to attempt to establish a model for monitoring piglet well-being.

CHAPTER 1: EFFECT OF SHORT- AND LONG-TERM FEEDING OF PLANT-EXTRACT (CAPSICUM) TO SOWS DURING GESTATION ON PIGLET BODY TEMPERATURE AND PHYSIOLOGICAL RESPONSES TO LPS CHALLENGE AT EITHER 24 OR 72 H OF AGE

ABSTRACT

Pre-weaning piglet mortality due to chilling is a major limiting factor of herd reproductive performance, thus a series of experiments were conducted to determine if feeding pregnant sows a standard gestation diet supplemented with capsicum (plant-extract) during gestation would improve neonatal piglet thermogenic and physiologic responses to lipopolysaccharide (LPS). In experiment 1, sows were randomly assigned a diet treatment: standard gestation diet (CONT) or standard diet supplemented with capsicum (TRT). The sows assigned TRT diet were switched 14 d prior to farrowing. In experiment 2, sows were randomly assigned to one of the 3 diet treatments: (CONT), TRT diet, or TRT diet fed for duration of gestation (TRT-LT). Eight piglets per litter were randomly assigned to either age treatment (24 h or 72 h) and 2 piglets were assigned to either saline or LPS (25 μg/kg of BW) challenge. Body temperature was recorded frequently via rectal and ear. Blood samples were taken 2 h post-injection. Data were analyzed using MIXED procedure of SAS. In experiment 1, piglets from sows fed TRT diet had greater rectal and ear temperatures ($P < 0.05$) than did piglets from sows fed CONT diet. Rectal and ear temperatures were greater ($P < 0.05$) for 72 h old piglets compared with 24 h piglets. Piglets from sows fed TRT diet had greater ($P = 0.08$) plasma IL-10 than did piglets from sows fed CONT diet. Plasma IL-12 was lower ($P = 0.07$) in piglets from sows fed TRT diet compared to piglets from sows fed CONT. Regardless of diet, plasma IL-10 and cortisol were greater ($P < 0.001$) in piglets

challenged with LPS compared with piglets injected with saline. Piglets challenged with LPS and from sows fed CONT diet tended to have greater (P = 0.14) plasma cortisol than did piglets from sows fed TRT diet. In experiment 2, there was a diet × LPS treatment × piglet age interaction for both rectal and ear temperatures. Those 24-h old piglets injected with LPS and from sows fed TRT diet had greater ($P < 0.05$) rectal temperature than did piglets from sows fed CONT diet. However, 72-h old piglets challenged with LPS and from sows fed TRT diet had lower ($P < 0.05$) rectal temperature compared with other treatment groups. Those piglets 24-h old and challenged with LPS and from sows fed TRT diet had greater ($P < 0.05$) ear temperature compared with piglets from sows fed TRT-LT diet. Piglets 72- h old from sows fed TRT-LT diet had lower ($P < 0.05$) ear temperature compared to all other dietary treatment groups. Also, among piglets treated with saline and from sows fed TRT diet ear temperature was greater ($P < 0.05$) compared with piglets from sows fed TRT-LT diet. Plasma cortisol was greater ($P <0.05$) among piglets injected with LPS and from sows fed TRT-LT diet compared with piglets from sows fed TRT diet. Plasma IL-10 was greater ($P <0.05$) for those piglets from sows fed CONT diet whereas plasma IL-12 was lower ($P <0.001$) in piglets from sows fed TRT diet compared with all other dietary treatments. Those ≤ 72 h of age piglets from sows fed TRT diet had greater IL-10 ($P < 0.01$) but lower IL-12 ($P < 0.01$) than did piglets from all other dietary treatments. Plasma IL-12 was greater ($P < 0.05$) in piglets that were injected with LPS compared with piglets injected with saline. These data indicate that feeding a gestation diet supplemented with the plant-extract capsicum to sows prior to farrowing has modulatory effects on the physiologic responses of their offspring, but capsicum supplemented diet does not modulate piglet thermogenic capacity.

INTRODUCTION

Pre-weaning piglet mortality is a major limiting factor of herd reproductive performance. Neonatal morbidity and mortality is a substantial economic loss to the swine industry and at times has reached 30% (English, 1984). In 1993, it was reported that about 15% of piglets born in the U.S. do not survive to weaning (Tubbs, 1993) and several factors attribute to this high neonatal loss including cold stress and infectious diseases. Neonatal susceptibility to thermal stressors is often a result of poor insulation due to low body fat and the inability of neonatal piglets to mount an effective febrile response to infective challenges (Heath, 1989).

Fever has long been recognized as a necessary aspect of the acute phase response of the immune system and is necessary to eliminate infective agents and reduce the negative consequences to the body (Kluger, 1991; Roberts, 1991). In pigs, low body fat and an immature immune system may contribute to the ineffective ability to thermoregulate or to mount an effective febrile response (Carroll, 2001; McCracken, 1981; Goelst, 1992). Moreover, piglets have a high effective environmental requirement, thus requiring a higher ambient temperature than older pigs. Management strategies or dietary manipulation that could result in a more robust piglet at birth could potentially improve neonate well-being and may have a significant economic impact on the swine industry.

Capsicum, a plant-extract derived from peppers has been shown to induce physiological changes that may offer thermogenic benefits. Previous unpublished research conducted by Archer Daniels Midland in cattle has demonstrated that feeding diets that contain capsicum increases peripheral blood flow, and others have reported an

effect on blood pressure in neonatal rats when treated with capsaicin, another plant-extract derived from peppers (Zeng, 2004). However, the effects of dietary inclusion of capsicum in swine diets have not been investigated

Pre-weaning piglet mortality due to low body weight and fat reserves and a diminished thermoregulatory capacity of neonates is a limiting factor of herd reproductive productivity, thus an experiment was conducted to determine the modulating effects of feeding sows a standard gestation diet supplemented with capsicum on their piglets' body temperature and physiologic responses to lipopolysaccharide (LPS) challenge. Therefore, the objectives of this study was to determine if feeding a gestation diet supplemented with capsicum to sows during late-gestation (TRT), or throughout gestation (TRT-LT), had an impact on (1) piglet birth body weight and temperature, and (2) piglet body temperature, cytokine concentrations, and total plasma cortisol levels in response to LPS challenge at either 24 or 72-h of age.

MATERIALS AND METHODS

Animals

Two experiments were conducted during the summer of 2005 (Experiment 1) and 2006 (Experiment 2) at the Decatur Research Center, Decatur, IN. Multiparous crossbred sows were used in this study, and standard management practices for both the gestational and lactational periods were followed. All sows received 2.3 kg of feed per day until ~d 95 of gestation, and then sows received 3.2 kg of experimental or control diet per day until farrowing. However, in experiment 2, a third diet treatment was added in which sows were fed the experimental diet throughout the entire gestational period.

The amount of feed was adjusted to maintain a sow body condition score of 3. Upon farrowing, all sows were switched to a standard lactation diet for the duration of the lactational period. Sows received water ad libitum. Cross-fostering did occur, but piglets were only cross-fostered to sows that were on same treatment. At birth, piglets were dried-off, sexed, identified, weighed and collected into a well-bedded, draft-free basket. Rectal temperature was taken within 5 min of birth. After 3 h of parturition or 10 piglets were born, the piglets were returned to the sow and allowed to suckle. Eight piglets were identified from within each litter to be used in the experiment. Sow rectal temperature was recorded at the end of the farrowing process.

Experimental Procedure

In experiment 1, all sows were fed a standard gestation diet until 14-day prior to the anticipated average farrowing date. Fourteen days prior to farrowing, sows were randomly assigned to remain on the standard gestation diet (CONT) or switched to the standard gestation diet supplemented with 25 ppm of capsicum (TRT). In experiment 2, sows were randomly assigned to 1 of 3 dietary treatments: standard gestation diet (CONT), standard gestation diet supplemented with capsicum fed short-term (TRT) or standard gestation diet supplemented with capsicum fed long-term (TRT-LT).

Eight piglets from each litter were randomly selected and assigned to age, treatment, and LPS-challenge. Four piglets were randomly assigned to either 24-h or 72-h age treatment. Within each age group, 2 piglets were assigned either saline or LPS injection. Piglets assigned to 24-h age treatment were removed at 13.5 h of age and those assigned to 72-h of age were removed at 61.5 h of age from their respective sows. Two

piglets from the same litter and same challenge treatment (either saline or LPS) were kept together during the experimental period. Piglets were placed in a draft-free, temperature controlled "plastic tub" within a standard nursery room. The tub temperature was ~93°F and tubs were kept 4" off the floor. Infrared thermometer was used to determine tub temperature. Following a 2-h adjustment period, piglets received an intraperitoneal injection of saline or LPS (25 μg/kg of BW; Sigma).

Rectal and tympanic temperatures were taken at 0 and 30 min and at 1, 1.5, 2, 2.5, 3, 4, 5, 6, and 8 h post-injection. Blood was also taken at 0 and 120 min post-injection via jugular puncture. At the end of the experimental period, piglets were returned to the sow.

Blood Sampling

Pigs were held in a supine position and < 5 mL of blood was collected from the anterior jugular vena cava. Blood samples were spun (900 x g, 20 min., 4 ºC), plasma removed and stored at -80 ºC until analyzed for total plasma cortisol and cytokines.

Plasma Cortisol

Plasma samples were assayed for cortisol using a Coat-A-Count cortisol kit, following the manufacturer's protocol (Siemens Medical Solutions Diagnostics, Los Angeles, CA). Briefly, in duplicate, 25 μL of sample or standard was added to antibody-coated tubes. Radiolabeled (I^{125}) cortisol was added to tubes and incubated 45 min at 37 ºC in a water bath. The liquid phase was decanted and radioactivity counted using a gamma counter (Cobra II, Perkin-Elmer, Boston, MA). A standard curve based on 0, 10, 50, 100, 200, and 500 μg/mL was used. A high (200 μg/mL) and low (10 μg/mL) control were used to determine intra- and interassay CV. Intra- and interassay CV was 25.1 and

(4.40), respectively. The minimal detectable cortisol concentration was approximately 2 ng/mL.

Cytokines

Peripheral levels of interleukin-10 and -12 were measured using a commercially available ELISA kit (R&D Systems, Minneapolis, MN) following manufacturer protocol. Briefly, plasma was diluted and standards were added in duplicate to 96-well antibody coated plates and incubated on a shaker for 2 hours. Plates were aspirated, washed, and conjugate was added to each well and plates were again incubated. Following a second aspiration, substrate solution was added to each well and the plate was protected from light and incubated on the bench for 30 min. Stop solution was added and plates were read on a spectrometer (Multiskan Plus, Fisher Scientific, Houston, TX). Optical density was analyzed and concentrations were converted to pg/mL. Intra- and interassay CV for IL-10 was 11.30 and 3.41 respectively. Intra- and interassay CV for IL-12 was 46.0 and 4.50 respectively.

Statistical Analysis

Statistical analyses were conducted using SAS version 9.1 (SAS Inst. Inc., Cary, NC). The model contained the fixed effects of DIET, AGE, and LPS treatment (LPS) and all interactions including DIET x AGE, LPS x AGE, and DIET x LPS. All variables were tested for departures from normality using Proc Univariate. Interleukins -10 and -12 as well as cortisol were normalized by natural log transformation. A Proc Mixed procedure with repeated measures was used to analyze all temperature measures (SAS). The random variable, pig, was included in the model for temperature measures. A

logarithmic transformation was applied to IL-10, IL-12, and cortisol. For all traits that were subjected to logarithmic transformation, means presented in tables and graphs are non-transformed means. Data was considered significant at $P < 0.05$, and trends were discussed at $P < 0.10$.

RESULTS

Experiment 1: Short-term feeding effects of capsicum on body temperature and immune function

Rectal and Ear Temperature

Ear and rectal temperatures were affected by an LPS treatment x AGE interaction ($P < 0.001$; Table 1-1). Piglets from sows fed the TRT diet had greater ($P < 0.01$; Table 1-2) rectal temperature than did piglets from sows fed CONT diet. Piglets from sows fed TRT diet had greater ($P < 0.01$; Table 1-2) ear temperature than did piglets from sows fed CONT diet. Regardless of diet or LPS treatment, piglets 72-h of age had greater ($P < 0.001$) rectal (103.1 °F ± 0.1) and ear temperatures (102.81 °F ± 0.09) than did piglets 24-h of age (100.5 °F ± 0.1 and 100.6 °F ± 0.1 respectively).

Plasma Cortisol and Cytokines

Plasma cortisol tended to be affected by an LPS treatment x AGE interaction ($P = 0.108$; Table 1-1). Diet or piglet age did not affect plasma cortisol; however, LPS-injected piglets (133.2 ng/mL ± 6.5) had greater ($P < 0.001$) cortisol levels than did those piglets injected with saline (51.0 ng/mL ± 6.4). There was a tendency for a DIET × LPS interaction for both plasma IL-10 and IL-12 ($P < 0.1$; Table 1-3), with LPS-injected piglets from sows fed TRT diet had greater IL-10 (115.0 pg/mL ± 16.3) than did piglets

from sows fed CONT (85.2 pg/mL ± 15.4). Plasma IL-12 tended ($P = 0.065$) to be greater among LPS-injected piglets from sows fed CONT (249.9 pg/mL ± 24.6) than for piglets from sows fed TRT diet (166.1 pg/mL ± 25.5). Plasma IL-10 was greater ($P < 0.001$) in piglets challenged with LPS (100.1 ± 11.2) compared to those piglets injected with saline (30.7 ± 11.3). Age of piglet had an effect on plasma IL-10 when challenged with LPS ($P < 0.1$; Table 1-1). Piglets 24-h of age and challenged with LPS (131.0 pg/mL ± 18.0) had greater ($P < 0.05$) plasma IL-10 levels than did those 72 h-old piglets injected with LPS (69.2 pg/mL ± 13.3).

Performance of Sows to Dietary Treatment

Piglet body weight at birth was affected by sow dietary treatment ($P < 0.05$; Table 1-4). Sows fed TRT diet gave birth to heavier piglets (1.35 kg ± 0.07) than did sows fed CONT (1.18 kg ± 0.10). Sows fed TRT diet tended ($P = 0.051$; Table 1-4) to have fewer piglets born alive per litter compared to sows fed CONT. Piglet rectal temperature at birth and sow temperature were not affected by sow dietary treatment.

Experiment 2: Short-term vs. long-term feeding effects of capsicum on neonatal piglet temperature and immune function

Rectal and Ear Temperatures

There was a DIET x LPS x AGE effect for rectal temperature ($P < 0.05$; Figure 1-1). Piglets ≤ 24 h old injected with LPS from sows fed TRT diet (101.6 °F ± 0.09) had greater rectal temperature than did piglets from sows fed CONT (101.3 °F ± 0.05). However, piglets injected with LPS and ≤ 72 h of age from sows fed TRT diet (102.4 °F ± 0.08) had lower rectal temperature than did both piglets from sows fed either CONT (103.2 °F ± 0.05) or TRT-LT diet (103.16 °F ± 0.07). Saline-injected piglets and ≤ 72 h of age from sows fed TRT-LT diet had the greatest rectal temperature (103.1 °F ± 0.07) compared to those piglets from sows fed CONT (102.5 °F ± 0.05) or TRT diet (102.3 °F ± 0.08).

Rectal temperature was also affected by DIET ($P < 0.05$; Table 1-6). Piglets from sows fed a TRT-LT diet (102.2 °F ± 0.03) had greater rectal temperature than did piglets from sows fed TRT diet (101.9 °F ± 0.04). Piglets from sows fed TRT-LT diet also had greater rectal temperature than did piglets from sows fed CONT (102.3 °F ± 0.03).

Piglets injected with LPS (102.2 °F ± 0.03) had greater rectal temperature compared with those piglets injected with saline (101.9 °F ± 0.03). Piglets ≤ 72 h old (102.8 °F ± 0.03) had greater rectal temperature than did piglets that were ≤ 24 h of age (101.3 °F ± 0.03).

