IMPACTS OF MICROENVIRONMENT VARIATION DURING BREEDING AND GESTATION ON THE REPRODUCTION AND WELL-BEING OF MATURE GILTS

BY

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THESIS

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ABSTRACT

The effects of room temperature and light intensity prior to breeding and into early gestation were evaluated on the reproductive performance and well-being of gilts housed individually in crates. In eight replicates, estrus was synchronized in mature gilts (n = 198) and after last feeding of Matrix were randomly assigned to a room temperature of 15 °C (COLD), 21°C (NEUTRAL) or 30 °C (HOT), and a light intensity of 11 (DIM) or 433 (BRIGHT) lx. Estrus detection was performed daily and gilts inseminated twice. Blood samples were collected before and after breeding for determination of immune measures and cortisol concentrations. Gilt ADFI, BW and body temperature were measured. On d 30 post-breeding, gilts were slaughtered to recover reproductive tracts to evaluate pregnancy and litter characteristics. There were no temperature x light intensity interactions for any response variable. Reproductive measures of follicle development, expression of estrus, ovulation rate, pregnancy rate (83.2%), litter size (14.3 ± 0.5), and fetal measures were not affected by temperature or lighting ($P > 0.10$). Gilts in COLD (37.6 °C) had a lower ($P < 0.05$) rectal temperature than those in NEUTRAL (38.2 °C) and HOT (38.6 ± 0.04 °C). Both BW gain and final BW were greater ($P < 0.0001$) for gilts kept in HOT than those in NEUTRAL or COLD environments. Gilts housed in the HOT environment made more postural changes than did those kept in either COLD or NEUTRAL temperatures. Gilts kept in the HOT temperature spent more total time lying and more time lying laterally compared to those gilts housed in the NEUTRAL or COLD rooms. Total white blood cells and the percentage of neutrophils, as well as neutrophil-to-lymphocyte ratio were all influenced ($P < 0.05$) by temperature but there was no effect of light or interaction with temperature on other immune cells or measures. These results indicate that temperatures in the range of 15 to 30 °C or light intensity at 11 to 433 lx do not impact reproduction during the follicular phase and into early

gestation for mature gilts housed in gestation crates. However, room temperature does impact physiological, behavioral, and immune responses of mature gilts and should be considered as a potential factor that may influence gilt well-being during the first 30-d post-breeding.

This work is dedicated to the memory of Richard "Papa" Salata- his enthusiasm and joy in life is an inspiration and will never be forgotten, even while 'chasing those pigs around'. In addition, I would like to dedicate this work to my parents, Jill and Kevin Canaday, and my brother, Conor Canaday, for their enduring love and support through my seemingly endless years of schooling. And finally to Vikram Chaudhery for his support during this project and for the courage to follow my dreams.

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1. Introduction

The pork industry has contributed immensely, not only to the food industry, but to the world economy and innovative biomedical research. For sustainable pork production, farms must be efficient to remain profitable in the face of rising production costs and enormous pressure from consumers and advocacy groups to change how swine are housed and managed. An approach to easing the effects of these detrimental pressures is to improve production rates by increasing reproductive efficiency and enhancing the well-being of the breeding herd. Typical breeding herds report unexplainable reproductive failure and health issues in facilities designed for efficiency and uniformity. These concerns could be related to inappropriate environmental conditions, caused by unequal distribution of heat, cooling and light sources.

Modern pork production makes a significant contribution to the world economy as well as to the food industry. The National Pork Board series "Pork Checkoff Quick Facts" (2009) stated that forty percent of the world's meat consumption is pork, more than any other single meat product. This Quick Facts brochure, available to producers and consumers, also describes the estimates of production and exportation for the U.S. and each state. In 2008, the U.S. produced roughly $14 billion in pork products and exported over 20% to other countries, including Japan, a major consumer of U.S. pork products. In particular, Illinois is the fourth largest pork producing state in the country with production of more than one billion pounds of pork per year. This results in a state economy boost of approximately $900 million per year (National Pork Board, 2009).

Swine production has also played a key role in areas other than food products, including medications, medical products and other household items. For example, porcine insulin has been implemented in the treatment of diabetes mellitus, valves from the hearts of pigs have been used

as replacements for humans with valvular diseases and other swine products have been used in the creation of adhesives, glass and chalks (Morihara et al., 1979; David et al., 1990). Furthermore, the use of swine for biomedical research has led to much advancement in health. This includes the replacement of pulmonary valves using the submucosa of porcine small intestine and the use of the pig as a model for many human diseases, such as hemorrhagic shock and acute myocardial infarctions (Kowalenko et al., 1992; Feng et al., 1998; Matheny et al., 2000).

The immediate goal of swine producers is to increase profitability while maintaining high standards of animal health. An animal that is not reproducing is neither healthy nor profitable. Through domestication and selection, the genetics of swine today are leaner and much better reproducers than those of earlier herds (Hagen and Kephart, 1980; van Vuren and Hedrick, 1989). Swine producers are taking advantage of this fact and consolidating herds for maximum profitability and efficiency, although the industry in itself continues to change by the year (Perelman, 1977). This move to intensive farming has been met with some criticism from consumers, the public and advocacy groups such as “Farm Forward”.

The move indoors and to more labor-efficient system led to the publishing of Ruth Harrison’s book, “Animal machines: The new factory farming industry” in 1964 which introduced concerns that are still holding strong in today’s society regarding the treatment of livestock and animal rights issues. Advocacy groups that “factory farming” causes unnecessary harm or discomfort to the animals. Because of this belief, laws have been introduced concerning the well-being of food animals housed indoors, including the Humane Methods of Slaughter Act enacted in 1958 to eliminate cruel slaughter acts of food animals and Proposition 2 and HB 5127 in California and Michigan, respectively, to ban the use of gestation crates, broiler cages and veal

crates. Further complicating the issues of animal use and rights is the confusion that the majority of the public have regarding such terms used in food production as ‘organic’ and ‘free-range’. It is possible that some production companies could potentially exploit this confusion with labeling changes to make their product more attractive to certain populations (Harper and Makatouni, 2002). Several studies have evaluated the public’s perception of similarities between humans and non-human animals, with an overwhelming tendency to advocate for animal rights when the subjects received higher scores of perceived similarities (Wuensch et al., 1991). Other factors that influence a human’s perceptions of animals and their rights can include education level, cultural background, past experiences with animals and religious attitudes (Serpell, 2004). This perception of well-being and attribution of human-like qualities to these animals with little scientific evidence are being incorporated into law and ultimately affecting producers’ bottom line. Legislation is passed yearly, although consumers support the laws less when presented with any economic impacts that would ensue, including increased pork prices and income taxes. A study indicated that consumers would support a ban that would cost them only up to $230 per year (Tonsor et al., 2009). Until a balance between science and human perception and motivation can be achieved, further investigation on the production and housing systems currently in place is required.