There was a DIET x LPS interaction ($P < 0.05$; Table 1-6), with those piglets injected with saline and from sows fed TR-LT diet having the greatest ($P < 0.05$) rectal

temperature (102.2 °F ± 0.01) compared to piglets from sows fed either CONT (101.8 °F ± 0.04) or TRT diet (101.8 °F ± 0.06). Among those piglets injected with LPS, rectal temperature was lowest ($P < 0.01$) in piglets from sows fed TRT diet (102.0 °F ± 0.06) compared to piglets from sows fed CONT (102.2 °F ± 0.04) or TRT-LT (102.3 °F ± 0.05) diets.

There was a DIET x LPS x AGE interaction for ear temperature ($P < 0.01$; Figure 1-2). Piglets ≤ 24 h of age from sows fed TRT diet and injected with LPS had greater ear temperature (100.6 °F ± 0.12) compared to piglets from sows fed either CONT (99.3 °F ± 0.07) or TRT-LT (98.9 °F ± 0.11) diets. Piglets ≤ 72 h of age from sows fed TRT-LT and injected with LPS (99.0 °F ± 0.13) had lower ear temperature compared to either piglets from sows fed CONT (99.8 °F ± 0.08) or TRT (100.0 °F ± 0.11) diets. Both 24 and 72 h-old piglets from sows fed TRT diet and injected with saline had greater ear temperature (100.4 °F ± 0.12; 99.9 °F ± 0.11 respectively) than did piglets from sows fed TRT-LT (98.7 °F ± 0.10; 98.8 °F ± 0.13 respectively) diet.

Diet affected ear temperature ($P <0.001$; Table 1-5), piglets from sows fed TRT diet had greater ear temperature (100.2 °F ±0.06) compared with piglets from sows fed TRT-LT (98.9 °F ± 0.06) diet. Statistically, piglets from sows fed TRT diet also had greater ear temperature than did piglets from sows fed CONT (99.3 °F ±0.04).

Plasma Cortisol and Cytokines

LPS-injected piglets (87.5 ng/mL ± 8.3) exhibited greater plasma cortisol ($P <0.05$) compared to piglets injected with saline (24.8 ng/mL ± 8.6). There was also a LPS x AGE interaction for plasma cortisol ($P < 0.05$). Piglets that were ≤ 24 h of age

and injected with saline (34.45 ng/mL ± 10.28) had greater plasma cortisol than did 72 h old piglets (15.1 ng/mL ± 12.6).

Plasma IL-10 was greatest ($P < 0.01$; Table 1-5) in piglets from sows fed CONT diet (67.7 pg/mL ± 13.6) compared to piglets from sows fed TRT (46.0 pg/mL ± 18.80) or TRT-LT (36.7 pg/mL ± 17.6) diets. Plasma IL-12 was lowest ($P < 0.001$; Table 6) in piglets from sows fed TRT diet (150.9 pg/mL ± 27.4) compared to piglets from sows fed CONT (241.0 pg/mL ± 19.3) or TRT-LT (269.6 pg/mL ± 22.9) diets. LPS-injected piglets (249.6 pg/mL ± 18.4) had greater ($P < 0.05$) plasma IL-12 levels than did saline-injected piglets (191.4 pg/mL ± 19.8). Independent of LPS treatment, both diet and piglet age affected IL-10 ($P < 0.01$) and IL-12 ($P < 0.01$) levels; for piglets ≤ 24 h old, plasma IL-10 was greatest in those piglets from sows fed CONT diet (101.8 pg/mL ± 16.1) compared to piglets from sows fed TRT (33.3 pg/mL ± 22.7) or TRT-LT (66.4 pg/mL ± 20.3) diets. Plasma IL-10 was greater ($P < 0.05$) in piglets ≤ 72 h of age from both sows fed CONT (33.6 pg/mL ± 21.9) or TRT (58.8 pg/mL ± 30.0) diets compared to piglets from sows fed TRT-LT (6.9 pg/mL ± 28.7) diet. Plasma IL-12 was lowest among 72 h old piglets from sows fed TRT diet (92.0 pg/mL ± 35.9) compared to piglets from sows fed CONT (303.3 pg/mL ± 28.9) or TRT-LT (333.7 pg/mL ± 32.1) diets. There was a DIET x LPS interaction for plasma IL-10 levels of piglets ($P < 0.05$; Table 1-6).

Performance of Sows to Dietary Treatment

Piglets from sows fed TRT diet (97.2 °F ± 0.5) had lower rectal temperature ($P < 0.001$; Table 1-4) than did piglets from sows fed CONT (99.3 °F ± 0.3) or TRT-LT (99.2

°F ± 0.5) diets. Dietary treatment had no effect on the number of piglets born alive, sow rectal temperature at end of farrowing, or piglet birth weight.

DISCUSSION

Maintaining body core temperature is difficult for neonatal pigs shortly after birth. It would be highly beneficial if sows could be fed a diet during gestation that would improve the thermogenic capacity of her piglets. Recent unpublished data from an Archer Daniels Midland experiment indicated that feeding a plant-extract called capsicum can increase blood flow to the mammary region in cattle. Another study showed that capsaicin, a lipophilic toxin produced in chili peppers that is similar to capsicum, also has direct effects on the circulatory system. A recent study by Zeng et al. (2004) showed that subcutaneous injection of 50 mg/kg of capsaicin to newborn Wistar rat pups for 2 days resulted in elevated blood pressure. To test our theory that feeding a diet containing capsicum to sows would improve the thermogenic capacity of her piglets, we conducted an LPS challenge and compared the physiologic responses of piglets at 24 h and 72 h of age.

In experiment 1, LPS-injected piglets regardless of diet at 72-h of age had greater rectal and ear temperature responses compared to 24-h pigs. Also, in experiment 2, of those piglets injected with LPS and from sows fed TRT diet, piglets 24-h of age and had lesser rectal temperatures compared to piglets at 72-h of age. These data agree with previous studies (Le Dividich 1983; Herpin, 2002) that showed piglets were not able to mount a febrile response to LPS challenge until about 3 days of life. There was also little if any difference in rectal temperature between piglets from sows fed CONT diet and

TRT diet at 24-h of age in both experiments, hence dietary treatment did not alter baseline body core temperature of a 24-h old piglet.

Gestation diet did affect ear temperature in response to LPS injection in experiment 2. At both ≤ 24 and ≤ 72 hours of age, LPS and saline-injected piglets from sows fed TRT diet had greater ear temperatures than did piglets from other dietary groups. Ear thermometers have proven to be a reliable measure of body core temperature in humans, but use of ear thermometers with neonatal piglets has not been evaluated. Perhaps the ear thermometers are not accurate measures of body core temperature, but instead record a peripheral temperature. Hence, perhaps exposure to capsicum in utero did, in fact, increase blood flow to the periphery as earlier unpublished Archer Daniels Midland reports suggest.

Gestation diet supplemented with capsicum had no effect on plasma cortisol, although in experiment 1 the plasma cortisol response to LPS injection seemed to be affected by piglet age. Piglets ≤ 24 h of age and injected with LPS tended to have greater plasma cortisol (144.0 ng/mL ± 9.9) than LPS-injected piglets ≤ 72 h of age (122.4 ng/mL ± 8.5). As expected, these experiments confirm previous findings that the HPA axis is indeed functional in piglets 24 h of age (Carroll, 2001); however, we failed to show a difference between dietary treatment groups, suggesting that feeding a diet containing capsicum to a sow will not have a significant effect on the HPA response of her piglets to a simulated infectious challenge. Why piglets at 24 h of age had an exaggerated HPA response to LPS injection is not clearly understood, but it correlates well with previous data showing that high neonatal mortality and morbidity is a common end result of infective challenges (Heath, 1989). The cortisol response to LPS injection

of 24 h pigs compared to 72 h pigs reinforces how important it is to produce a more robust pig at birth that is less susceptible to stress.

To examine the overall immune response to LPS we measured plasma IL-12, an inflammatory cytokine released by macrophages (Webel, 1997; Scheicher, 1995) as well as plasma IL-10 levels, an indicator of anti-inflammatory, or regulatory, immune function. Feeding sows diet supplemented with capsicum for 14 days prior to farrowing impacted piglet cytokine profile in response to LPS. These piglets had greater IL-10 but lower IL-12 compared to those piglets injected with LPS from sows fed CONT diet. Acute administration of capsaicinoids, dihydrocapsaicin, and capsaicin decreased the number of acquired immune cells (Akimoto, 2009). Hence our data potentially support that the consumption of plant extracts, such as capsicum, by sows can affect the leukocyte population and cytokine profile of the offspring. Perhaps 100 days of gestation to farrowing is a period of time when the developing immune system is susceptible to change. While we only measured interleukin-12 to gauge the inflammatory response to LPS, the decreased levels of IL-12 and often increased levels of IL-10 in piglets from sows fed TRT diet could be indicative of a more developed acquired immune system.

Another possibility for a shift in cytokine profile would be an increase in cortisol. Glucocorticoids have been shown to drive a shift towards Th2 cytokines (Blotta, 1997); however, cortisol was relatively unaffected by diet. Therefore, the shift in immune profile towards anti-inflammatory cytokines and humoral defense might be due to diet directly. A balanced state of the immune system is important for adequately responding to pathogens (Elenkov, 1999). Feeding a diet with capsicum for 2 weeks prior to farrowing seems to favor a Th2 shift and thus an immune-protective state.

Feeding diet supplemented with capsicum to sows resulted in heavier piglets at birth in one of the trials. However, sows fed TRT diet tended to give birth to fewer live piglets. Piglets born with greater body mass would have less surface area relative to size. This may give these piglets an advantage being heavier. They likely are born with more brown fat to help with temperature regulation which would decrease piglet mortality due to chilling. On the contrary, this might not be desirable if it comes at the cost of the number of piglets.

In summary, it seems that feeding a diet including capsicum to sows did seem to affect piglet cytokine profiles though it is unclear whether the changes in IL-10 and IL-12 levels would be beneficial to the neonatal pig. The benefit of farrowing a heavier pig after feedingInclusion of capsicum in sow gestation diet does not appear to enhance thermogenic ability of neonatal piglets nor does it affect piglet birth performance. Inclusion of capsicum in sow diets may provide some benefits to the neonatal piglet, but further research is necessary to elucidate those benefits.

Table 1-1. Effects of age (24 h) or (72 h) after parturition on piglet physiologic measures during acute LPS challenge in Experiment 1.[1]

	Treatment x Age				P-value
Item	24 h		72 h		Treatment x Age
	Saline	LPS	Saline	LPS	
Ear, °F	100.84 ± 0.13[c]	100.28 ± 0.13[d]	102.66 ± 0.13[b]	102.95 ± 0.13[a]	<.001
Rectal,°F	100.75 ± 0.15[c]	100.32 ± 0.15[d]	102.86 ± 0.15[b]	103.25 ± 0.15[a]	<.001
Cortisol, ng/mL	44.90 ± 15.07	144.00 ± 49.20	57.08 ± 58.27	122.37 ± 39.24	0.108
IL-10, pg/mL	45.24 ± 18.04	131.00 ± 18.04	16.06 ± 13.69	69.21 ± 13.32	0.073
IL-12, pg/mL	194.27 ± 25.89	206.46 ± 26.80	187.64 ± 23.80	209.56 ± 23.16	0.159

[a-d]Within a row, means without common superscript differ. ($P < 0.05$)
[1]Means (non-transformed) ± SE

Table 1-2: Effects on piglet physiologic measures following feeding capsicum treated gestation diet to sows for 14 days prior to farrowing.[1]

	Diet		P-value
Item	Control	TRT	Treatment
Ear, °F	101.6 ± 0.1[b]	101.8 ± 0.1[a]	0.002
Rectal, °F	101.6 ± 0.1[b]	102.0 ± 0.1[a]	0.001
Cortisol, ng/mL	95.1 ± 7.4	89.1 ± 5.4	0.566
IL-10, pg/mL	58.2 ± 11.3	72.5 ± 11.2	0.113
IL-12, pg/mL	216.4± 17.6	182.6 ± 17.7	0.139

[a-b]Within a row, means without common superscript differ. ($P < 0.05$)
[1]Means (non-transformed) ± SE

Table 1-3. Effects on piglet physiologic measures following feeding capsicum treated gestation diet to sows for 14 days prior to farrowing and acutely challenging piglets with LPS.[1]

	LPS Treatment x Diet				P-value
Item	Control		TRT		LPS x Diet
	Saline	LPS	Saline	LPS	
Ear, °F	101.7 ± 0.1	101.5 ± 0.1	101.9 ± 0.1	101.7 ± 0.1	0.749
Rectal, °F	101.6 ± 0.2	101.6 ± 0.2	102.0 ± 0.1	101.9 ± 0.1	0.703
Cortisol, ng/mL	48.5 ± 33.4	141.7 ± 49.57	53.5 ± 48.64	124.7 ± 41.3	0.241
IL-10, pg/mL	31.3 ± 16.6	85.2 ± 15.4	30.0 ± 15.4	115.0 ± 16.3	0.084
IL-12, pg/mL	182.8 ± 25.2	249.9 ± 24.6	199.1 ± 24.6	166.1 ± 25.5	0.065

[a-b]Within a row, means without common superscript differ. ($P < 0.05$)

[1]Means (non-transformed) ± SE

Table 1-4. Piglet body weight (PBW), piglet rectal temperature (pRectal), sow rectal temperature (sRectal), and number of piglets born alive (PBA) at farrowing following feeding of capsicum treated gestation diet for 14 days prior to farrowing or throughout gestation.[1]

	Diet		P-value
Item	Control	TRT	Treatment
PBW, kg	1.18 ± 0.1[b]	1.35 ± 0.07[a]	0.013
pRectal, °F	96.8 ± 0.4	97.2 ± 0.3	0.509
sRectal, °F	102.0 ± 0.3	102.2 ± 0.2	0.720
PBA	13.2 ± 1.1	10.4 ± 0.7	0.051

[a-b]Within a row, means without common superscript differ. ($P < 0.05$)
[1]Means (non-transformed) ± SE

Table 1-5. Piglet body weight (PBW), piglet rectal temperature (pRectal), sow rectal temperature (sRectal), and number of piglets born alive (PBA) at farrowing following feeding of capsicum treated gestation diet for 14 days prior to farrowing or throughout gestation.[1]

	Diet			P-value
Item	Control	TRT	TRT-LT	Treatment
PBW, kg	1.45 ± 0.2	1.38 ± 0.20	1.37 ± 0.34	0.534
pRectal, °F	99.3 ± 0.3[a]	97.2 ± 0.5[b]	99.2 ± 0.5[a]	0.001
sRectal, °F	102.0 ± 1.1	101.7 ± 1.7	102.2 ± 0.8	0.523
PBA	11.1 ± 2.7	11.6 ± 2.4	11.2 ± 3.4	0.854

[a-b]Within a row, means without common superscript differ. ($P < 0.05$)
[1]Means (non-transformed) ± SE