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2. Microenvironments and Gestation Facilities

2.1 History of Swine Housing Facilities and the Gestation Crate

Housing in swine production has changed dramatically since the introduction of the gestation crate in the 1960s. Animals were kept outdoors, exposed to the elements, variable seasonal temperatures, intense sunlight, predators and microbiotic and zoonotic diseases. For example, Callaway et al. (2005) determined that outdoor swine wallows persistently contained over 49 bacterial species of *Salmonella* that continually cycled between wallows. Similarly, parasitic infections have been noted to occur at a much higher rate in outdoor facilities than indoor facilities, a concern for both producers and consumers (van der Giessen et al., 2007). The transition to indoor facilities has ultimately led to better animal health and reproduction of these animals through heightened husbandry and favorable conditions year-round. A study by Karg and Bilkei (2002) in Hungary evaluated the mortality rates of pigs housed indoors compared to those housed in outdoor facilities. They discovered that the mortality rate of sows was roughly 7% higher in the outdoor facilities than the indoor facilities (Karg and Bilkei, 2002). This was due to various reasons in either location, including higher rates of urogenital tract infections, locomotor problems and heart failure in outdoor facilities compared to more reproductive infections and abdominal torsion in indoor animals. Results of another study indicated significant decreases in reproductive success in sows housed outdoors compared with those housed indoors (Akos and Bilkei, 2004). This leads one to conclude that although animals housed indoors can also suffer from infections or incur injuries, the average lifespan and productivity of a sow indoors can be much greater than those housed outside- a blow to the idealized picture of animal husbandry. It is possible then that the ideal location for this country's swine production is actually indoors with incorporations of ideas and suggestions of many

groups of people, including farmers, scientists, consumers and advocacy groups.

With the movement of animals into indoor facilities, production manuals began to be distributed and readily available to producers. These manuals are based on current and reasonable research and 'tried-and-true' methods and served to provide consistent suggestions on several aspects of housing, including what temperature to maintain thermostats at and how bright facilities need to be. Recommendations, such as those provided in the Pork Checkoff "Swine Care Handbook", are implied for the specific physiology of pigs at one particular point in natural history (National Pork Board, 2002). Also influencing suggested environmental settings were the requirements and safety for human workers (Tripp et al., 1998), placement and perception of environment control devices and subtle differences in the environmental requirements of differently aged and gestating animals (Nichelmann, 1983). A sow stands much shorter than human workers checking the thermostat at a readable level and maintaining cleanliness is not always possible in dim conditions. As most producers know, younger animals, or those with less accumulated subcutaneous fat, have a significantly higher thermal neutral zone (National Pork Board, 2002). Even though gilts used as culled sow replacements are of reproductive age, they remain much leaner and smaller in stature than older sows housed in the same facility.

Typical gestation facilities implement gestation crates- 0.6 x 2.1 m enclosures that allow sows and replacement gilts to sit, stand, lie down, eat, and drink but do not permit fighting between animals (McGlone, 2001). Because of the harmful impact fighting for group dominance among sows has on reproduction and health, crates are used to prevent pregnancy loss and injury during pregnancy (Simmins, 1993). Unfortunately, with the benefit of fight prevention follows a consequence of group behavioral thermoregulation elimination. After hierarchies have been established, sows and gilts often use huddling in cooler temperatures to help maintain body heat,

an action that is barred in gestation crates (Waiblinger et al., 2004). These crates are aligned in rows inside of a single barn to maximize efficiency and consequently productivity, with large fans, cool cells, heaters, and lights placed at strategic locations within the facility. These environmental control devices are utilized to maintain the room as a whole at a specific temperature and light intensity (Baxter, 1984). This gives one the impression that each crate within a facility houses a sow or gilt at the appropriate, recommended setting. However, when measuring the actual temperature or light intensity at different locations in the barn, it is common to see extreme variation in both measures depending on the relative distance from the environmental control devices. This leads to variable microenvironments that average to achieve the recommended settings but with areas of drafts and inappropriate insulation (Jacobson, 2011). Another method of sow housing is in groups- one large pen containing 3-120 animals. These groups allow for thermoregulatory and social behaviors, however, can lead to fighting and serious injuries among sows (Luescher et al., 1990; Harris et al., 2006).

2.2 Current Challenges in Swine Gestation Facilities

In today's gestation facilities, unexplained causes of reproductive failure and health issues continue to negatively impact profitability and animal well-being. The variable microenvironments throughout the facilities, in combination with several factors could be the reasons for these physiological upsets. The development of leaner animals through selection may result in less cold tolerance of these gilts and sows, and therefore, placement too near a cool cell or draft without an increase in feed intake may be detrimental to reproduction and health. Baker (2004) described the increased amount of feed being shuttled into use for thermoregulation could be up to 0.03 kg per degree below the lower critical temperature for a smaller sow. Also described here are the different components that make up an individual pig's

effective environmental temperature, including convection, conduction, and radiation heat loss, as well as evaporative coolers, behavioral adaptations, and changes in feed intake (Baker, 2004) . This can be further compounded by simply housing differently sized gilts and sows at the same environmental conditions. As a result, producers put one group at a disadvantage when determining the temperature of the facility due to discretely differing thermal neutral zones. Furthermore, the configuration and implementation of gestation crates prevent the behavioral thermoregulation or light intensity choices normally performed by pigs housed in larger group pens, possibly leading to increased heat requirements compared with group-housed sows (Geuyen et al., 1984). Concurrently affected for individually-housed sows is the suboptimal light intensity that may occur within some areas of a barn. The recommendation for light intensity in farrowing, breeding and gestation buildings is roughly 160 lx. In the nursery, at least 107 lx and in growing/finishing buildings, roughly 54 lx is required (Stetson et al., 2010). These requirements are effective only if uniformly present throughout the area: a goal that may be lofty depending on the layout of the facilities, producing spaces that are more or less intensely lit. As with the costs that accompany regulating temperature in a facility, lighting costs can also greatly impact a producer's profit and the 'carbon footprint' of a swine barn. To reduce the cost of maintaining a barn at a specific temperature and light intensity, producers would benefit from striking a balance between low energy consumption and ideal settings for swine health and production, as well as employee safety. Research is needed to provide insight on the impact that the environmental variation within a facility has on the reproduction and health of the animals housed there.

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3. Well-being of Swine in Production

3.1 Measurements of Well-Being

At the heart of animal husbandry lies the fundamental compassion for and attention to animal well-being. However, well-being, the state of existence when all needs are met, has long been disputed both in production systems and in legislation. David Fraser (1993), a Canadian animal researcher, stated that animal well-being involves 3 levels of functioning including: high biological functioning, freedom from negative experiences such as hunger, pain, or thirst and positive experiences such as comfort or contentment. The first and second levels, maintaining animals disease-free, well-fed and uninjured, are easier to achieve and are much more tangible than the third which also includes positive experiences. This third level has led to much debate that viciously continues due to the inevitable lack of psychological evaluations of the animals themselves. Through recent history and the evolution of companion animals, the general public has developed a much more anthropomorphized view of animals and their needs. An article by Hirschman et al. (1994) analyzed the various roles that companion animals play in the lives of humans, and the intense bonds that some people share with non-human animals. Because of this humanized outlook on the way companion animals affect the population, food animals are similarly being anthropomorphized and legislation regarding the state of well-being has been introduced to prevent diminished well-being of these animals. Unfortunately, the legislation has very little scientific background incorporated and what results are laws that do not necessarily heighten animal well-being, depending on the needs of the animals. For example, the banning of farrowing crates, seen by the majority of the public as inhumane, results in the death of significantly more piglets than open designs (Marchant et al., 2000). It is for this type of reason that well-being laws and regulations necessitates the study of several aspects of animal health to

use the best possible judgments when possibly changing the majority of housing systems globally. Areas of study that can be used in concert to demonstrate a valuable picture of the situation could include: physiology, behavior, immune function, and reproduction (Stott, 1981).

Some physiological attributes, such as stress hormones, can be significantly altered when an animal perceives a threat to its survival. These stress hormones prep the body for quick escape or coping mechanisms. If the changes in physiology are significant enough, an animal then initiates changes in its behavior to try to alleviate the higher levels of stress hormones (Moberg, 2000). Behavioral responses are the easiest, least costly changes an animal can perform to return to a state of homeostasis. If the behavioral changes are undesirable or ineffective, other systems of the body begin to be affected, such as physiological measures and immune function. Higher levels of stress due to imbalanced homeostasis can cause a change in immune cell function and the ability to fight off diseases. This change is often affected by stress type and duration- acute versus chronic stressors- as well as animal experience, genetics and age (Beerda et al., 1997). Lastly, reproduction in food animals is depressed when the animal encounters severe or prolonged stress. This change is also dependent on the stressor type and duration, but is of the most concern to producers.

Intensive farming has developed to meet the needs of a growing population worldwide. Because of the nature of the production system and the differences between the way animals are domestically raised and the way they would survive in the wild, animals are exposed to many different stressors than encountered naturally. In the wild, these food animals would have to contend with environmental elements such as heat, cold, precipitation and extreme weather conditions, as well as predators and diseases (Martin, 2009). These are all very rigorous threats, however, the stresses evident in modern production systems can range from milder, but still

noticeable environmental elements to handling stresses when being maintained in a facility (Von Borell, 2001). Handling stress can be mild such as moving a sow from one area of a barn to another, to severe such as snaring or transporting. Also a major concern is group mixing which stimulates fighting for dominance among all-aged pigs. The fighting lasts for several days and oftentimes severe injuries result (Arey and Edwards, 1998).

3.2 Thermal Comfort in Swine Production

The way that most swine facilities are set up is that animals of the same age groups travel through the system together (Floyd et al., 1994). For example, piglets are weaned in groups and kept in the nursery until moved into the growing facility as a whole. This allows producers to be more efficient at feeding and health maintenance, as well as gives them the ability to keep differently aged animals at different ambient temperatures. Piglets, young animals and old animals all have very different levels of thermal comfort zones- piglets perform best when kept between 26.6- 32.2°C, finishing pigs between 10.0- 23.9°C and larger sows between 15.6- 23.9°C (National Pork Board, 2002). A thermal comfort zone is the temperature range in which the animal can easily maintain core body temperature without needing to consume significantly more food (Curtis, 1983). Because piglets and younger animals have much less fat content and body mass, their thermal comfort zone is much higher than older animals. Most age groups could potentially survive lower or higher temperatures, but changes in feeding and drinking would be inevitable.

The thermal comfort zones are just one of the methods used by producers in order to maintain high herd health and the well-being of their animals. Although many systems have been evaluated for the different processes involved with maintaining this level of well-being, several of them are coming under fire for their perceived impact on the animals. Because both

producers and the general public seem to have the same interest at heart, more research and integration of several aspects of animal well-being is absolutely required to determine as best as possible the most effective way to achieve food animal well-being.

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4. Stress and its Implications in Swine

4.1 Stress Physiology

Stress, like well-being, has long been debated and is often difficult to define. It is much more tangible within our own species as we know how to describe the perceived cognitive impacts, however, when attempting to attribute the same psychological and physiological effects to animal stressors, it is nearly impossible to determine. Researchers have painstakingly pieced together stress response pathways and effects, the physiological profile and types of stressors that actually affect animals (Levine, 2005). Unfortunately, as with the effects of human perception of animal well-being, animal stress must also rely solely on physiological measures. The stress response is a comprehensive pathway that initiates several methods of coping with an unbalanced homeostasis (Moberg, 2000). This pathway is complex and often leads to most systems of the body being impacted in some way by the downstream effects of a stressor.

Homeostasis is a key component of the stress pathway. The end result of activating this pathway is to counteract the event that tipped homeostasis out of balance (Romero, 2009). By activating the stress pathway, an animal can initiate changes that will most likely help to restore their physiological systems. The main causes of stress for animals in production facilities are heat, cold, limited feed, mixing, social stresses, aggression, crowding, handling, transportation, weaning and restraint (Hemsworth et al., 1986; Francisco et al., 1996; Grandin, 1997; Hicks et al., 1998; Hyun et al., 1998; Rosochacki et al., 2000). These types of stressors are abundant and can all lead to the activation of the stress pathway.

The stress pathway never begins until a stress is actually perceived by an animal. Because of this crucial feature, every animal perceives a stressor differently depending on age, sex, breed, prior experiences and environment (Angelier et al., 2007). Once a stimulus is

perceived as a stressor, the pathway is initiated (Herman and Cullinan, 1997). The perception takes place in the cerebrum and is channeled to the hypothalamus where corticotrophin-releasing hormone (CRH) is released into the hypophyseal portal system, a collection of blood vessels connecting the hypothalamus and the pituitary gland. Once CRH has reached the anterior pituitary, corticotropes- a subpopulation of cells in the pituitary- are stimulated to release adrenocorticotropic hormone (ACTH) (Abel and Majzoub, 2005). ACTH targets cells in the cortex of the adrenal gland, stimulating the release of glucocorticoids, specifically, cortisol (Castro et al., 2011). Cortisol is a steroid hormone that has many target tissues throughout the entire body. Other hormones that are released in a stress response are catecholamines, released from sympathetic nervous system stimulation of the adrenal medulla. Catecholamines, such as epinephrine and norepinephrine, are AA hormones, not steroid hormones, but are also main effectors of the 'flight or fight' phenomena (Jansen et al., 1995). Cortisol also has negative feedback regulation both at the level of the hypothalamus stimulating CRH, and at the level of the pituitary releasing ACTH (Bamberger et al., 1996). This negative feedback is critical in keeping the reaction from becoming uncontrollable and permanently affecting the physiology of the animal.

Cortisol can potentially affect every cell in the body. It achieves its effect by entering the nucleus and becoming a transcription factor, up-regulating the production of specific proteins needed to return the body to homeostasis. Effects of cortisol are vast, ranging from altering metabolism to maturing the lungs of fetuses before parturition. Effects on metabolism include increasing gluconeogenesis which results in higher blood glucose for use in the body tissues, AA mobilization from muscle to be used in gluconeogenesis, and stimulating fat breakdown for energy use (Van Der Boon et al., 1991; Brillon et al., 1995). Aside from direct effects on

metabolism, cortisol can also detrimentally affect the absorption of glucose from the intestines (Li et al., 2009). Glucocorticoids like cortisol can also have profound effects on the immune system and these effects are specific to the type and duration of a stressor- a topic that will be discussed in Chapter 7. Some mineralocorticoids, such as aldosterone, can potentially have some glucocorticoid effects, although their major effects are in the kidneys to help regulate blood pressure (Funder, 1993). The effects that glucocorticoids and stress have on the areas of behavior, physiology and reproduction will be discussed in subsequent chapters.

4.2 Temperature and Light Intensity on Stress Physiology

As discussed previously, there are many types of stressors that a production animal can perceive, even after its domestication. These include extreme cold or heat, handling and transportation stresses and social stress. Temperature has been studied extensively in order to determine the extreme ranges in which animals can remain healthy and productive. Results of several studies have shown that swine have increased cortisol or ACTH responses to elevated temperatures, but not decreased temperatures- as long as feed is provided *ad libitum* (Marple et al., 1972; Siegel, 1983; Bate and Hacker, 1985; Klemcke et al., 1989). The importance of these findings is that a producer may be able to avoid the detrimental effects of low ambient temperatures if pigs are allowed *ad libitum* access to feed. If not, however, or if the pigs are maintained at a temperature that is significantly above their thermal comfort zone, there could be increases in cortisol levels and a negative impact on reproduction and health.