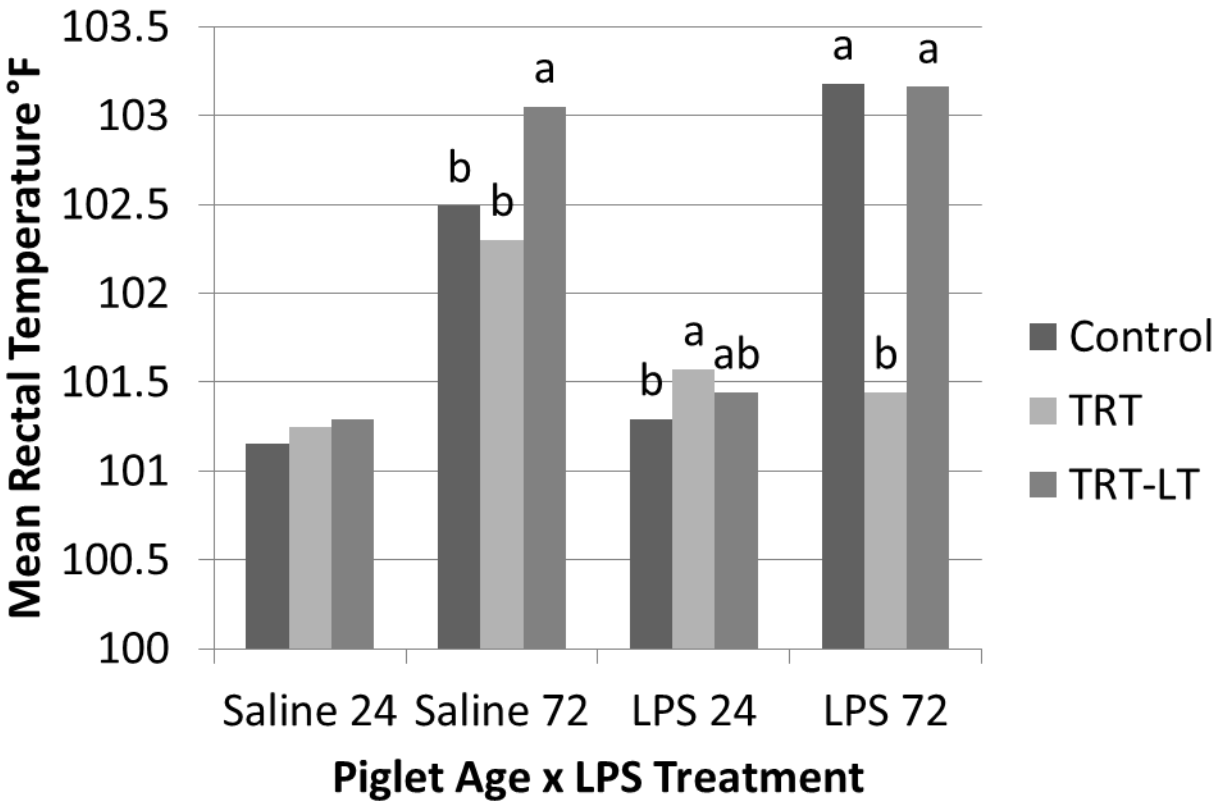


Figure 1-1. Least square means of effect of piglet age and gestation diet on piglet rectal temperature during acute LPS challenge.

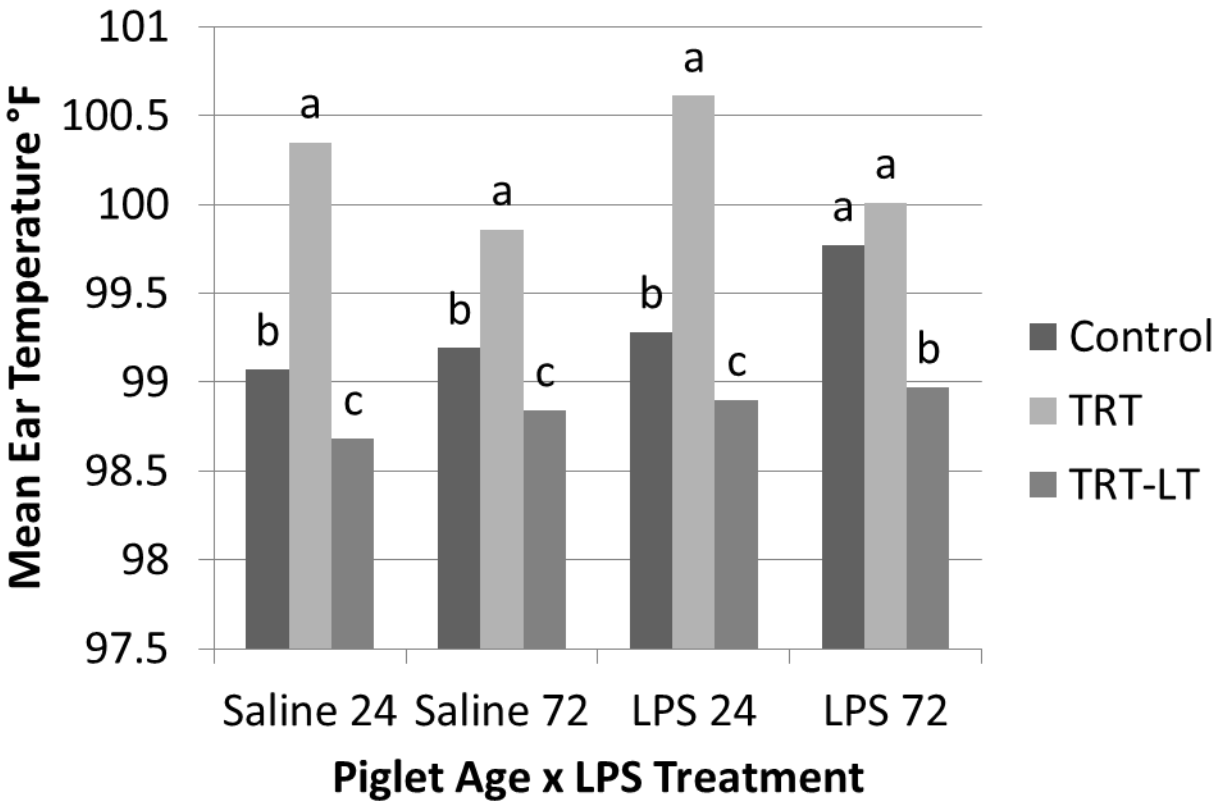


Figure 1-2. Least square means of effects of piglet age and gestation diet on piglet ear temperature during acute LPS challenge.

Table 1-6. Effect on piglet physiologic measures following feeding of capsicum treated gestation diet to sows for 14 days prior to farrowing or throughout gestation.[1]

	Diet			P-value
Item	Control	TRT	TRT-LT	Diet
Ear, °F	99.3 ± 0.1[b]	100.2 ± 0.1[a]	98.9 ± 0.1[c]	0.001
Rectal °F	102.0 ± 0.1[a]	101.9 ± 0.1[c]	102.2 ± 0.1[b]	0.001
Cortisol, ng/mL	59.1 ± 5.5	40.3 ± 13.8	67.0 ± 10.2	0.137
IL-10, pg/mL	67.7 ± 13.6[a]	46.0 ± 18.8[b]	36.7 ± 17.6[b]	0.005
IL-12, pg/mL	241.0 ± 19.3[a]	150.9 ± 27.4[b]	269.6 ± 22.9[a]	0.001

[a-c]Within a row, means without common superscript differ. ($P < 0.05$)

[1]Means (non-transformed) ± SE

Table 1-7. Effects on piglet physiologic measures following feeding capsicum treated gestation diet to sows for 14 days prior to farrowing or through gestation and acutely challenging piglets with LPS.[1]

	Diet x LPS treatment						P-value
Item	Control		TRT		TRT-LT		LPS x Diet
	Saline	LPS	Saline	LPS	Saline	LPS	
Ear, °F	99.1 ± 0.1	99.5 ± 0.1	100.1 ± 0.1	100.3 ± 0.1	98.8 ± 0.1	98.9 ± 0.1	0.173
Rectal, °F	101.8 ± 0.1[b]	102.2 ± 0.1[a]	101.8 ± 0.1[b]	102.0 ± 0.1[b]	102.2 ± 0.1[a]	102.3 ± 0.1[a]	0.002
Cortisol, ng/mL	29.2 ± 8.0	89.1 ± 7.5	16.7 ± 19.3	64.0 ± 19.3	28.6 ± 14.8	109.4 ± 13.7	0.508
IL-10, pg/mL	87.6 ± 19.0	37.7 ± 25.4	47.8 ± 19.4	24.1 ± 7.1	54.4 ± 27.8	49.2 ± 22.4	0.058
IL-12, pg/mL	218.6 ± 103.8	263.3 ± 95.3	101.1 ± 94.6	200.7 ± 170.2	254.5 ± 169.8	284.7 ± 104.8	0.466

[a-b]Within a row, means without common superscript differ. ($P < 0.05$)

[1]Means (non-transformed) ± SE

CHAPTER 2: TEMPERATURE AND CORRELATED PHYSIOLOGIC RESPONSES AS INDICATORS OF WELL-BEING

ABSTRACT

Young pigs are often subjected to stressors that may impact their well-being particularly at weaning. Pigs respond to stress through a variety of physiologic and behavioral changes to maintain or return to homeostasis. Based on preliminary studies, implantable microchip thermo-sensors were implanted in the jugular and/or flank regions of young pigs. In three experiments, weanling pigs were exposed to different stressors. Experiment 1, a preliminary study, was designed to further evaluate the reliability of the implantable sensors temperature in various body regions using acute LPS challenge and experiment 2 was to develop a chronic LPS model. Finally, experiment 3 was to assess the effects of cold stress, social status, and LPS challenge on temperature, cortisol, behavior, and immune status of young pigs. Data were analyzed with MIXED procedure of SAS.

In experiment 1, littermates were challenged intraperitoneally with either saline or LPS (25 ug/kg). Jugular and flank temperatures were measured via implantable sensors and rectal using digital thermometer. Rectal temperature was greater ($P < 0.05$) in LPS-injected pigs compared to saline-injected pigs at 45 min until 4 hours post-injection. Jugular temperature was also greater ($P < 0.05$) in LPS-injected pigs compared to saline-injected pigs but not until 2 h post-injection.

In experiment 2a, weaned pigs (n=24) were video recorded and social status was determined. Pairs of pigs were injected with either saline or LPS on d 0, 2, 4, and 7. On d 0, 2, and 4 pigs were bled prior to injection and at 1, 3, and 4-h post-injection. In

experiment 2b, pigs (n=22) were mixed and social status was determined. Pairs of pigs were injected with either peanut oil or LPS + peanut oil (LPS-O) on d 0, 3, and 6. Pigs were bled at 0 and 45 min, and at 1.5, 4, 8, 24, and 48 h.

Rectal and jugular temperatures were similar between pigs injected with LPS or saline; however, pigs injected with LPS-O had greater ($P < 0.05$) rectal and jugular temperatures at 2, 4, 8, and 24 h, post-injection compared to pigs injected with peanut oil. LPS-O injected pigs had greater ($P < 0.05$) rectal and jugular temperatures than did peanut oil-injected pigs on d 0, but not on d 3 and 6 post-injection.

Percentage of neutrophils was greater ($P < 0.05$) in LPS-injected pigs compared to SALINE-injected pigs at 4 h post-injection. Pigs injected with LPS-O also had greater ($P < 0.05$) percentage of neutrophils at 4 h as well as at 8 h compared to pigs injected with peanut oil. Pigs injected with saline and peanut oil alone had greater ($P < 0.05$) lymphocytes at 4-h post-injection compared with pigs injected with LPS and LPS-O, respectively.

In experiment 2a, IL-12 was greater ($P <0.05$) in LPS-injected pigs on d 0 than saline-injected pigs and no differences were detected between treatment groups for IL-12. Moreover, plasma cortisol was greater ($P < 0.05$) in LPS-injected pigs at 3 h post-injection compared to saline-injected pigs. However, in experiment 2b, piglets injected with LPS-O tended to have greater ($P = 0.09$) plasma cortisol at 1.5 h and 4 h compared to peanut oil-injected pigs on d 0 only. In experiment 2b, dominant pigs tended to have greater plasma cortisol compared to submissive pigs, and neutrophil chemotaxis to both c5a and IL-8 were greater ($P < 0.05$) in submissive pigs than in dominant pigs.

Moreover, chemotaxis tended to be greater ($P < 0.10$) in submissive pigs injected with LPS-O compared to dominant pigs injected with LPS-O.

In experiment 3, there were few interactions between cold and mixing stress. Pigs subjected to cold stress at 2 h and 4 h after mixing had lower ($P < 0.05$) jugular, whereas rectal and jugular temperatures were lower ($P < 0.05$) at 2 h and 4 h for pigs kept in COLD compared to TNT. For immune status, there were no differences. However, plasma cortisol levels were greater among pigs challenged with LPS and kept in COLD ($P < 0.05$). Also, intermediate pigs challenged with LPS had greater ($P < 0.05$) rectal temperature compared to dominant and submissive pig. Rectal ($P < 0.05$) and jugular ($P < 0.10$) were greater in pigs injected with LPS at 4 h post-injection compared to pigs injected with saline. Among cold stressed pigs, dominant and submissive pigs injected with LPS had greater ($P < 0.05$) jugular temperature than did intermediate pigs.

Pig behavior was affected by LPS treatment, cold stress, and dominance ($P < 0.05$). At 1 h and 2 h post-injection, LPS-injected pigs spent less time ($P < 0.05$) drinking than did saline-injected pigs. Dominant pigs spent more time ($P < 0.05$) drinking than did intermediate pigs. Eating behavior was also affected by dominance x treatment x ambient temperature ($P < 0.05$). Pigs injected with LPS ate less ($P < 0.05$) than saline-injected pigs, whereas pigs in COLD spent greater time eating ($P < 0.05$) compared to pigs in TNT. Pigs injected with LPS fought less ($P < 0.05$) compared to pigs injected with saline. Stand behavior was also affected by LPS; with those pigs injected with LPS spending less ($P < 0.05$) time standing compared with saline-injected pigs. Also, pigs kept in COLD spent more time ($P < 0.05$) standing than did pigs in TNT.

There was dominance x treatment x ambient temperature interaction for lying behavior; DOM (573.8 sec/h ± 171.3) and SUB (847.4 sec/h ± 171.3) pigs injected with LPS and kept in COLD spent more time ($P < 0.05$) lying in contact with both wall and pig than did INT pigs (0.67 sec/h ± 171.3). Conversely, of pigs injected with LPS and kept in COLD, the INT pigs (985.0 sec/h ± 171.0) spent more time ($P < 0.05$) lying in contact with pig compared to SUB pigs (400.2 sec/h ± 171.0). Pigs kept in COLD spent more ($P < 0.01$) time sitting in contact with pig at 1, 2, 3, and 4 h post-injection compared to pigs kept in TRT. There was an LPS treatment x ambient temperature x time interaction ($P < 0.05$) for sitting in contact with pig behavior.

Body temperature of weanling pigs appears to be a relatively good indicator of exposure to stressful situations. However, changes in body temperature do not necessarily indicate that a pig's well-being is compromised, as they could be exhibiting a normal physiologic response to a stressor that may enable them to cope. When challenged repeatedly with LPS, pigs responded initially with an increase in body temperature, but subsequent injections resulted in a blunted physiologic response. This individual tolerance was not altered by delivery system of LPS. Plasma cortisol levels and immune parameters were dependent on stressor. In summary, pig body temperature in conjunction with behavior observations could be an easy and effective means of monitoring changes in pig well-being.

INTRODUCTION

Weaning is a common stressful event that results in abrupt social, nutritional, and environmental changes that causes an "acute" stress response. Increased morbidity and mortality is often the result of this stress response which is an indication of decreases in piglet well-being (Puppe, 1997). In conventionally-raised pigs, weaning has a negative effect on growth rate following mixing and relocation (Davis, 2006). Weaning, as well as thermal stressors, have negative effects on immune function (Blecha, 1981; Blecha, 1983; Blecha, 1985). While more physiologically competent than neonatal pigs, weanling pigs are still sensitive to environmental changes and possess a relatively naïve immune defense against common pathogens. Cytokine levels, including interleukin-2, have been shown to decrease following weaning, an indication that the immune system is impaired at this time (Bailey, 1992). This impairment is further supported by Wattrang et al. (1998) in which they reported a higher prevalence of viral and bacterial infections following compared to before weaning as indicated by increased interferon-alpha, higher circulating neutrophils, and decreased IL-2.