Lighting has been studied in various forms in animal production for the advancement of puberty attainment, improved breeding outcomes and even lactation optimization. The more studied aspect of light is photoperiod, or the amount of time that light is on or the sun is up. Scotoperiod is the term used for darkness or when the sun is down. The amount of time an

animal spends in the light compared to darkness seems to have major effects in many areas of function, including stress hormone concentration, physiology, reproduction and behavior. For example, a study by Minton and Cash (1990) determined that cortisol follows a circadian rhythm that is uninterrupted by cranial sympathectomy. Furthermore, data have shown conflicting effects of changing photoperiod on the serum cortisol concentration of lactating sows (Kraeling et al., 1983). Other areas of light that impacts animal physiology and reproduction that tend to be less studied are light intensity, phototransitional quality and spectrum effects (Stoskopf, 1983). A study performed by Griffith and Minton (1992) demonstrated that the circadian profiles of cortisol and ACTH did not depend on light intensity, but only on photoperiod. There is theoretically an intensity of light that is required to perceive a difference between the photophase and scotophase in order to produce these hormonal circadian rhythms, shown especially in a study of blackheaded buntings, a species of bird, although this has not been as clearly demonstrated in pigs (Kumar et al., 1992). More studies should be done to determine the influence of light intensity on the cortisol concentrations of swine, and of particular importance, those that are gestating.

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5. Behavior of Production Animals

5.1 Behavior and Well-Being

Behavior plays an important role in the well-being of animals. It is a process that allows them to express freely their drives and motivations. With domesticated animals, however, it is unknown what exactly the drives and motivations are and whether or not these behaviors have been altered through the process of domestication from the known behaviors of their feral predecessors (Graves, 1984). Because of this, behavior is evaluated most often as a supplement to other measures such as health and physiology or reproduction. One study by Lindberg et al. (1994) determined that reproductive problems in captive zoo animals can be successfully evaluated and alleviated using behavioral changes in the animal. Another study demonstrated that boars in collection facilities would need roughly 5.3 m^2 of space to perform well, based solely on measurements of specific behaviors in the facilities (Rohrmann and Hoy, 2005). The ethogram of domestic swine, a set of behaviors normally displayed by an animal, is often somewhat redefined as new genetic lines or unique housing systems are introduced (Stolba and Wood-Gush, 1989). Even though this measure is often obscure or vague, it provides valuable information of extreme situations. For example, a producer can easily detect if an animal is content or in severe distress by vocalizations or postures or ill by demonstrating a decrease in activity or feeding behavior. However, to differentiate the well-being status between two animals that are both healthy and not currently in distress is significantly more difficult. A change in behavior is often the first action taken when an animal encounters a stimulus, and generally consequences are severe when these actions fail to alleviate any negative impacts (Moberg, 2000).

5.2 Stress and Behavior

The importance of behavior as a measure of well-being is often highly underestimated. Charles Snowdon (2007), ex-president of the Animal Behavior Society, once stated that "behavior is the link between organisms and environment and between the nervous system and the ecosystem"- a profound summary of the importance of this field in the well-being of all animals. When an animal perceives a stimulus as a stressor, their reactions- vocalizations, postures and positions- are all cues to what type of stressor it is. Psychological and physical stressors have very different responses, although ultimately can have the same consequences (Sutherland et al., 2006). By simply moving away from the stressor, long-term effects can be avoided. However, if it is impossible to move away or change positions to alleviate the stressor, the body's homeostasis is affected (Schulkin, 2004). Acute and chronic stress brings about differing behavioral reactions, described by Ted Friend (1991). Depending on whether the animal is fearful, in pain, frustrated or in a strange location, it may have completely different responses. Animals that are fearful may crouch, defecate, tremble, or sweat, those in pain can vocalize or become aggressive. Frustration leads to behaviors that are displaced, or blocked, and can include chewing on non-food items, an action that prevents an increase in norepinephrine. Finally, chronic stressors can lead to adaptation behaviors, learned helplessness intensification of drives or the absence of normal animal behaviors (Friend, 1991). Other examples of the impacts that stress has on animal behavior include a review on studies that involve maternal stress 'priming' the offspring for the current environment by having a change in hormone profiles during gestation (Kaiser and Sachser, 2005). Furthermore, maternal stress may produce postpartum depression-like behaviors in rats, which lead to decreased care for the offspring- creating a vicious cycle for altered behaviors (Smith et al., 2004).

5.3 Temperature and Lighting Effects on Behavior

Modern production systems often limit the amount of movement of animals in confinement, such as gilts or sows in typical gestation facilities. This can be beneficial to limit aggressive behaviors that can lead to injury or lameness, but also limits expression of behaviors of all types. Sows in gestation crates are able to stand, sit, and lie down in different positions, eat, drink, and play with feeders, waterers, and metal bars. The amount of social interactions in these facilities, however, is limited. Also constrained is the ability to use behavior as a method of thermoregulation. When animals in group pens are cold, they huddle together, maximizing skin to skin contact (Hrupka et al., 2000). When they are hot, pigs lay far apart from one another, maximizing surface area exposed to the air. In gestation crates, producers need to rely on other cues such as panting or the position in which the animals are lying down. Studies using pigs trained to operate thermal panels with their snouts demonstrated that depending on the ambient temperature, and subsequently the amount of heat lost to the environment, pigs chose to either activate the heat panel or huddle with pen mates in order to regain the heat lost (Baldwin and Ingram, 1968).

Feral pigs are very different from domestic pigs. The wild ancestors of modern-day domestic swine are sometimes generally nocturnal animals and seasonal breeders (Graves, 1984). Because swine species lack sweat glands, thermoregulation is performed through panting or implementing wallows to cover themselves in cool mud (Graves, 1984). Domesticated pigs still use panting; however, few facilities provide wallowing areas. These animals use group thermoregulation and change postures, such as lying ventrally or laterally to adjust the amount of contact they have with cool, concrete flooring or metal crate bars (Spoolder et al., 2012). Lateral lying is generally implemented when the animals are warm, whereas ventral lying is favored in

cool conditions. When ad libitum access to water and feed is provided, swine will increase water intake and decrease food intake in hot ambient temperatures (Quiniou et al., 2000). Results are often reversed in cold ambient temperatures, with animals attempting to maintain body temperature by increasing the amount of catabolic reactions taking place after eating (Quiniou et al., 2001).

The effect of light on behavior has been widely studied, however, with most domestic animals, studies tend to focus on photoperiod and not necessarily the intensity of the light provided. It is known that a drop in light intensity is required to initiate release of melatonin and to signal sleep patterns (Zeitzer et al., 2000). This specific caveat of light effect, as previously discussed, requires a certain difference between the photoperiod and the scotoperiod. If the difference in intensity is not significant enough between the two time periods, the animal does not perceive any change, and therefore, melatonin is not released. In humans, for example, the minimum light intensity needed to prevent the release of melatonin for 30 minutes is 393 lux (Aoki et al., 1998). This is important because many producers keep lights on for a specific length of time and then they are shut off. If animals in a dark area of the facility do not perceive a difference from lights on to off, they will not be on the same cycle as the other animals. A study performed by Tast et al. (2001) demonstrated that a light intensity >40 lx was enough for domesticated pigs to inhibit melatonin production, and interestingly, there was no difference in melatonin concentration during the scotophase of photophase light intensities of either 200 lx or 10,000 lx. Photoperiod has also been implicated in the feeding behavior of weanling pigs, showing increased feed intake with extended photoperiod lengths (Bruininx et al., 2002; Reiners et al., 2010). The impact that light intensity has on behavior has been less studied, although darkness and shadows have been shown to frighten piglets (Tanida et al., 1996). Finally, periods

of darkness has led to minimizing the amount of cannibalistic behaviors in growing pigs (Jensen, 1971).

5.4 Stereotypies of Production Animals

Many studies performed on production animals have encountered anomalous behaviors called stereotypies. These behaviors are motions that have been deemed unnecessary to animal survival, but are often performed in patterns and incessantly (Hurnik et al., 1988). Stereotypies are unique to different species, to particular animals and develop due to completely distinctive circumstances during the development of an animal (Mason, 1991a). Examples of these in swine are bar biting or sham chewing, the reasoning behind which has yet to be fully explained (Terlouw et al., 1991). These motions are thought to occur as a result of stress or frustration, although little evidence is available to prove this hypothesis and other theories include activation of the dopamine reward pathway (Mason, 1991a). Several examples in other animals, once thought to be a result of captivity, resulted in discovery of adapted systems for maintaining good health. For example, crib-biting in horses has been thought to be detrimental to the teeth and necks of horses, however, studies show some evidence for an anti-ulcer effect of this behavior (Nicol et al., 2002). Horses chew on inanimate objects, increasing alkaline saliva flow and combat the acidic conditions of the gastric system. Stereotypies may not be fully understood now, but should be evaluated and recorded nonetheless. Individuals not familiar with the current production system may interpret these actions as indicators of diminished well-being, although the true reasons for these behaviors are unknown. Pigs have very strong oral and olfactory senses, which lead some researchers to believe that bar biting and sham chewing is a way for them to experience their surroundings. Others believe that because of these strong senses, pigs undergo a release of endorphins when these senses are stimulated (Krebs, 2012). And still others

believe that this is a sign of stress, boredom and frustration from being housed in a confined system (Mason, 1991b). Behavior is an underutilized tool on many animal production farms. By using the established ethograms of domesticated swine and encouraging more research on the nature and effects of stereotypies, livestock well-being and production rates will likely increase.

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6. Physiology of Production Animals

6.1 Physiological Measures of Well-Being

Several aspects of physiology are crucial to producers and the development of individual animals as well as the herd. These physiological parameters include body weight, feed intake and body temperature, measures of an animal's ability to sustain basal metabolic reactions and homeostasis (Fraser, 1993). Age significantly impacts these measures as younger animals need excess nourishment for growth, gestating and lactating animals require a surplus to provide for developing fetuses and newborns, and older animals simply require an energy balance to maintain weight and body temperature (Britt, 1986; Dourmad, 1991; Owens et al., 1993). These differences control how producers manage their animals and shape the guidelines for dietary requirements (National Pork Board, 2002).

Body weight (BW) is a critical component to animal production. Animals grown for food are taken to market at a specific weight, ideal for meat quality and profitability. Overweight animals have greater concentrations of fat and may be less desirable in the food market (Marcoux et al., 2007). Underweight animals fail to be profitable given the amount of food consumed (a loss for producers) and the return received from slaughter. BW is also essential in non-market animals, such as the breeding stock (Rozeboom et al., 1996). Both over- and underweight animals have difficulties reproducing, are sensitive to inappropriate environments, are more susceptible to disease, and incur more injuries. Animal well-being is greatly affected by body weights that are sub-optimal because of the impact the inappropriate condition has on other aspects of health and reproduction.

To maintain proper BW, an animal must be eating properly. Feed efficiency is a measure of how much weight is gained for the amount feed provided (Kennedy, 1984). This can

be impacted by several factors including environmental, breed, age and housing condition, although it completely relies on feed intake. Feed intake, a measure of motivation to eat, is used to assess animal well-being in several different ways. Animals that are ill, uncomfortable or injured tend to not eat as much as healthy, calm animals. Tooth and mouth problems, leg and foot lameness, illness, air quality and genetics can all greatly impact feed intake (Williams, 1995; Koenen et al., 1996; Johnson et al., 1999). With reduced feed intake consequently comes lowered BW.

6.2 Physiological Measures of Production Animals and Stress

Healthy mammals have a core body temperature that is adjusted to stay at the same level. This aspect of homeostasis is critical in the maintenance of most biochemical reactions (Sawyer and Schlossberg, 1933). It is dependent on many aspects of production, including circadian rhythm, ambient environment and amount of feed available (Refinetti, 1997; Collin et al., 2002). Homeostasis of this ideal core temperature is sometimes shifted when ambient temperatures are too much to handle, either cold or hot, and when easing elements are not available (Morrison and Mount, 1971). One way that animals combat this is through adjusting feed intake. If an animal needs to produce more heat, they eat more, and when they are producing too much, they tend to eat less. This is due to the significant amount of heat produced during normal metabolism and breakdown of nutrients in the body (Refinetti, 2003).

Stressful conditions have long been cited as an obstruction to normal animal physiology. Cortisol released can channel nutrients to critical parts of functioning, starving the less crucial components to immediate survival such as digestion. The type of stressor and the duration in which it is applied greatly impacts the outcome of nutrient channeling, and subsequently, the effect it has on all areas of physiology- including BW, temperature, and feed intake. As

previously discussed, glucocorticoids can have many effects on metabolism and directly on absorption of glucose from the intestines (Li et al., 2009).

6.3 Temperature and Lighting Effects on Physiology

Extreme temperatures are not only stressful to animals, but significantly impact weight gain and core body temperature, as well as respiratory rates and feed and water intake. High ambient temperature can increase core body temperature and depress feed intake, a method of alleviating the production of metabolic heat from digested food (Huynh et al., 2005). High temperatures have also been shown to cause increased rectal temperature and respiratory rates in different breeds of pregnant ewes (Ross et al., 1985). Food intake increases in animals kept in cold environments versus hot environments and are influenced by previous housing situations and thyroid hormone concentrations (Macari et al., 1986). Low ambient temperatures can decrease core body temperatures if excess feed is not available to raise the internal heat production (Koskela et al., 1997). In modern production systems, most animals are limit fed, a method to keep animals at roughly the same size and to keep animals from becoming overweight (Okine et al., 2004). Because of this practice, animals that are in cooler areas of a facility need more of the allowed feed for heat production and can no longer use it for growth.

Light intensity effects on body temperature are not well-known, but a study in humans demonstrated a greater decrease in body temperature under dim lights (50 lux) compared to bright lights (5,000 lux) (Badia et al., 1991). Photoperiod has been shown to increase body weight in newborns, however, light intensity alone is not thought to be a cause (Bruininx et al., 2002). Studies in fish have shown that light intensity impact the size and transparency of prey and amount consumed, although it is not certain whether this is directly related to appetite or simply the ability to perceive the prey (Kestemont and Baras, 2001). Similarly, in human

medicine, the depression symptoms of seasonal affective disorder are known to involve increased food intake during times of lower light intensity and shorter photoperiods (Terman et al., 1989). Research in the area of light intensity and temperature on the physiology of gestating pigs may prove beneficial to producers to optimize well-being and health of their animals.

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7. Immune Function of Production Animals

7.1 Immunology and Well-Being

The health of the breeding herd is important to maintain often vigorous schedules and production deadlines, as well as to ensure optimal animal well-being. Health is evaluated in modern facilities by absence of disease, a method that lacks in pro-activity but is the most efficient. The economic impact of disease is considerably, demonstrated by the fact that illness caused by the Porcine Reproductive and Respiratory virus (PRRSV) alone can cost the country's producers up to $686 million per year in lost revenue and additional cost (Kliebenstein, 2009). Not only does this affect producers and the national economy, but it results in the diminished well-being of the herd. Properly functioning immune systems can greatly improve the proportion of healthy animals and is achieved by proper environmental and physical conditions (Straw et al., 1989).