Body temperature is regulated primarily by a control center in the hypothalamus (Germann, 2002) which activates both behavioral and physiologic responses in order to maintain a homeostatic temperature. It is necessary for homeothermic animals such as pigs to maintain their body temperature to support normal body functions. While slight diurnal variations in body temperature are common in pigs (Hanneman, 2005), wide divergences from the normal "set point" can be indicative of problems such as fever during infection or social stress following physical exertion during mixing. One model used to induce fever is challenge with bacterial lipopolysaccharide (LPS). Not only does

it reliably induce increases in body core temperature (Carroll, 2001), but it is an appropriate stressor at weaning when fecal shedding of *E. coli* has been shown to increase (Jones, 2001).

Lipopolysaccharide challenge has long been known to activate the immune system and other physiologic responses. Cortisol and ACTH are both increased in response to LPS challenge while mitogen induced lymphocyte proliferation is decreased (Kanitz, 2002). In weaned piglets at 14 d of age, growth hormone was decreased; while lymphocyte proliferation was increased (Liu, 2003). Acute LPS challenge also leads to an increase in inflammatory cytokines such as TNF-α, IL-6 (Webel, 1997). While it is known that macrophages secrete IL-8, a potent chemoattractant, to antigen recognition, the functional migratory ability of neutrophils to LPS challenge and mixing has not been well.

A series of experiments were conducted to determine correlations between piglet body core temperature and other physiologic and behavioral measures in response to social, thermal, and LPS stressors. These findings would potentially determine whether changes in body core temperature alone were a good physiologic measure to indicate changes in pig well-being or if other physiologic measures in conjunction with temperature would be more appropriate for painting a picture of pig well-being.

MATERIALS AND METHODS

Animals

70 cross-bred pigs from multiparous sows were used during 3 experiments at the Imported Swine Research Laboratory and the Environmental Research Laboratory at the University of Illinois Urbana-Champaign. Experiments took place between summer

2006 and spring 2009. Pigs were allowed ad libitum access to water and maintained on nursery diets appropriate for their age.

Experimental Design

Pigs were weaned at ~21 days of age. In experiments 1 and 2 pigs were moved to nursery pens and housed in litters for up to 2 weeks in order to acclimate to their new environment. In experiment 3, at weaning, pigs were transported from the Imported Swine Research Laboratory to the Environmental Research Lab. Shortly after weaning in all experiments, a temperature sensor (Digital Angel) was implanted near the anterior jugular vena cava following the manufacturer's recommendations using a 13 gauge syringe. In trials 1 and 2 a temperature sensor was also implanted in the flank fold. Rectal temperature and sensor temperatures were recorded 2 or 3 times daily after weaning in order to better acclimatize pigs to their environment and to the researchers. Pigs were weighed prior to mixing and LPS challenges. At mixing, unfamiliar pigs of similar body weight were randomly assigned to a nursery pen. All mixing, LPS, and temperature challenges were initiated at 0700 h unless otherwise noted and will be referred to as 0 h.

In experiment 1, 6 siblings were sex paired and housed across 6 nursery pens. After a 2-wk acclimation period, pigs were challenged with either LPS or saline. Three pens of pigs were injected intraperitoneal at 1400 h using an 18g needle with 25 ug/ kg body weight of LPS diluted in 3 mL of saline. The other 3 pens were injected with 3 mL of saline. Rectal, jugular, and flank fold temperatures were recorded at 0, 15, 30, and 45

min, and at 1, 1.5, 2, 2.5, 3, 3.5, and 4 h post-injection. Seven days later, each pair of pigs received the opposite treatment that it had received a week earlier.

In experiment 2, weanling pigs were challenged chronically with i.p. LPS injection that was dissolved in either saline or peanut oil. Pigs were weaned at 21 d of age and housed in nursery pens by litter. The digital thermosensors were implanted next to the anterior jugular vein and flank fold at weaning. Rectal and thermosensor region temperatures were recorded multiple times per day for 6 days to acclimatize pigs to handlers. Six days later, unfamiliar pairs of pigs were mixed and moved to new pens. Pigs were video-recorded to determine social status. Following mixing, pigs were allowed to recover for several days before LPS injection.

In experiment 2a, pigs were injected i.p. on day 0, 2, 4, and 7, with either 3 mL of 0.9% saline (n=6) or 25 ug/kg body weight LPS delivered in saline (LPS; n=6). Rectal and flank temperatures were recorded at 0, 0.5, 0.75, 1.5, 2, 2.5, and 3.5 h (TIME) post-injection. Blood samples were taken at 0, 1, 3, and 4 h post-injection.

In experiment 2b, pigs were injected i.p. on day 0, 3, and 6, with either peanut oil (n=12) or 25 ug/kg body weight LPS delivered in peanut oil (LPS-O; n=10). Administration of a substance in a lipophilic vehicle was used by Molitor et al. (1991) to maintain levels of morphine for up to 4 days. While their experiment utilized a constant rate infusion, it is hypothesized that the LPS would be released slower in a single injection if delivered in peanut oil. Rectal and flank temperatures were recorded at 0, 0.25, 0.5, 1, 2, 4, 8, 24, and 48 h post-injection. Blood was drawn at 0 and 45 min, and at 1.5, 4, 8, 24, and 48 h following LPS administration. The effects of TIME and injection

day (DAY) were analyzed with repeated measures. LPS treatment and dominance interactions were also analyzed.

In experiment 3, 2 trials were conducted with 12 female weanlings each. Pigs were weaned at ~21 d of age, transported to the Environmental Research Laboratory, and placed in 4 environmentally controlled chambers, by litter. Pigs were mixed into unfamiliar pens with unfamiliar pen mates and assigned to one of four treatments: thermo neutral ambient temperature (TNT) and saline (n=6), thermoneutral ambient temperature and LPS (n=6), cold ambient temperature (COLD) and saline (n=6), or cold ambient temperature and LPS (n=6). Immediately after mixing, the ambient temperature in COLD pens was reduced to 15 °C and remained there until 2 days later. Based on behavioral cues such as level of shivering, temperature was lowered again on d 2 to 10 °C for the remainder of the experiment. Piglets were bled via jugular vena puncture at 0, 2, and 4 h after mixing and again 48-h later. After 72-h post-mixing, pigs were challenged with LPS (50 μg/kg) dissolved in saline 0.9% or an equivalent amount of saline. Pigs were bled just prior to LPS injection as well as 2 and 4 h post-injection. Behavior was measured from -1 h to 5 h post-injection during acute LPS challenge.

Blood Sample Collection

Pigs were held in a supine position, and up to 10 mL of blood were collected into vacutainers containing heparin and/or EDTA via anterior jugular vena puncture (blood sampling lasted < 2 min). Heparin coated tubes, <5 mL, were used to collect whole blood for leukocyte differentials, and total white blood cell (WBC) count as well as

plasma for peripheral cortisol and cytokine analysis. In experiments 2 and 3, 7 mL of blood was collected into EDTA coated tubes for cell isolation.

White Blood Cell and Differential Counts

Whole blood was evaluated to determine total WBC count and leukocyte differential percentages. Total WBC were counted using an electronic Coulter Z1 Particle Counter (Beckman Coulter, Miami, FL) at 1:1,000 dilution, and red blood cells were lysed before counting. Whole blood was smeared and fixed in methanol and stained with Hema-3 staining system (Fisher Scientific, Houston, TX) to determine differential percentages of neutrophils, lymphocytes, eosinophils and monocytes. Slides were viewed under a light microscope and 100 cells per slide were visually counted.

Cell Isolation

Porcine neutrophils and lymphocytes were isolated from 7 mL of whole blood by density gradient centrifugation using Histopaque-1077 (density = 1.077 g/mL; Sigma) and Histopaque-1119 (density = 1.119 g/mL; Sigma). 5 mL of whole blood was layered over Histopaque-1077 and -111) (Sigma), then centrifuged at 700 x g for 30 min at room temperature. Lymphocytes were collected from the 1077 layer, washed twice in RPMI, resuspended, and counted. Neutrophils and red blood cells were removed from the 1119 layer. Red blood cells were lysed using cold endotoxin-free water, and isotonicity was restored using 5 x PBS. Neutrophils were centrifuged for 10 min at 475 x g, and the supernatant was decanted, and the pellet was washed twice and resuspended in RPMI.

Plasma Cortisol

Plasma samples were assayed for cortisol using a Coat-A-Count cortisol kit, following the manufacturer's protocol (Siemens Medical Solutions Diagnostics, Los Angeles, CA). Briefly, in duplicate, 25 μL of sample or standard was added to antibody-coated tubes. Radiolabeled (I^{125}) cortisol was added to tubes and incubated 45 min at 37 °C in a water bath. The liquid phase was decanted and radioactivity counted using a gamma counter (Cobra II, Perkin-Elmer, Boston, MA). A standard curve was based on 0, 10, 50, 100, 200, and 500 μg/mL was used. A high (200 μg/mL) and low (10 μg/mL) control were used to determine intra- and interassay CV. Intra- and interassay CV was 24.9 and 4.40, respectively. The minimal detectable cortisol concentration using this assay was approximately 2 ng/mL.

Neutrophil Chemotaxis

Neutrophil migration ability was measured according to the procedure described by Salak et al. (1993). A modified Boyden chamber (Neuro Probe, Cabin John, MD) was used to measure the migration of neutrophils toward RPMI-1640 medium (control; random migration), recombinant human complement C5a (rhC5a; Sigma), or porcine interleukin-8 (IL-8; Sigma) [chemotaxis (CHTX); directed migration]. Briefly, in duplicate, medium, hC5a (10^{-7} *M*), and pIL-18 (100 ug/mL) were added to the bottom wells of a 48-well microchemotaxis chamber (Neuroprobe, Gaithersburg, MD), and neutrophils adjusted to 3 x 10^6 cells/mL in RPMI were added to the top wells of the chamber and incubated for 45 min. The polyvinylpurolidone-free filter (pore size 5 um; Neuro Probe) was fixed and stained using the Hema-3 system (Fisher Scientific). Five

fields per well were counted in blind fashion using a light microscope at 100× magnification.

Cytokines

Peripheral levels of interleukin -10 and 12 were measured using a commercially available ELISA kit (R&D Systems, Minneapolis, MN) following manufacturer protocol. Briefly, plasma was diluted accordingly and along with standards was added to 96-well antibody coated plates along with assay diluent and incubated on a shaker for 2 hours. Plates were aspirated, washed, and conjugate was added to each well and again incubated. Following a second aspiration, substrate solution was added to each well and the plate protected from light and incubated on the bench for 30 min. Stop solution was added and plates were read on a spectrometer (Multiskan Plus, Fisher Scientific, Houston, TX). Optical density was analyzed and concentrations were converted to pg/mL. Intra- and interassay CV for IL-10 was 33.33 and 2.01 respectively. Intra- and interassay CV for IL-12 was 62.5 and 2.52 respectively.

Behavior

To establish social status, piglets were video-recorded for the first 8 h after the initiation of mixing. Video-records were viewed in real time. The winner or loser of each agonistic encounter was determined using previously described methodology (McGlone, 1985). Social status was assigned based on the outcomes of agonistic encounters, and pigs were identified as dominant (DOM), intermediate (INT), or submissive (SUB). DOM pigs won the majority of their fights, whereas INT pigs lost

more fights to the DOM pig. Piglets were identified as SUB based on submissive postures displayed towards other pigs, including losing most conflicts.

Behavior during the Cold/LPS trial was also recorded. Behaviors that were recorded include standing, sitting (SIT), sitting in contact with other pigs (SIT-C), eating, drinking, fighting with other pigs, lying in contact with the wall (LYW), lying in contact with other pigs (LYP), lying in contact with both a wall and other pigs (LYWP), lying without contact (LYNC). Video recordings began at -1 h prior to LPS injection and continued throughout 5 h post-injection period. Behaviors were recorded in seconds.

Statistical Analyses

Data were analyzed using SAS version 8.0 (SAS Inst. Inc., Cary, NC). The model for experiment 1 contained the main effects of treatment (TRT), time after injection (TIME), temperature measurement location, rectal, jugular, or flank fold (LOCATION). Gender was also analyzed to determine if there was any effect. In experiment 2, the chronic LPS challenge model included the fixed effects TRT, TIME, social status (SOC), and injection day (DAY). In experiment 4, Cold/Mixing challenge included the main effects ambient temperature (TEMP), SOC, and TIME. The Cold/LPS model included the fixed effects TRT, TIME, TEMP, and SOC. PIG was used as a random variable. All variables were tested for departures from normality using Proc Univariate. When necessary, variables were normalized by natural log transformation. A Proc Mixed procedure was used to analyze all data, and when appropriate, repeated measures were incorporated. A logarithmic transformation was applied to IL-10, IL-12, and cortisol. For all traits that were subjected to logarithmic transformation, means presented in tables

and graphs are non-transformed means. Data was considered significant at $P < 0.05$, and trends were discussed at $P < 0.10$.

RESULTS

Experiment 1: Effects of Acute LPS administration on rectal and sensor temperature

There was a TIME x TRT effect for both rectal and jugular temperatures ($P < 0.05$; Fig. 2.1); with rectal temperature being greater in LPS-injected pigs from 45 min until 4 h post-injection compared with saline-injected pig. Pigs injected with LPS had greater jugular ($P < 0.05$) temperature from 2 to 4-h post-injection compared with saline-injected pigs. Overall, LPS-injected pigs had greater ($P < 0.05$) rectal and jugular temperatures than did saline-injected pigs. There were no significant gender differences.

Experiment 2: Effects of Chronic LPS administration on weaned piglet temperature, immune and physiologic measures

Rectal and Jugular Temperature

In experiment 2a, pigs were injected with either SALINE or LPSS repeatedly for several days. Jugular temperature was less ($P = 0.046$) in pigs injected with LPS (101.8 °F ± 0.2) at 45 min post-injection compared with saline-injected pigs (102.5 °F ± 0.3). Rectal and jugular temperatures were similar post 45 min.

For those pigs treated with peanut oil or LPS + peanut oil in experiment 2b, there was a TRT x TIME interaction ($P < 0.05$; Fig. 2.2) for rectal temperature. By 1.5 h post-injection, rectal temperature was greater in LPS + peanut oil-injected pigs (103.2 °F ±

0.1) compared with pigs injected with peanut oil (103.6 °F ± 0.1) and remained elevated until 24 h post-injection. There was a TRT ($P = 0.013$) and TRT x TIME ($P < 0.01$; Fig. 2.3) interaction for jugular temperature. Jugular temperature was greater ($P < 0.05$) in LPS + peanut oil-injected pigs between 2 and 24-h post-injection compared to pigs injected with peanut oil. There was also a TRT x DAY interaction for both rectal and jugular sensor temperature ($P = 0.001$); on d 0, LPS-O-injected pigs had greater rectal (104.0 °F ± 0.1) and jugular temperatures (103.3 °F ± 0.2) than pigs injected with peanut oil on day 0 for rectal (103.3 °F ± 0.1) and jugular temperatures (102.2 °F ± 0.2). There was also a DAY x DOM ($P = 0.04$) interaction for rectal temperature; rectal temperature was lower in DOM pigs on d 0 (103.5 °F ± 0.1) and 3 (103.3 °F ± 0.1) compared to SUB pigs on d 0 (103.8 °F ± 0.1) and 3 (103.5 °F ± 0.1).

Cell and Differential Measures

For pigs in experiment 2a, percentage of neutrophils, lymphocytes, and N:L ratio differed (TRT x TIME; $P < 0.03$; Table 2-1). At 4-h post-injection on injection days, percentage of neutrophils (31.3% ± 2.9) and N:L ratio (0.72 ± 0.09) were greater for LPS-injected pigs than pigs injected with saline (19.0% ± 3.2) and (0.28 ± 0.10) respectively. Conversely, percentage of lymphocytes was less at 4-h post-injection for LPS-injected pigs (58.9% ± 3.2) compared to saline-injected pigs (74.6% ± 3.6). Percentage of lymphocytes and N:L ratio were different (TRT x DAY; $P < 0.05$; Table 2-2). Saline-injected pigs had a greater percentage of lymphocytes (76.1% ± 3.1) and consequently lower N:L ratio (0.24 ± 0.09) compared to LPS-injected pigs on day 2

(64.8% ± 2.9; 0.53 ± 0.08), whereas percentage of neutrophils tended to differ on d 2 (TRT x Day: $P = 0.087$).