The immune system of mammals is made up of two halves: the innate and the acquired. The innate immune system is that which an animal is born with, physical barriers, mucous membranes and several primed immune cells that attack anything foreign, such as neutrophils or macrophages (Mestecky et al., 2005). The acquired immune system, however, is much more precise and accurate, although it takes time and exposure to develop (Straw et al., 1989).

The innate system is an army at the ready. Any particle displaying a foreign antigen or 'signal' will immediately trigger action by these cells. Dead or decaying tissue or foreign debris is degraded by macrophages which then release signals such as chemokines or cytokines to attract other cells such as the neutrophils (Strieter et al., 1996). Cytokines are small pieces of proteins that are released to attract other immune cells. Neutrophils and macrophages can display the antigens using receptors and major histocompatibility (MHC) receptors to acquired

immune cells such as T or B cells, and vice versa, creating the link between innate and acquired immunity (Hoebe et al., 2004). The innate cells also cause inflammation which increases blood flow to the infected area (Hawiger, 2001). The inflammation brings more cells to the area in order to help fight off the infection (Granger and Kubes, 1994).

The acquired immune system is a complex system of immune cells that rely on the ability to proliferate after encountering a specific antigen. The cells of this system are primarily the lymphocytes, cells that are produced in the bone marrow and matured either in the bone marrow or the thymus (Thomis et al., 1995). Lymphocytes are divided into T cells and B cells, each with its own responsibility for mounting an effective immune response. B cells mature in the bone marrow and primarily help stimulate other effector cells or provide the memory to a specific antigen for faster, more effective responses in the future (Sallusto et al., 2004). T cells, on the other hand, mature in the thymus to hone proper responses to non-self proteins. This maturation process presents the immature T cells with antigens from elsewhere in the body (Tissue-specific antigens, or TSA) and destroys the cells that have too strong or too weak of a response to self-antigens or MHC receptors (Sprent and Kishimoto, 2001). After maturation, each T and B cell carries antibodies with receptors that match a certain antigen. When the foreign antigen and the corresponding antibody link together, the rapid proliferation and subsequent destruction of the source of the antigen begins if proper co-stimulatory signals are present (Linsley et al., 1991). This eventually leads to a heightened attack by the immune system on the invading foreign particles or microbes.

Many factors impact immune function, particularly species, age, sex and genetics. Some mammals are born with their acquired immunity, others get antibodies passively from their dam during lactation (Brambell, 1958). Pigs specifically inherit acquired immunity during the first

milking where they consume colostrum (Rooke and Bland, 2002). Age affects the immune system in that very young and very old animals tend to have more difficulty fighting off infections than grown, healthy animals (LeMaoult et al., 1997). Different genetic lines of animals also have very different responses to pathogens, even within the same species. Swine breeds may vary with the function and number of immune cells (Sutherland et al., 2005).

7.2 Stress and Immune Function

Stress plays a major role in the functioning of immune cells, and especially on lymphocyte subpopulations. T lymphocytes can further be classified into T helper cells, T cytotoxic cells, T memory cells and T regulatory cells (Broere et al., 2011). Furthermore, the helper T cells can be considered one of 2 significant subtypes, depending on relative concentrations of interferon-gamma (INF-γ) or interleukin-4 (IL-4), -10 (IL-10) or -12 (IL-12): T helper type 1 (Th1) and T helper type 2 (Th2). Th1 cells tend to secrete specific cytokines that stimulate phagocytic activity of macrophages and neutrophils (Spellberg and Edwards, 2001). These cytokines include interleukin-2 (IL-2) and INF-γ. Th2 cells secrete cytokines that increase the activity of B cells to produce antibodies against the specific target (Spellberg and Edwards, 2001). There is, however, some overlap between the two subtypes, as each has either a stimulatory or inhibitory effect on the other branch.

Glucocorticoids, produced in higher amounts during stressful periods, tend to decrease the amount of interleukin-5 (IL-5), interleukin-6 (IL-6), IL-12 and IFN-γ and tumor necrosis factor-alpha (TNFα) and increase the amount of IL-10 (Salak-Johnson and McGlone, 2007). This change causes a shift in the ratio of Th1 and Th2 cells by causing a negative feedback on macrophages and IL-12, causing an up-regulation in the action of Th2 cells, decreasing inflammation (Elenkov, 2004; Li et al., 2007). Interestingly, IL-12 has a modulatory effect on

both IL-4 and IFN-γ- its inhibition leading to the up-regulation of IL-4 and down-regulation of IFN-γ (Wiegers et al., 2005). Results of some studies show heightened immune function in times of increased stress while others show diminished function as well as alterations in innate immune system functioning (Dhabhar 2002, 2009; Stanger et al., 2005; Borghetti et al., 2006; Stolte et al., 2008). The difference between these studies is that the stressor type and length is varied. Acute stress has been shown to increase immune system functioning, while chronic stressors depress it (Dhabhar, 2009). Psychological stressors tend to have more lasting effects so their effects are more similar to the chronic stressors whereas physical stressors can be acute or chronic depending on the length of the applied stimulus (O'Leary, 1990; Segerstrom and Miller, 2004). Because the factors affecting both stress and immune function vary so greatly, producers and consumers should be aware of the types of stress that can negatively and positively impact immune function as well as the effects both have on an animal's well-being.

7.3 Temperature and Lighting Effects on Immune Function

Temperature is a physical stressor that has, for the most part, been studied as a stressor in addition to another stimulus. High ambient temperature, along with other types of stress, has been shown to increase immune functioning as evidence of an acute stress. Results of several studies demonstrated decrease immune functioning with subsequent lowered white blood cell counts, blunted antibody production, and elevated mortality rates of chickens housed in high temperature (Ferket and Qureshi, 1992; Zulkifli et al., 2000; Mashaly et al., 2004). Likewise, cold temperatures- not balanced for by increased feed intake- can have detrimental effects on immune function due to the shunting of available energy to maintaining body heat and metabolism instead of the ability to mount an appropriate immune response (Demas and Nelson, 1998).

Light impacts on the immune function of swine has studied extensively in lactation and weaning periods, the majority of studies focused on the photoperiod or seasonal effects of light. Photoperiod has been shown to alter the immune response of sows during late gestation and lactation, changing lymphocyte proliferation to a specific antigen in sows in short day versus long day photoperiods (Niekamp et al., 2006). Furthermore, studies describing the role of melatonin in immune function have demonstrated that melatonin can help to coordinate reproduction and immunology in order to achieve optimal energy shunting to immunological needs during times of low seasonal fertility (Nelson and Demas 1997; Nelson et al. 1998; Nelson, 2004). Conversely, light intensity has been less widely studied, with notable studies attributing any effect of light intensity on immune function to possible changes in melatonin secretion with varied light levels in some animals (Park et al., 1999; Blatchford et al., 2009).

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8. Reproduction of Production Animals

8.1 Reproductive Physiology of Swine

In food animal production systems, reproduction is the cornerstone of progress. When severe reproductive issues affect the breeding herd, the result is less product and output, and consequently, revenue. Swine have a very complex reproductive system, but it has been fine-tuned through selection to meet certain requirements for a successful breeding life. These requirements have led to advancements in breeding and gestation, both with timing strategies and with hormone adjustment products (Whittemore, 1998). However, problems still arise, with causes sometimes unknown to producers. These problems may be due to the animal itself or other, outside factors such as physiological or environmental stressors.

The estrous cycle of swine, or the rhythm in which a female fluctuates between high and low fertility, is roughly 21 d (Knobil and Neill, 2006). During this time, the sow develops several follicles on each ovary and ovulates the contained oocytes into the oviduct where they are met by sperm if inseminated during the period of receptivity (Perry, 1946). Once the oocytes are fertilized, a signal is released to ensure the estrous cycle pauses to allow development of the oocytes into embryos. If the oocytes released are not fertilized, fail to implant or do not produce the pregnancy signal, the estrous cycle restarts to allow another chance for fertilization (Knobil and Neill, 2006).

Estrus is an important time point for effective production systems because of the critical breeding procedures necessary for herd sustainment. Fortunately, sows give producers excellent, visible cues to the status of their receptivity (Coffey et al., 2002). Day 0 of the estrous cycle is considered the day of ‘first standing estrus’. When in estrus, a sow prepares for breeding and fertilization by a boar, and displays this by stiffening all voluntary muscles (Cronin et al., 1982).

This stance signals to the boar that she is capable of handling a mounting weight and is receptive to breeding at this time. Standing estrus normally lasts roughly 50 hours in sows and 40 hours in gilts, allowing for insemination to occur just before the ovulation of eggs from follicles (Diehl et al., 1998). Since swine are polytocous animals, each ovary releases several eggs into the oviduct. Once released into the oviduct, the eggs are met in the oviduct by sperm cells if the sow was inseminated within the specific timeframe of standing estrus, and in some cases, just after standing estrus. The behavior seen by producers at this particular time is the result of complex hormonal changes during the estrous cycle (Andersson et al., 1984). Swine have large ovaries that develop many follicles in response to follicle stimulating hormone (FSH), each containing a single oocyte (Knobil and Neill, 2006). Some follicles begin to develop with increasing amounts of fluid accumulating, while others begin to undergo atresia, or regression, in a process called selection. The dominant follicles produce estradiol along with another hormone called inhibin to suppress the concentration of FSH circulating (Knobil and Neill, 2006). This lowering of FSH prevents an unlimited number of follicles from developing. Follicles help the oocyte to mature and contain two different cell types: granulosa and theca cells (Knobil and Neill, 2006). These cells provide nutrients to the oocyte and produce the steroids necessary for ovulation. Theca cells are stimulated by luteinizing hormone (LH) from the anterior pituitary to begin producing androgens. The androgens are then shuttled into the neighboring granulosa cells where they are synthesized into estrogens. This accumulation of estrogens initiates a large increase in LH called the LH surge. The surge in LH causes ovulation of the follicles that are ovulatory-sized and stimulates the release of oocytes from follicles. This surge in LH typically occurs between 8 hours before until 12 hours after the onset of behavioral estrus (Tilton et al., 1982). A study by Almeida et al. (2000) developed an equation to determine the time of ovulation in gilts based on

the duration of estrus (Time of ovulation= (duration of estrus)*0.409 + 22.7) in order to provide an optimal time for insemination.

After ovulation, the follicles that ruptured and released oocytes undergo dramatic changes from follicles to corpora lutea (CL). CL develop when theca cells become luteinized and begin to produce progesterone, a hormone necessary to sustain pregnancy, after an alteration in enzyme expression. If the eggs are successfully fertilized, they implant into the uterine horns and begin to proliferate both in embryonic and placental size. This proliferation and subsequent estrogen elevation, signals to the newly developed CL to continue making progesterone (Niswender et al., 2000). The CL are progesterone-producing structures that form in the place of the ruptured follicles. This transition from estrogen-producing follicle to progesterone-producing corpus luteum occurs after ovulation and is under the control of theca and granulosa cells. If no developing embryos and placental cells (trophoblasts) give a signal to the CL, they regress through PGF_2 and lead to the elevation of gonadotropin releasing hormone (GnRH) from the hypothalamus, in turn, elevating both FSH and LH from the pituitary (Sammelwitz et al., 1961; Spencer and Bazer, 2004). This causes the estrus cycle to continue and the sow returns to estrus in roughly seven days (Coffey et al., 2002). Considering the role of CL in pregnancy sustainment, a study by Spies et al. (1959) determined that supplemental exogenous progesterone after mating resulted in significantly more solid CL, however, the weights of these CL were significantly lower.

Pregnancy can be detected by a variety of methods, including behavioral changes (lack of return to estrus), ultrasonography, or blood tests for progesterone or PGF_2 (Flowers and Knox, 2003). The gestation length of swine is approximately 114 d, split into early, mid and late periods. During early gestation, the embryos implant and begin to proliferate, sending the signal

to the CL to continue producing progesterone (Knobil and Neill, 2006). This is a very critical time period and producers are generally advised to minimize the amount of stress animals at this point of gestation endure (Einarsson et al., 1996). During gestation, embryos either develop into fetuses or are absorbed, which may be due to genetic factors or spatial organization within the uterine horns (Chen and Dziuk, 1993; Marsteller et al., 1997; Vonnahme et al., 2002). Fetuses continue to develop until parturition, or birth, usually to an average litter size of roughly ten piglets (Gillespie and Flanders, 2010).

After parturition, the sow begins lactation, the length of which is generally determined by producers to be between 21 and 28 d (National Pork Board, 2002). The time periods of gestation and lactation keep progesterone elevated, which suppresses the production of estradiol. At weaning, the progesterone block is released and follicles begin to develop (Stevenson and Britt, 1980). This marks the beginning of a new estrous cycle. Producers use this phenomenon to help synchronize sows to come into estrus within the same timeframe. Gilts, younger animals that have not yet given birth to a litter, do not undergo lactation and therefore, do not have this effective synchronization tool. As a result, producers use MATRIX (altrenogest), a synthetic progesterone, as a feed additive for 14 d to mimic a lactation period. This causes gilts on the regimen to come into estrus between 5 and 7 d after the last feeding of this product, giving the producers the ability to synchronize the entire breeding herd (Cassar, 2009).

8.2 Current Challenges in Swine Reproduction

Because of the complexity of the reproductive system, several problems can arise during the production-manipulated processes of breeding, gestation and lactation. During breeding, producers use different methods of estrus detection, including boar exposure or artificial pheromones, the back pressure reflex test and/or observing mounting in group-housed sows

(Diehl et al., 1998). However, some sows do not respond in the same way to boar presence, or if the boar is housed too close to the sows that are being observed, they may become accustomed to the pheromones and fail to stand at the proper time (Evans et al., 1994). Leg or foot problems can hinder the back-pressure reflex test.

The vast majority of production systems use artificial insemination (AI) to service their breeding herd, not natural service seen in some smaller, family-owned systems. An ejaculate is collected from a boar, on-site or from a boar stud facility, and diluted into doses for several sows (Levis et al., 1998). For sows that are in standing estrus, a catheter is inserted and locked into the cervix and a dose of extended semen is injected into the cervix or uterus (Levis et al., 1998). Several obstacles can arise with this procedure, however the risk of sexually transmitted infections (STI) is dramatically lower compared to natural service (Smith and Dobson, 1992). If the catheter is not locked into the cervix properly, or if the sow is not in a full standing estrus, the semen dose can leak back out of the vagina (Levis et al., 1998). This can lead to an unsuccessful insemination and a non-pregnant sow, reducing the efficiency and productivity of the breeding herd.

Gestation systems differ dramatically depending on the long term goals of the producers. The majority of modern producers house their sows in gestation crates to minimize the amount of stressors the sows are subject to during the critical time points of gestation. Other housing facilities include tether stalls, group housing and outdoor sites. Because of the controversy with housing systems, some states have been required to change their facilities, with possible increases in stress during gestation.