There was a TRT x DAY interaction for total WBC ($P = 0.002$; Table 2-3); on d 2, total WBC was greater in LPS-injected pigs ($21.1 \times 10^7 \pm 1.3$) than in saline-injected piglets ($15.6 \times 10^7 \pm 1.3$).

For pigs treated with peanut oil or LPS + peanut oil, percentage of lymphocytes and neutrophils, and N:L ratio were affected by a TRT x TIME interaction ($P < 0.01$; Table 2-4) and a TRT x DAY interaction ($P < 0.01$; Table 2-5). Percentage of lymphocytes were greater in peanut oil treated pigs at 4-h (64.4% ± 2.4) and 8-h (64.7% ± 2.4) compared to pigs injected with LPS + peanut oil (55.5% ± 2.8) and (54.1% ± 2.67). Conversely, percentage of neutrophils and N:L ratio was greater ($P < 0.05$) in pigs injected with LPS + peanut oil compared with pigs injected with peanut oil alone at 4 and 8-h post-injection.

Percentage of lymphocytes was less ($P < 0.05$) in pigs injected with LPS + peanut oil (59.9% ± 2.3) on 0 d than pigs injected with peanut oil (67.9% ± 2.1). Percentage of neutrophils and N:L ratio were greater ($P < 0.05$) in pigs injected with peanut oil alone compared with pigs injected with LPS + peanut oil.

Plasma Cortisol

In experiment 2b, plasma cortisol was not affected by the main effects LPS or TIME; however, a TRT x TIME interaction existed ($P = 0.002$). At 3-h post-injection, LPS-injected pigs (115.9 ng/mL ± 14.2) had greater ($P < 0.05$) plasma cortisol levels than saline-injected pigs (67.7 ng/mL ± 15.4). Plasma cortisol response to LPS was decreased

after the first day regardless of TRT ($P < 0.05$) though there was a TRT x DAY interaction ($P < 0.05$; Table 2-3). Cortisol was greater for LPS-injected (115.3 ng/mL ± 11.5) pigs on d 0 compared to saline-injected (73.2 ng/mL ± 12.8) pigs on d 0.

In experiment 2b, DOM pigs tended to have greater plasma cortisol (38.8 ng/mL ± 2.5) compared with SUB pigs (35.3 ng/mL ± 2.5). Pigs injected with LPS + peanut oil at 1.5-h (46.3 ng/mL ± 5.0) and 4-h (52.8 ng/mL ± 5.2) post-injection tended to have greater plasma cortisol levels as compared to pigs injected peanut oil at 1.5-h (32.1 ng/mL ± 4.7) and 4-h (30.59 ng/mL ± 4.81) post-injection. Plasma cortisol levels in all pigs tended to be greater on day 0 (43.3 ng/mL ± 2.5) compared with day 3 (34.7 ng/mL ± 2.6) or with day 6 (34.2 ng/mL ± 2.6) suggesting that any response to the first LPS injection might have muted the HPA response to subsequent LPS injections.

Plasma Cytokines

Plasma cytokines were only analyzed during experiment 2a. Plasma IL-12 differed across DAY ($P = 0.026$) and there was a TRT x DAY interaction ($P < 0.01$; Table 2-3); plasma IL-12 was greater in LPS-injected pigs (843.0 ± 72.5) on day 0 compared with pigs injected with saline (462.6 pg/mL ± 75.4). Plasma IL-10 levels tended to differ across DAY ($P = 0.067$) and there was also a TRT x DAY interaction ($P = 0.002$; Table 2-3); plasma IL-10 was lesser (10.0 pg/mL ± 11.3) in LPS-injected pigs on day 2 compared with pigs injected with saline (41.8 pg/mL ± 11.8). Even though there was no statistical difference, IL-12 was greater in LPS-injected piglets at 3-h (772.6 pg/mL ± 79.0) and 4-h (717.2 pg/mL ± 75.2) post-injection compared to saline-injected pigs (502.1 ± 86.0; 542.4 ± 87.26 respectively).

Chemotaxis

Neutrophil chemotaxis was measured during experiment 2a. Neutrophils of DOM pigs injected with LPS (84.6 ± 23.0) tended to have lesser ($P = 0.075$) chemotactic response to c5a than did SUB pigs injected with LPS (174.0 ± 22.4). Neutrophil chemotaxis to IL-8 also tended to be lower ($P = 0.068$) in DOM pigs injected with LPS (60.0 ± 20.9) compared with SUB pigs injected with LPS (116.5 ± 20.4). Neutrophil chemotaxis to c5a was greater ($P < 0.006$) in SUB pigs (163.6 ± 15.7) compared with DOM pigs (110.6 ± 15.5). Chemotaxis of neutrophils to IL-8 was also greater ($P < 0.034$) for SUB pigs (120.0 ± 14.3) than for DOM pigs (79.0 ± 14.1).

Experiment 3: Effects of Cold and LPS on weaned piglet temperature, immune and physiologic measures

Temperature

In the cold/mixing challenge, rectal temperature was greater ($P < 0.001$) in pigs housed in TNT (103.6 °F ± 0.1) than pigs housed in COLD (103.1 °F ± 0.1).

In the cold/LPS challenge, INT pigs challenged with LPS had greater ($P < 0.05$) rectal temperature (104.1 °F ± 0.2) compared with DOM (103.3 °F ± 0.2) and SUB pigs (103.5 °F ± 0.2). At 4 h post-injection, pigs injected with LPS (104.7 °F ± 0.2) had greater ($P < 0.05$; Fig. 2-4) rectal temperature compared to pigs injected with saline (103.0 °F ± 0.2). Pigs injected with LPS (103.6 °F ± 0.1) had greater ($P < 0.05$) rectal temperature compared with pigs injected with saline (103.2 °F ± 0.1). Ambient

temperature affected pig rectal temperature as pigs in TNT (103.8 °F ± 0.1) had greater ($P < 0.05$) rectal temperature compared with pigs in COLD (103.1 °F ± 0.1).

Jugular sensor temperature was affected by the interaction social status (SOC) x TRT x TEMP (Fig. 2-5). For those pigs housed in COLD and injected with LPS, DOM (102.2 °F ± 0.7) and SUB pigs (102.0 °F ± 0.7) had greater ($P < 0.05$) jugular temperature compared to INT pigs (98.7 °F ± 0.7). The opposite was seen in those pigs housed in TNT and injected with LPS as INT pigs (104.1 °F ± 0.7) had greater ($P < 0.05$) jugular temperature compared with SUB pigs (100.6 °F ± 0.7). At 4-h post-injection, pigs injected with LPS tended to have greater ($P = 0.07$) jugular temperature compared to pigs injected with saline (101.6 °F ± 0.5). Of pigs housed in COLD, DOM pigs (101.8 °F ± 0.5) had greater ($P < 0.05$) jugular temperature compared with INT pigs (100.1 °F ± 0.5) and SUB pigs (100.2 °F ± 0.5). Overall, pigs housed in TNT (100.7 °F ± 0.3) had greater ($P < 0.05$) jugular temperature compared with pigs housed in COLD (100.7 °F ± 0.3). DOM pigs (102.3 °F ± 0.3) had greater ($P < 0.05$) jugular temperature compared with SUB pigs (100.8 °F ± 0.3).

Cell and Differential Measures

In the Cold/Mixing challenge, percentage of lymphocytes, neutrophils, N:L ratio, and total WBC, lymphocyte and neutrophil counts differed across TIME ($P <0.001$). Percentage of lymphocytes tended to differ due to social status ($P = 0.095$). Percentage of eosinophils was lesser ($P < 0.001$) at-2 h (0.61 % ± 0.26) and 4-h (0.42 % ± 0.25) and on d 6 (1.17 % ± 0.25) compared to 0 h (2.33 % ± 0.25) regardless of TEMP or SOC.

Percentage of lymphocytes, neutrophils, monocytes, and N:L ratio were not affected by LPS/Cold challenge. At 4 h post-injection, percentage of banded neutrophils tended to be greater ($P = 0.09$) in pigs injected with LPS (1.75 % ± 0.35) compared to pigs injected with saline (0.92 % ± 0.35). Total WBC count was less ($P < 0.05$) at 2 h ($1.95 \times 10^7 \pm 0.23$) and 4 h ($1.50 \times 10^7 \pm 0.22$) post-injection compared with 0 h ($2.97 \times 10^7 \pm 0.22$).

Plasma Cortisol

Ambient chamber temperature did not affect circulating plasma cortisol during the Cold/Mixing challenge. However, there was a trend for a SOC x TIME interaction ($P = 0.095$); on d 6, DOM pigs (25.2 ng/mL ± 4.3) tended to have greater cortisol than SUB pigs (13.8 ng/mL ± 4.3)

In the Cold/LPS challenge, LPS-injected pigs (71.5 ng/mL ± 11.0) had greater ($P = 0.024$) plasma cortisol than saline-injected pigs (52.0 ng/mL ± 10.9). Cortisol also increased over TIME ($P <0.001$) regardless of TRT or TEMP and was greater at 2-h (83.5 ng/mL ± 13.1) and 4-h (79.9 ng/mL ± 13.7) than before treatment was administered (21.9 ng/mL ± 13.4).

Neutrophil Chemotaxis

Neutrophil CHTX in response to RPMI, hc5a, and IL-8 differed over time ($P = 0.001$) during the Cold/Mixing challenge. In the Cold/LPS challenge, neutrophil CHTX in response to RPMI was less ($P= 0.019$) in pigs in COLD (17.1 ± 4.8) compared with pigs in TNT (33.7 ± 5.4) at 4-h post-injection.

Eating and Drinking Behavior

At -1 h, pigs injected with saline and housed in COLD (11.8 sec/h ± 6.9) spent considerably less ($P < 0.05$) time drinking than did pigs injected with saline and kept in TNT (40.8 sec/h ± 6.9). At 0 h, LPS-injected pigs (36.7 sec/h ± 4.9) spent greater ($P < 0.05$) time drinking than saline-injected pigs (19.8 sec/h ± 4.9). At 1 and 2 h post-injection, LPS-injected pigs (2.4 sec/h ± 4.9; 2.0 sec/h ± 4.9) spent less time ($P < 0.05$) drinking than did saline-injected pigs (30.9 sec/h ± 4.9; 26.9 sec/h ± 4.9). DOM pigs (22.2 sec/h ± 2.5) spent more time ($P < 0.05$) drinking than did INT pigs (14.7 sec/h ± 2.5).

Eating behavior was affected by DOM x TRT x TEMP interaction ($P < 0.05$; Fig. 2-6). Pigs injected with LPS ate less ($P < 0.05$) at 1, 2, 3, and 4-h post-injection compared to pigs injected with saline. Except 3 h post-injection, pigs in COLD ate more ($P < 0.05$) than pigs in TNT. Pigs injected with saline (1187.4 sec/h ± 47.9) spent more ($P < 0.05$) time eating than did pigs injected with LPS (607.0 sec/h ± 47.9). Pigs in COLD (1219.4 sec/h ± 47.9) spent more time eating ($P < 0.05$) than did pigs in TNT (575.0 sec/h ± 47.9).

Fighting Behavior

Pigs injected with saline (54.2 sec/h ± 6.1) spent more time ($P < 0.05$) fighting than did pigs injected with LPS (12.6 sec/h ± 6.1). Pigs injected with saline spent more ($P < 0.05$) time fighting at -1 h and 4 h post-injection. Pigs in COLD fought less ($P < 0.05$) than pigs in TNT at -1 h and 4 h post-injection. Those pigs injected with saline and kept in TNT at -1 h and 4 h (336.0 sec/h ± 21.2; 9.7 sec/h ± 21.2) spent more time

fighting ($P < 0.05$) compared with pigs injected with saline and kept in COLD (140.0 sec/h ± 21.2; 12.0 sec/h ± 21.2).

Standing Behavior

Pigs kept in COLD (1149.5 sec/h ± 60.2) spent more time ($P < 0.05$) standing compared to pigs kept in TNT (889.7 sec/h ± 60.2). More specifically, at 1-h post-injection, pigs kept in COLD (1466.9 sec/h ± 147.5) tended to spend more time standing ($P = 0.05$) than did pigs kept in TNT (671.5 sec/h ± 147.5). At 2 and 3-h post-injection, saline-injected pigs (1123.0 sec/h ± 147.5; 938.8 sec/h ± 147.5) spent more time standing ($P < 0.05$) than did LPS-injected pigs (671.3 sec/h ± 147.5; 458.8 sec/h ± 147.5).

Lying Behavior

Lying behavior was broken down into 4 specific categories: lying in contact with a pig (LYP), lying in contact with a wall (LYW), lying in contact with both a wall and a pig (LYWP), and lying no contact (LYNC). LYNC behavior did not differ between treatment groups. Pigs injected with LPS spent greater time ($P < 0.05$) LYW (686.7 sec/h ± 110.1) or LYWP (574.0 sec/h ± 69.9) compared with saline-injected pigs (221.0 sec/h ± 110.1; 343.6 sec/h ± 69.9). Pigs kept in TNT spent greater time ($P < 0.05$) LYW (822.4 sec/h ± 110.1) or LYWP (636.6 sec/h ± 69.9) compared with pigs kept in COLD (83.2 sec/h ± 110.1; 280.9 sec/h ± 69.9). Of those pigs injected with LPS, pigs kept in TNT spent more time ($P < 0.01$; Table 2.6) LYWP and LYP at 0 h compared to pigs kept in COLD. Of those pigs injected with saline, pigs kept in TNT spent more time ($P < 0.01$; Table 2.6) LYW and LYP at 3-h post-injection compared with pigs kept in COLD.

For pigs injected with LPS, those in COLD tended to spend more time LYP at 2 and 3-h post-injection (1366.7 sec/h ± 241.8; 1469.7 sec/h ± 241.8) compared with pigs in TNT (441.2 sec/h ± 241.8; 273.8 sec/h ± 241.8). There was a SOC x TRT x TEMP interaction for LYP ($P < 0.05$; Fig. 2-7). Of those pigs injected with LPS and housed in COLD, DOM pigs (573.8 sec/h ± 171.3) and SUB pigs (847.4 sec/h ± 171.3) spent more time ($P < 0.05$) LYWP compared with INT pigs (0.7 sec/h ± 171.3). Conversely, of pigs injected with LPS in COLD, INT pigs (985.0 sec/h ± 171.0) spent more time ($P < 0.05$) LYP compared to SUB pigs (400.2 sec/h ± 171.0).

Sitting Behavior

Pig sitting behavior was divided into sitting in contact with another pig (SITC) and sitting without contact (SIT). Pigs kept in COLD (266.2 sec/h ± 23.0) spent more time ($P < 0.01$) SITC than did pigs kept in TNT (23.2 sec/h ± 23.0). Pigs kept in COLD (91.8 sec/h ± 18.0) also spent more time ($P < 0.01$) SIT compared with pigs kept in TNT (37.4 sec/h ± 18.0). Pigs injected with LPS (89.8 sec/h ± 18.0) tended ($P = 0.05$) to spend more time SIT compared with pigs injected with saline (39.5 sec/h ± 18.0). Pigs kept in COLD spent more time ($P < 0.01$) SITC with other pigs at 1, 2, 3, and 4 h post-injection compared to pigs kept in TNT ($P < 0.05$; Table 2-6). Pigs injected with LPS (109.8 sec/h ± 56.4) spent less time ($P < 0.05$) SITC at 3-h post-injection compared with pigs injected with saline (276.8 sec/h ± 56.4); however, at 4-h post-injection pigs injected with LPS (472.3 sec/h ± 56.4) spent more time ($P < 0.05$) SITC compared with pigs injected with saline (124.7 sec/h ± 56.4). There was a TRT x TEMP x TIME interaction for SITC behavior ($P < 0.05$; Table 2-6).

At 4-h post-injection, those pigs injected with LPS and kept in COLD (385.5 sec/h ± 62.6) spent more time ($P < 0.05$) SIT compared with pigs injected with LPS and kept in TNT (21.5 sec/h ± 62.6).

Discussion

Young pigs are subjected to many stressors that may negatively impact their well-being. It would be highly beneficial to the pig and to the producer if there was an easily recorded physiologic measure, or collection of measures, to accurately monitor piglet well-being. Body core temperature is one such measure that has been well studied and is known to be affected by multiple stressors. For example, rectal temperature in young pigs decreases in colder environments (Carroll, 2001) and is greater when ambient temperature increases (Sutherland, 2007). The body also responds to systemic infection by increasing body core temperature in the form of a fever.

In the first experiment, the rectal temperature response of piglets that were challenged with LPS (25 μg/kg) mirrored previous studies as expected. Decades of work have shown that LPS is effective at activating a febrile response which can be detected via rectal temperature (Carroll, 2001; Matteri, 1998). Other studies have found thermo sensors to be valuable tools for monitoring body core temperature including Chen et al. (2006) who evaluated several thermometry techniques and determined that microchip sensors implanted between the shoulder blades of rabbits closely resembled rectal temperature. However, a recent study in equids showed conflicting results as similar microchip sensors were sensitive to fever detection at 87.4% in one trial but only 58.3% in another (Robinson, 2008). The results of experiment 1 indicated that the thermo

sensors at the jugular vein and the flank fold were not equal to rectal temperature, and thus could not be considered accurate measures of body core temperature. The thermosensors, either at the flank fold or at the jugular vein did not detect changes to LPS until at least an hour later than the rectal temperature. This might have contributed to this issue, but the scanner was not able to read some of the flank sensors leading to a smaller sample size and perhaps a lack of statistical power. Despite these discrepancies with the sensors, we continued to use a sensor implanted near the jugular in both the chronic LPS challenge as well as the cold challenges.

Unfortunately, comparing the temperature results of repeated injection of LPS + peanut oil compared to LPS + saline was difficult because injection of LPS + saline did not elevate rectal and jugular temperature as expected. Administration of LPS + peanut oil did extend the rectal and jugular response as long as 24 h post-injection on the first injection day. However, this effect was not significant following subsequent injections of LPS and likely the product of desensitization in the HPA as also reported by Grinevich (2001). Other measures, including percentage of neutrophils and lymphocytes, as well as plasma cortisol (though not significant), were also affected following the first injection but not subsequent ones. Thus it can be assumed that delivering LPS in peanut oil i.p. does not circumvent the tolerance to endotoxin.

What can be gleaned from experiment 2 is that injection of both pigs with LPS + peanut oil and LPS + saline did affect circulating leukocyte populations. Though not affected by ambient temperature, leukocyte populations were also affected by mixing in the Cold/Mixing challenge. This suggests that in the face of a single acute stressor, monitoring shifts in neutrophils and lymphocytes would, in fact, be a good measure of a

stress response. This makes sense because neutrophil populations will demarginate from the vascular endothelium and begin circulating in response to spikes in cortisol (Barger, 2010). The increase in cortisol that we saw in pigs injected with LPS is similar to previous studies that showed HPA activation by 2 h post-injection of 5 ug/kg LPS (Webel, 1997). However, it has become more accepted that social status plays a strong role in the response to challenge with a pathogen.

It was suggested by Walker (1992) that the HPA axis of rats is based on individual differences. More recent studies in pigs point to dominance status as one of those characteristics that dictate the individual response to stress (Hicks, 1998; Sutherland, 2006). In experiment 2, dominance affected neutrophil chemotaxis to IL-8 and c5a. Sutherland et al. (2006) found that among stressed pigs, dominant pigs had enhanced immune parameters, but neutrophil chemotaxis was not one of those parameters. However, it has recently been suggested that tolerance to LPS does not diminish neutrophil response (Natarajan, 2008) lending some validity to the results in experiment 2.

Dominance did not have a profound effect on many of the physiologic measures in experiment 3, including neutrophil chemotaxis, leukocyte differential, or plasma cortisol. Temperature response to LPS was affected by dominance though not consistently. While INT pigs had a stronger rectal temperature response to LPS, they also had a lesser jugular temperature when challenged with cold and LPS. Overall, the pigs that were categorized as DOM had presumably an easier time thermo-regulating in the COLD chamber compared to INT and SUB pigs. While some of these data conflict,

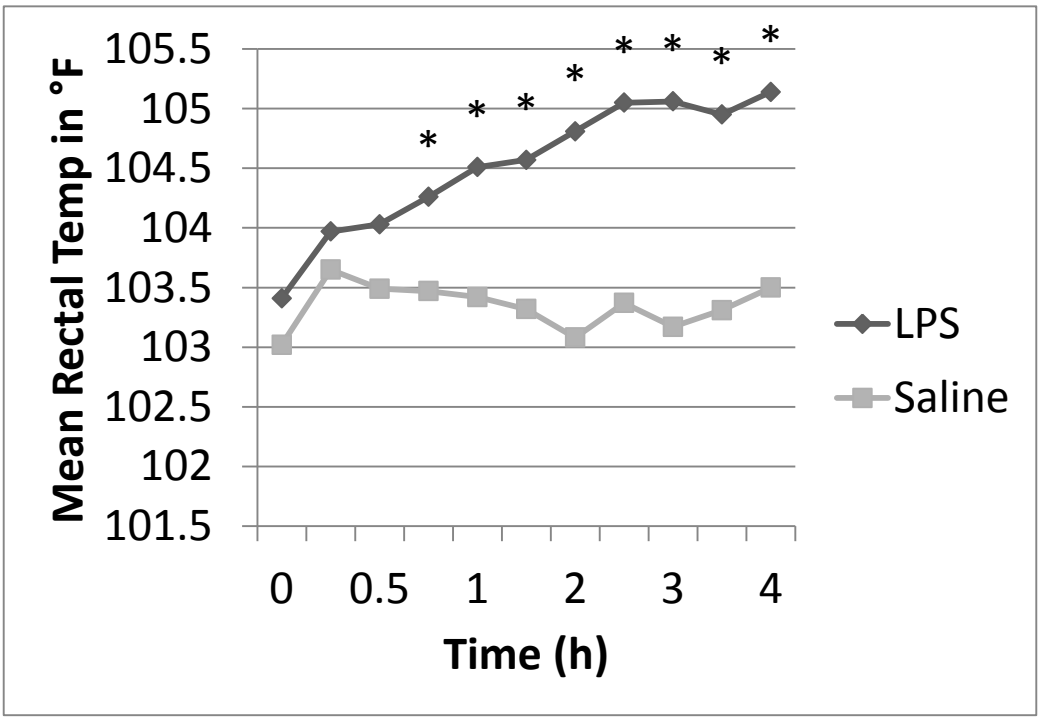
INT pigs seem to have greater fluctuation in jugular and rectal temperature when faced with various stressors.

Dominance status affected how pigs adjusted their behavior in response to Cold/LPS challenge. When challenged with cold and LPS, submissive pigs spent more time eating than their pen mates, perhaps indicating that they did not experience the same level of sickness behavior. Diminished activity as well as a dramatic reduction in eating behavior has been witnessed in rats (Skinner, 2008) as well as pigs (Johnson, 1994) and over time can be indicative of impingements on animal well-being through observed weight loss. There were similar findings in this study. Within 1 hour following injection of LPS, pigs reduced eating and drinking behavior regardless of ambient temperature. Consequently, sedentary behaviors such as sitting and lying increased in LPS-injected piglets. Whether they were lying in contact with a wall, a pig, or both was a product of their dominance status. INT pigs spent less time LB when challenged with Cold and LPS, perhaps another indication of their social status as well as why their jugular temperature was much lower. Furthermore, lipopolysaccharide injected piglets in the neutral chambers spent more time out of contact with their pen mates whereas those in cold chambers huddled more in order to maintain body core temperature. Pigs also spent more time SP in COLD chambers, as opposed to in NEUT chambers.

As discussed by Kelley (2003), "sickness behavior" is a result of pyrogenic cytokine activity in the brain. We did not measure those cytokines considered to be pyrogenic, but in experiment 2, we did measure IL-12. Interleukin-12 is given off by macrophages when their surface receptors come in contact with LPS (Janeway, 2005). This explains why IL-12 was greater in LPSS-injected pigs at 3 h compared to saline-

injected pigs. However, whether it was due to elevated glucocorticoids blocking IL-12 release from macrophages (DeKruyff, 1998; Blotta, 1997) or the overall desensitization of repeated LPS administration, IL-12 levels were not significant on subsequent injection days. IL-10 levels were not strongly affected by LPS injection.

In summary, measuring body core temperature is a relatively sensitive indicator of a pig's response to various stressors and thus their current state of being. However, other measures such as behavioral changes, including eating, drinking, and posture, as well as physiologic measures such as leukocyte percentages and cortisol would together paint a better picture of an animal's state of being and their ability to maintain or return to homeostasis. For a producer, monitoring changes in body temperature in conjunction with accurate identification of behavioral changes could be an effective means of identifying changes in pig well-being.



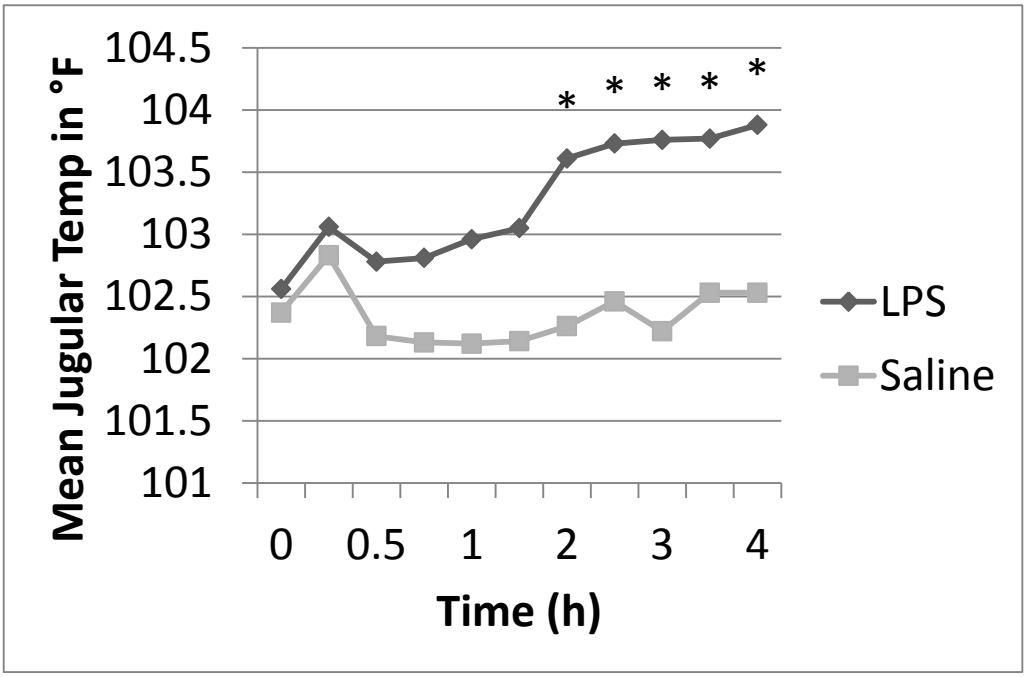


Figure 2-1: Least square means comparison of rectal and jugular temperature for pigs injected with saline (n=6) and LPS (n=6) in Experiment 1.

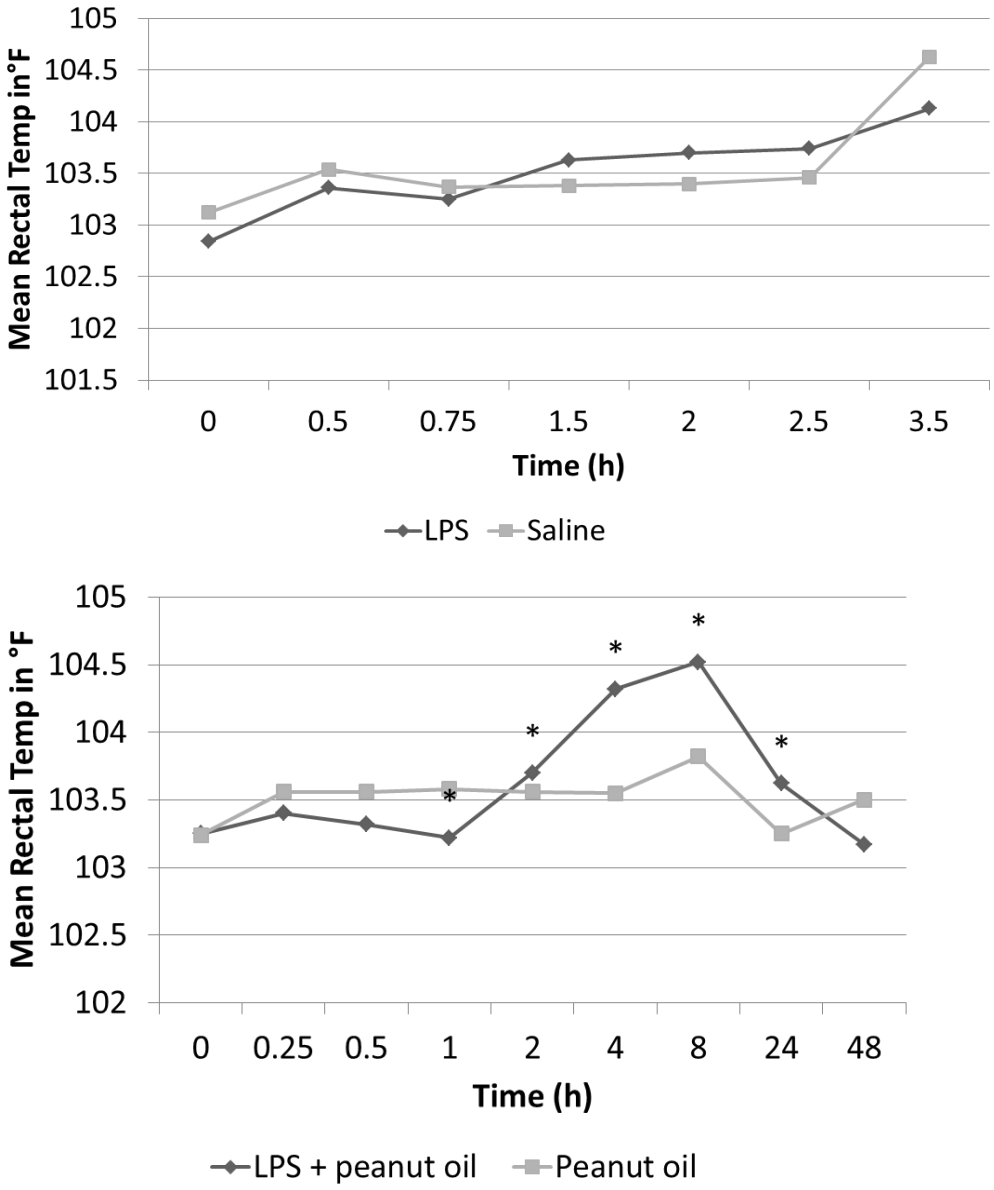


Figure 2-2. Least square means comparison of rectal temperature between piglets chronically injected with saline (n=6), LPS dissolved in saline (n=6), peanut oil (n=10), and LPS dissolved in peanut oil (n=12). Means without common superscript differ ($P < 0.05$)

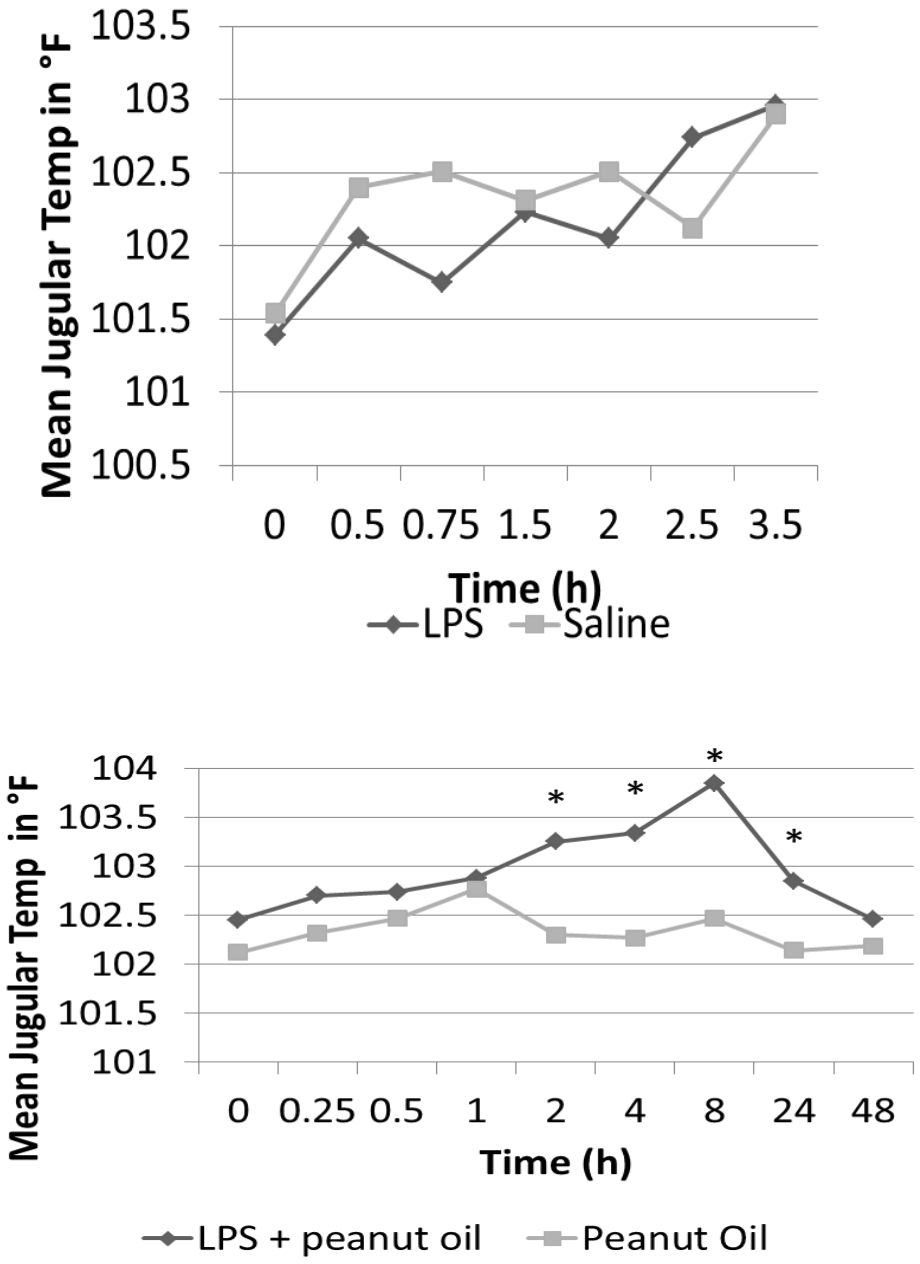


Figure 2-3. Least square means comparison of jugular temperature between piglets chronically injected with saline (n=6), LPS dissolved in saline (n=6), peanut oil (n=10), and LPS dissolved in peanut oil (n=12). Means without common superscript differ ($P < 0.05$)

Table 2-1. Leukocyte percentages for Saline (n=6) and LPS + Saline (n=6) injected pigs across time during chronic LPS challenge.[1]

		LPS Treatment		*P*- value
	Hour	Saline	LPS + Saline	Trt x Time
Neutrophils, %				
	0	17.2 ± 3.2	20.2 ± 3.2	
	1	17.8 ± 3.1	15.7 ± 3.2	
	3	28.2 ± 3.7	23.7 ± 2.9	
	4	19.0 ± 3.2[b]	31.3 ± 2.9[a]	0.027
Lymphocytes, %				
	0	73.9 ± 3.7	69.4 ± 3.6	
	1	76.6 ± 3.5	78.7 ± 3.6	
	3	66.9 ± 4.2	70.8 ± 3.4	
	4	74.6 ± 3.6[a]	58.9 ± 3.2[b]	0.007
Eosinophils, %				
	0	1.5 ± 0.4[b]	2.8 ± 0.4[a]	
	1	1.9 ± 0.4	2.3 ± 0.4	
	3	1.0 ± 0.5	0.9 ± 0.4	
	4	1.4 ± 0.4	0.7 ± 0.3	0.017
Monocytes, %				
	0	6.9 ± 0.9	7.2 ± 0.9	
	1	3.3 ± 0.9	4.0 ± 0.9	
	3	4.4 ± 1.2	4.6 ± 0.8	
	4	4.6 ± 0.9	5.9 ± 0.8	0.286
N:L ratio				
	0	0.3 ± 0.1	0.3 ± 0.1	
	1	0.3 ± 0.1	0.2 ± 0.1	
	3	0.4 ± 0.1	0.4 ± 0.1	
	4	0.3 ± 0.1[b]	0.7 ± 0.1[a]	0.007

[a-b]Within a row, means without common superscript differ. ($P < 0.05$)
[1]Means (non-transformed) ± SE

Table 2-2. Leukocyte differential percentages for Saline (n=6) and LPS + saline (n=6) treated pigs across Day[1] during chronic LPS challenge.[2]

		LPS Treatment		*P*- value
	Day	Saline	LPS	Trt x Day
Neutrophil, %				
	0	23.2 ± 6.1	34.0 ± 5.7	
	2	17.5 ± 5.5	40.8 ± 5.7	
	4	19.3 ± 6.8	28.7 ± 5.7	0.418
Lymphocyte, %				
	0	66.6 ± 6.3	58.2 ± 5.7	
	2	78.2 ± 5.7	55.0 ± 5.7	
	4	76.3 ± 7.0	63.7 ± 5.7	0.442
Eosinophil, %				
	0	1.6 ± 0.4	0.2 ± 0.4	
	2	1.3 ± 0.4	0.5 ± 0.4	
	4	1.0 ± 0.4	1.3 ± 0.4	0.083
Monocyte, %				
	0	7.4 ± 1.4	7.7 ± 1.2	
	2	3.0 ± 1.3	3.7 ± 1.3	
	4	4.5 ± 1.6	6.3 ± 1.3	0.843
N:L ratio[3]				
	0	0.37 ± 02	0.60 ± 0.2	
	2	0.19 ± 0.2	1.00 ± 0.2	
	4	0.29 ± 0.2	0.56 ± 0.2	0.314

[a-b]Within a row, means without common superscript differ. ($P < 0.05$)
[1]Blood measures were taken at 4 h post-injection of LPS or saline on specified days.
[2]Means (non-transformed) ± SE
[3]N:L = neutrophil : lymphocyte ratio

Table 2-3. Plasma cortisol, cytokines, and total white blood cell (WBC) count for saline (n=6) and LPS + saline (n=6) treated pigs across Day[1] during chronic LPS challenge.[2]

		LPS Treatment		*P*- value
	Day	Saline	LPS + saline	Trt x Day
Cortisol, ng/mL				
	0	61.3 ± 31.0	151.8 ± 25.1	
	2	64.8 ± 27.4	50.6 ± 25.1	
	4	45.1 ± 31.0	92.6 ± 25.1	0.172
IL-10, pg/mL				
	0	10.7 ± 5.1	12.7 ± 4.2	
	2	15.2 ± 4.6	4.7 ± 4.2	
	4	14.3 ± 5.1	4.8 ± 4.2	0.191
IL-12, pg/mL				
	0	126.6 ± 32.0[b]	257.9 ± 26.4[a]	
	2	152.3 ± 29.1	120.4 ± 26.4	
	4	178.8 ± 32.4	159.4 ± 26.4	0.024
Total WBC, 10^7/mL				
	0	1.59 ± 0.30	1.11 ± 0.27	
	2	1.10 ± 0.30[b]	2.13 ± 0.27[a]	
	4	1.96 ± 0.33	1.94 ± 0.27	0.038

[a-b]Within a row, means without common superscript differ. ($P < 0.05$)

[1]Blood measures were taken at 4 h post-injection of LPS or saline on specified days.

[2]Means (log transformed for IL-10 and IL-12) ± SE

Table 2-4. Leukocyte percentages and total white blood cell (WBC) count for peanut oil (n=10) and LPS + peanut oil (n=12) treated pigs across time during chronic LPS challenge.[1]

		LPS Treatment		*P*- value
	Hour	Peanut Oil	LPS + peanut oil	Trt x Time
Neutrophils, %				
	0	27.7 ± 2.2	27.7 ± 2.5	
	1.5	36.4 ± 2.4	32.1 ± 2.7	
	4	32.6 ± 2.2[b]	40.9 ± 2.6[a]	
	8	31.3 ± 2.2[b]	42.9 ± 2.4[a]	
	24	24.6 ± 2.2	24.4 ± 2.5	
	48	25.9 ± 2.2	22.9 ± 2.4	0.001
Lymphocytes, %				
	0	68.2 ± 2.4	68.8 ± 2.7	
	1.5	61.1 ± 2.6	66.3 ± 2.8	
	4	64.4 ± 2.4[a]	55.5 ± 2.8[b]	
	8	64.7 ± 2.4[a]	54.1 ± 2.7[b]	
	24	71.7 ± 2.4	71.6 ± 2.7	
	48	69.8 ± 2.4	73.4 ± 2.7	0.001
Eosinophils, %				
	0	2.4 ± 0.3	2.0 ± 0.3	
	1.5	1.1 ± 0.3	0.7 ± 0.4	
	4	1.2 ± 0.3	0.8 ± 0.3	
	8	2.4 ± 0.3	1.2 ± 0.3	
	24	2.0 ± 0.3	3.1 ± 0.3	
	48	1.8 ± 0.3	1.7 ± 0.3	0.003
Monocytes, %				
	0	1.7 ± 0.3	2.1 ± 0.3	
	1.5	1.8 ± 0.3	1.5 ± 0.3	
	4	1.9± 0.3	2.4 ± 0.3	
	8	1.6 ± 0.3	2.6 ± 0.3	
	24	1.7 ± 0.3	1.5 ± 0.3	
	48	2.1 ± 0.3	2.5 ± 0.3	0.081
N:L ratio[2]				
	0	0.5 ± 0.1	0.4 ± 0.1	
	1.5	0.7 ± 0.1	0.5 ± 0.1	
	4	0.6 ± 0.1	0.9 ± 0.1	
	8	0.5 ± 0.1[b]	0.9 ± 0.1[a]	
	24	0.4 ± 0.1	0.4 ± 0.1	
	48	0.4 ± 0.1	0.3 ± 0.1	0.002
Total WBC, 10^7/mL				
	0	2.9 ± 0.2	2.9 ± 0.2	
	1.5	2.7 ± 0.2	2.4 ± 0.2	
	4	3.0 ± 0.2	3.0 ± 0.2	
	8	3.0 ± 0.2	3.3 ± 0.2	
	24	2.5 ± 0.2	2.8 ± 0.2	
	48	2.5 ± 0.2	2.7 ± 0.2	0.178

[a-b]Within a row, means without common superscript differ. ($P < 0.05$)

[1]Means (non-transformed) ± SE

[2]N:L = neutrophil : lymphocyte ratio

Table 2-5. Leukocyte percentages and total white blood cell (WBC) count for peanut oil (n=10) and LPS + peanut oil (n=12) treated pigs across Day[1] during chronic LPS challenge.[2]

		LPS Treatment		*P*- value
	Day	Peanut Oil	LPS + peanut oil	Trt x Day
Neutrophils, %				
	0	31.5 ± 3.8[b]	56.7 ± 4.4[a]	
	3	32.4 ± 3.8	29.2 ± 4.4	
	6	34.3 ± 4.0	42.7 ± 4.2	0.006
Lymphocytes, %				
	0	65.5 ± 3.8[a]	39.5 ± 4.5[b]	
	3	64.5 ± 3.8	67.7 ± 4.5	
	6	62.9 ± 4.0	54.7 ± 4.2	0.005
Eosinophils, %				
	0	1.1 ± 0.4	0.7 ± 0.4	
	3	1.1 ± 0.4	0.8 ± 0.4	
	6	1.4 ± 0.4	0.4 ± 0.4	0.614
Monocytes, %				
	0	3.1 ± 0.6	3.1 ± 0.6	
	3	2.1 ± 0.5	2.3 ± 0.6	
	6	1.6 ± 0.6	2.2 ± 0.6	0.697
N:L ratio[3]				
	0	0.5 ± 0.2[b]	1.7 ± 0.2[a]	
	3	0.6 ± 0.2	0.5 ± 0.2	
	6	0.6 ± 0.2	1.0 ± 0.2	0.001
Total WBC, 10^7/mL				
	0	2.7 ± 0.3	3.2 ± 0.3	
	3	3.0 ± 0.3	2.8 ± 0.3	
	6	3.3 ± 0.3	2.9 ± 0.3	0.234

[a-b]Within a row, means without common superscript differ. ($P < 0.05$)
[1]Blood measures were taken at 4 h post-injection of LPS or saline on specified days.
[2]Means (non-transformed) ± SE
[3]N:L = neutrophil:lymphocyte ratio

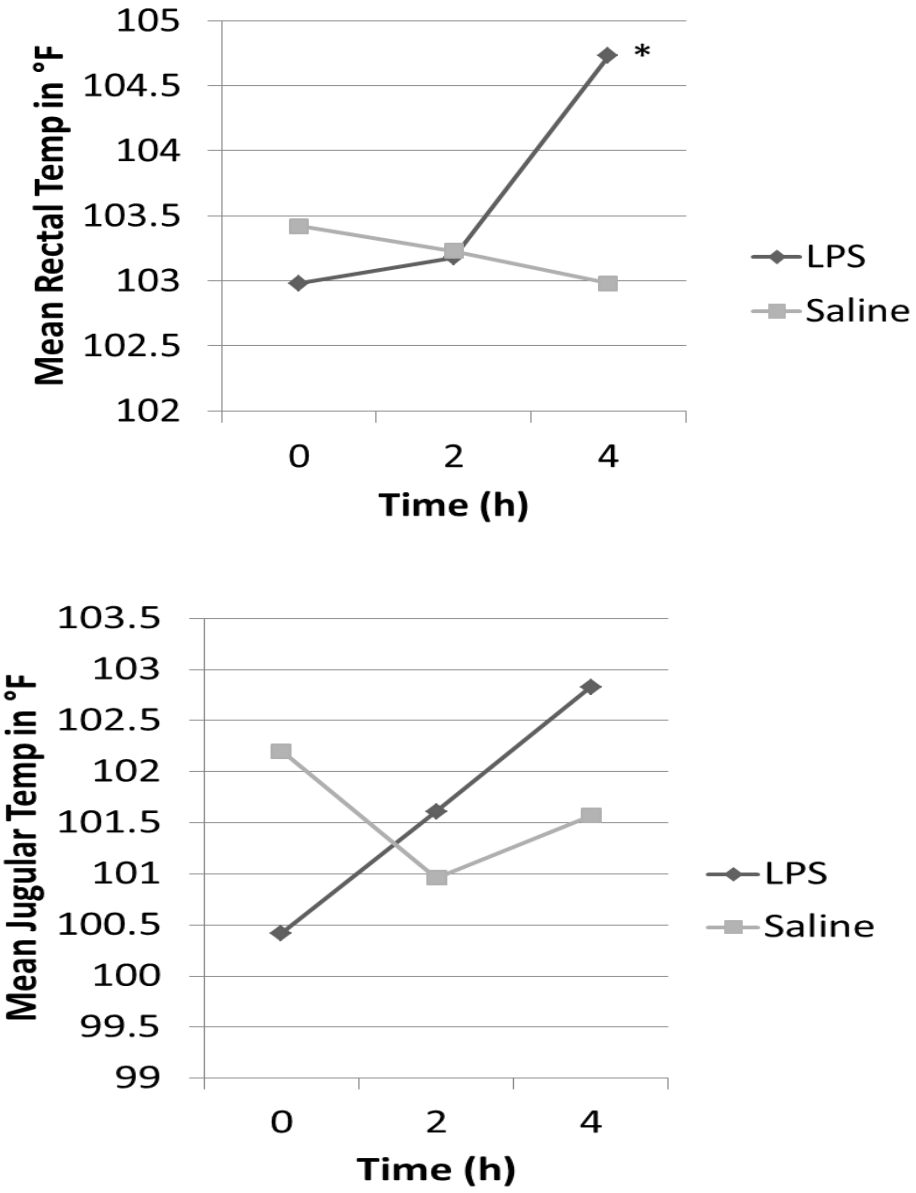


Figure 2-4. Least square means comparison of rectal and jugular temperatures between pigs acutely challenged with saline (n=12) or LPS (n=12) during chronic cold stress challenge. Means without common superscript differ ($P < 0.05$)

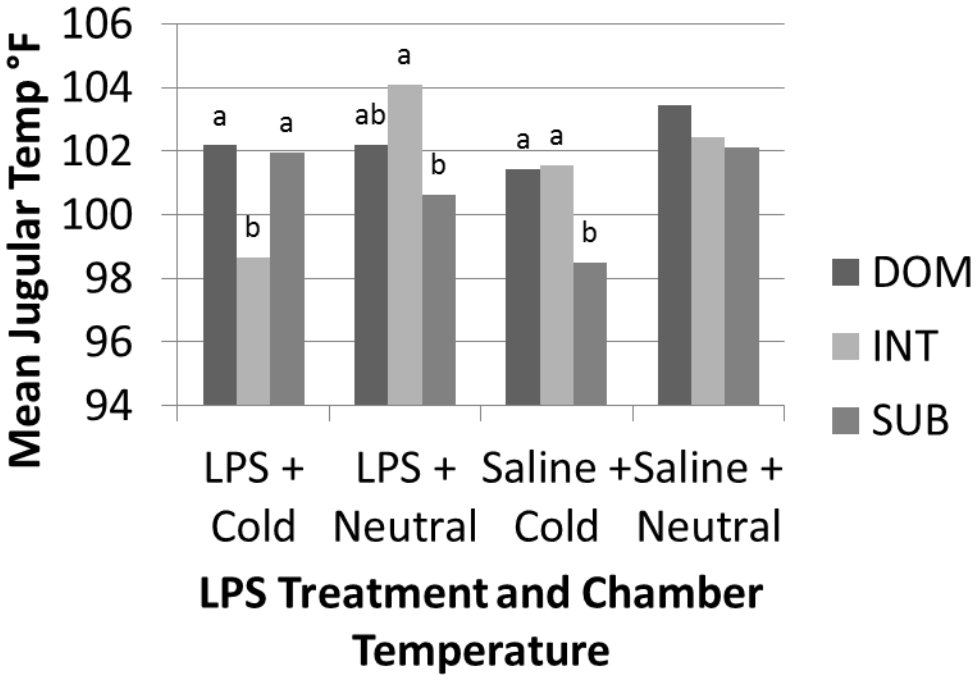


Figure 2-5. LPS treatment and ambient temperature, means without common superscript differ ($P < 0.05$)

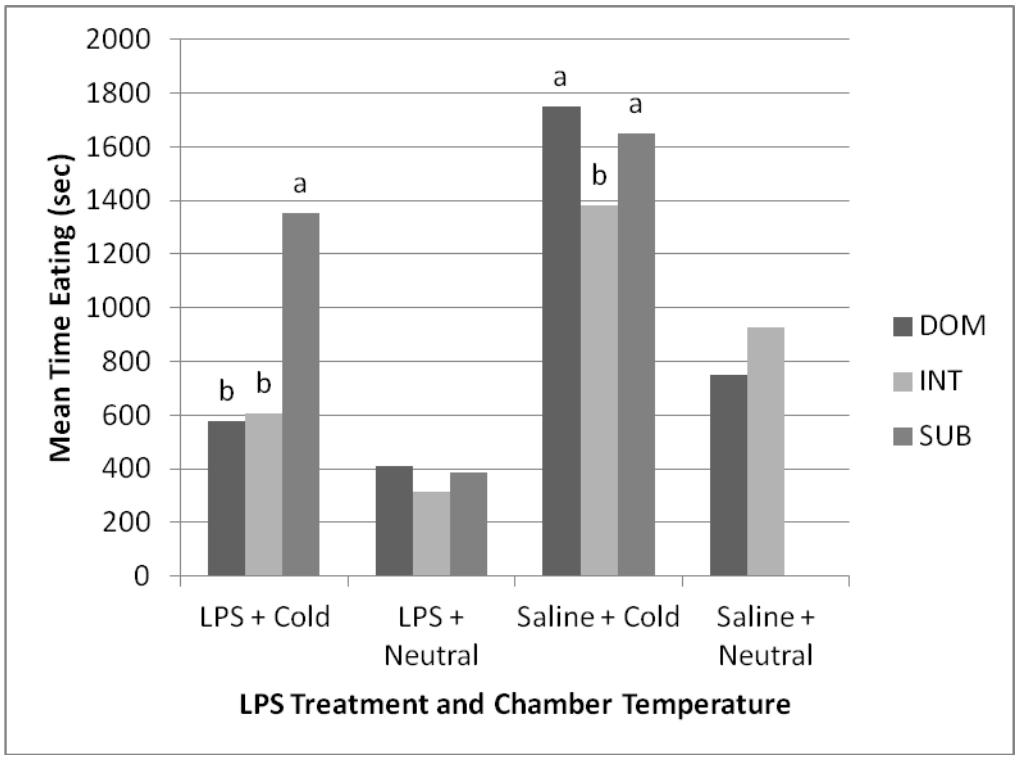


Figure 2-6. Least square means of effect of dominance status on pig eating behavior between pigs acutely challenged with saline (n=12) or LPS (n=12) during chronic cold stress challenge. Within LPS treatment and ambient temperature, means without common superscript differ ($P < 0.05$)

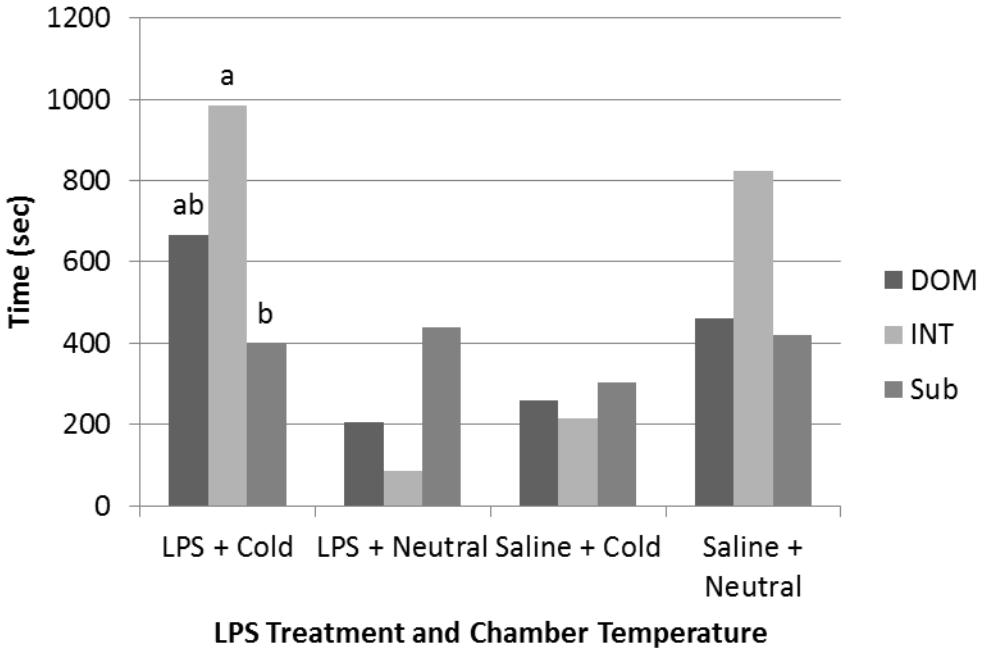


Figure 2-7. Least square means of effect of dominance status on time spent lying in contact with a pen mate between pigs acutely challenged with saline (n=12) or LPS (n=12) during chronic cold stress challenge. Within LPS treatment and ambient temperature, means without common superscript differ ($P < 0.05$)

Table 2-6. Effect of LPS challenge and chamber temperature on piglet lying and sitting behavior over time. LYP = Lying in contact with pig, LYWP = lying in contact with wall and pig, SITC = sitting in contact with pig, SIT = sitting no contact.[1]

		Saline		LPS		P-value
	Hr	Neutral	Cold	Neutral	Cold	Trt x Temp x Time
LP						
	-1	270.3 ± 241.8	161.8 ± 241.8	87.3 ± 241.8	69.0 ± 241.8	
	0	377.8 ± 241.8	594.0 ± 241.8	414.7 ± 241.8	126.2 ± 241.8	
	1	590.7 ± 241.8	0.0 ± 241.8	69.2 ± 241.8	497.3 ± 241.82	
	2	319.8 ± 241.8	231.8 ± 241.8	441.2 ± 241.8	1366.7 ± 241.8	
	3	1299.8 ± 241.8	703.5 ± 241.8	273.8 ± 241.8	1469.7 ± 241.8	
	4	337.5 ± 241.8	72.3 ± 241.8	177.2 ± 241.8	573.7 ± 241.8	0.083
LB						
	-1	194.3 ± 242.3	160.2 ± 242.3	39.3 ± 242.3	116.0 ± 242.3	
	0	568.3 ± 242.3	162.5 ± 242.3	1518.8 ± 242.3[a]	0.0 ± 242.3[b]	
	1	674.5 ± 242.3	0.00 ± 242.3	224.0 ± 242.3	177.3 ± 242.3	
	2	613.2 ± 242.3	150.7 ± 242.3	839.8 ± 242.3	1077.3 ± 242.3	
	3	1003.7 ± 242.3[a]	54.3 ± 242.3[b]	592.7 ± 242.3	1241.3 ± 242.3	
	4	541.3 ± 242.3	0.0 ± 242.3	829.2 ± 242.3	231.7 ± 242.3	0.006
SP						
	-1	20.0 ± 79.8	35.3 ± 79.8	34.0 ± 79.8	61.0 ± 79.8	
	0	5.7 ± 79.8[b]	238.5 ± 79.8[a]	29.7 ± 79.8	124.7 ± 79.8	
	1	2.8 ± 79.8	89.3 ± 79.8	8.8 ± 79.8[b]	338.5 ± 79.8[a]	
	2	11.8 ± 79.8[b]	355.5 ± 79.8[a]	61.8 ± 79.8	87.3 ± 79.8	
	3	0.8 ± 79.8[b]	552.8 ± 79.8[a]	20.7 ± 79.8	198.8 ± 79.8	
	4	2.2 ± 79.8[b]	247.2 ± 79.8[a]	79.7 ± 79.8[b]	865.0 ± 79.8[a]	0.001
SNC						
	-1	15.8 ± 62.7	63.5 ± 62.7	20.2 ± 62.7	3.33 ± 62.7	
	0	14.8 ± 62.7	87.0 ± 62.7	15.0 ± 62.7	116.7 ± 62.7	
	1	19.3 ± 62.7	28.8 ± 62.7	68.5 ± 62.7	114.8 ± 62.7	
	2	35.7 ± 62.7	94.2 ± 62.7	200.0 ± 62.7	96.8 ± 62.7	
	3	5.3 ± 62.7	81.0 ± 62.7	21.3 ± 62.7	13.7 ± 62.7	
	4	11.8 ± 62.7	16.5 ± 62.7	21.5 ± 62.7[b]	385.5 ± 62.7[a]	0.065

[a-b]Within a row, means without common superscript differ. ($P < 0.05$)

[1]Means (non-transformed) ± SE

General Discussion

Monitoring and improving pig well-being is a challenge that has yet to be perfected. Advancements in a number of areas including nutrition have benefited pigs. Also, while we know much about the physiologic responses to stress, it has not been well analyzed what measures would be most effective for monitoring changes in pig well-being. Previous research has shown that body core temperature, for example, is altered by thermal changes, social stress, and infectious challenge. Activation of the HPA axis, changes in immune function, including altered cytokine levels and leukocyte populations, and behavioral changes are also initiated as a means of coping with stress. When animals cannot effectively cope, their well-being becomes compromised. Thus, the initiation of the stress response is the first opportunity to intervene, and recognizing these changes is crucial. Science and technology have yielded great advancements for improving piglet well-being as well as developing monitoring systems that allow for easy identification with limited handling. Therefore, these studies were conducted to explore the thermogenic effects of including a particular plant-extract in sow diets on their offspring as well as validating the effectiveness of microchip thermo-sensors during stress and other physiologic measures as indicators of well-being.

In Chapter 1, the effect of including a plant-extract "capsicum" in sow diets on neonatal thermogenic capacity was explored. Previous studies by ADM have shown that including capsicum in animal diets can increase peripheral blood flow.increased peripheral blood flow following feeding of a diet including capsicum. Other extracts such as capsaicin have shown similar stimulatory effects on circulation. In the present study, piglets from sows fed "capsicum" were injected with LPS, which is known to

initiate fever, and the temperature response between 24 h and 72 h old piglets was compared was recorded. Regardless of the duration that the experimental diet was fed to sows, neonatal piglets < 24 hours of age did not show a dramatic difference in their temperature response to this endotoxin challenge. This is not completely unexpected as piglets are born with a relatively naïve immune system. One possibility is that the capsicum may not have reached the piglets *in utero*. It is unknown whether capsicum would even cross the placenta intact. It would have been useful to observe the sow more closely to see if there were any direct effects on the sow when fed a diet containing capsicum. This would better explain if any effects to the fetus were a result of the physiologic response of the sow to capsicum or due to the capsicum directly.

In Chapter 2, we might have been too quick to write off the usefulness of the flank fold sensor. Based on the data in the first experiment, both sensor locations should have been able to detect changes in body temperature, though not necessarily body core temperature, in response to LPS injection. It's entirely possible that the sensors were misplaced or the scanner was not reading the sensors properly. While it is certainly not feasible to use these sensors in all piglets that are weaned, they could perhaps be useful in females that are meant to be replacement gilts. It would be interesting to see the efficacy of the sensors when the pigs have grown to an adult weight. We were also able to re-use them, making the cost of monitoring pig well-being more feasible from a financial standpoint.

Another issue that arose included a need to handle the pigs frequently. Knowing that pig stress physiology and behavior can be changed due to handling we explored the use of non-surgical jugular catheters during the chronic challenge as described by Damm

et al. (2000); however, due to technical complications this method was abandoned. It would have also decreased handling time if the sensors had been equivalent to rectal temperature in measuring body core temperature. It is impossible to determine the effect that handling had on the data, but we limited jugular sticks to under 2 minutes to minimize handling.

In all, this series of experiments served to show that body core temperature, if effectively monitored, could be a good indicator of pig well-being if paired with other non-invasive techniques such as regular observation of pig behavior.

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