8.3 Stress and Reproduction

Reproduction can be impacted by several different stressors abundant in a production

facility. This includes mixing groups and handling and environmental stressors. Some studies have reported an effect of stress on the gametes of animals including spermatozoa quality and slowing of cleavage of the oocyte after fertilization (Einarsson et al., 2008; Schreck et al., 2001). Stress is thought to increase the action of opioid-producing cells and subsequently decrease GnRH pulse frequency and increases suppression of LH by norepinephrine (Dobson et al., 2003). This could lead to inhibition of the estrous cycle and decreased fertility. Psychological stressors in humans and other animals have been studied extensively, demonstrating significant infertility in the overworked, captive or traumatized and in environmentally-stressed animals (Sandler, 1968; Hemsworth et al., 1986; Johnson et al., 1991; Negro-Vilar, 1993; Sanders, 1997; Rideout et al., 2005). Some studies have shown a difference in effect of stress during pregnancy depending on the point in gestation in which the stress occurs. A study by Razdan et al. (2004) demonstrated an increased resistance to pregnancy loss during the time of maternal recognition (roughly 13-14 d gestation). Early gestation has been a controversial time period for stress of animals. Some studies report increased embryonic losses, whereas others state there is no impact during this time of gestation (Edwards et al., 1968; van Wettere et al., 2008). Mid gestation tends to be much more stable and has been shown to be resilient to many major stressors (Kattesh et al., 1980; Omtvedt et al., 1971). Late gestation is more sensitive to outside influences and stressors as the sow becomes heavier and the physiological demand increases, however, some studies have shown a high resistance to stress during late gestation (Omtvedt et al., 1971). Piglet development is also sensitive during late gestation because of the growth and changes in hormone-sensitive organs such as the adrenal glands (Kanitz et al., 2006). There are some instances in different animal species where a stress response is biologically resisted in order to promote reproductive success, such as animals with very short breeding windows, very

aged animals and in social groups with small breeding windows for submissive animals (Wingfield and Sapolsky, 2003). This phenomenon has not been described in domesticated animals.

8.4 Temperature and Lighting Effects on Reproduction

Temperature has long been studied as a key component to effective reproduction. In animals that are housed above their thermoneutral zones, reproduction losses can occur in many ways. Puberty in swine has been shown to be affected by elevated ambient temperature with increased age at puberty and higher incidence of cystic follicles at the time of puberty, possibly as a result of diminished LH, FSH and GnRH secretion (Flowers et al., 1989; Flowers and Day, 1990). After puberty, heat stress can impact reproduction by altering the concentrations of several reproductive hormones including estradiol and progesterone. Progesterone has been shown to be decreased in heat-stressed versus control gilts, and estradiol concentrations higher in heat-stressed (Hoagland and Wettemann, 1984). Heat stress can also have an effect at ovulation, with a study by d'Arce et al. (1970) demonstrating a decrease in ovulation rate with increased duration of heat stress (Teague et al., 1968). During pregnancy, heat stress, like stress of other origins, can have variable effects depending on when the heat stress occurs. In early gestation, some studies have shown a delay in estrus expression and decrease in embryos when gilts are heat-stressed for the first 15 d post-breeding, especially from d 1-5 post-breeding (Warnick et al., 1965; Tompkins et al., 1967; Edwards et al., 1968). Mid gestation was shown to be the most resistant to heat stress in a study by Omtvedt et al. (1971). One study regarding the performance of sows in late gestation under heat stress determined that sows are more likely to die of heat exhaustion than lose a litter, however, with the increased stress on pregnant sows, the incidence of mortality is not insignificant (Heitman et al., 1951). Heat stress can also play a major role in

lactation with a reduction of milk production, feed intake and weaning weight of sows and an increased interval to 'return-to-estrus' (Prunier et al., 1997; Johnston et al., 1999). These effects have been shown to be alleviated by providing evaporative cooling systems, and by increasing fat content and decreasing fiber content in feed given to lactating sows (Black et al., 1993).

Cold temperatures have been less extensively studied as many reproductive problems could be prevented by providing an adequate amount of feed to maintain all bodily processes as well as the pregnancy (Noblet et al., 1990, 1997). If sows and gilts are allowed to make contact with neighbors, the effects are even more minimal (Verstegen and Curtis, 1988).

Light is a critical component for seasonal breeders and has been studied mostly in photoperiod experiments. Light intensity has not been well studied in reproductive experiments. Longer photoperiods have been shown to shorten the age at puberty of gilts with less LH pulses (Ntunde et al., 1979; Diekman and Hoagland, 1983; Paterson et al., 1992). A study by Perera et al. (1984) examined the effects of long days on the duration of estrus and determined that behavioral estrus was prolonged during longer photoperiods. Another study demonstrated that although sows and gilts are usually housed under artificial lighting regimens, they still may display some seasonal fertility variation (Hälli et al., 2008). Interestingly, one melatonin receptor present in swine has been linked to the variation in litter sizes seen across different seasons (Ramírez et al., 2009). Lactation has been shown to be sensitive to several outside influences such as ambient environment and photoperiod. In a study performed by Stevenson et al. (1983), lactating sows were shown to have increased post-weaning litter weights and well as more synchronized return-to-estrus after 16 h supplemental light. Light intensity alone has not been widely studied in swine reproduction as the light intensity needed to produce a difference between light and dark is very low and supplemental lighting does not cause production of

higher concentrations of melatonin (Griffith and Minton, 1992; Tast et al., 2001). Light and temperature have also been studied together, however, the overwhelming conclusion is that temperature has much more of an effect than photoperiod on litter performance and gonadotrophin secretion (Prunier et al., 1994, 1996).

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9.0 Conclusions and Future Outlooks

In order to ensure the sustainment of the pork industry- a valuable asset to many global economies- the cause of the unexplainable reproductive losses and health issues need to be evaluated. Because of the design of modern day gestation facilities, one possibility is that the variation in each sow's microenvironment within the facility causes some to be housed in inappropriate environmental conditions. Research needs to be done to determine if variation in the temperature or light intensity, or an interaction between the two environmental components is the cause of these failures. Without a clear cause or logical solutions, the fate of this industry is unpromising.

10. Effect of variability in lighting and temperature environments for mature gilts housed in gestation crates on measures of reproduction and animal well-being[1].

10.1 Abstract

The effects of room temperature and light intensity prior to breeding and into early gestation were evaluated on the reproductive performance and well-being of gilts housed individually in crates. In eight replicates, estrus was synchronized in mature gilts (n = 198) and after last feeding of Matrix were randomly assigned to a room temperature of 15 °C (COLD), 21°C (NEUTRAL) or 30 °C (HOT), and a light intensity of 11 (DIM) or 433 (BRIGHT) lx. Estrous detection was performed daily and gilts inseminated twice. Blood samples were collected before and after breeding for determination of immune measures and cortisol concentrations. Gilt ADFI, BW and body temperature were measured. On d 30 post-breeding, gilts were slaughtered to recover reproductive tracts to evaluate pregnancy and litter characteristics. There were no temperature x light intensity interactions for any response variable. Reproductive measures of follicle development, expression of estrus, ovulation rate, pregnancy rate (83.2%), litter size (14.3 ± 0.5), and fetal measures were not affected by temperature or lighting ($P > 0.10$). Gilts in COLD (37.6 °C) had a lower ($P < 0.05$) rectal temperature than those in NEUTRAL (38.2 °C) and HOT (38.6 ± 0.04 °C). Both BW gain and final BW were greater ($P < 0.0001$) for gilts kept in HOT than those in NEUTRAL or COLD environments. Gilts housed in the HOT environment made more postural changes than did those kept in either COLD or NEUTRAL temperatures. Gilts kept in the HOT temperature spent more total time lying and more time lying laterally compared to those gilts housed in the NEUTRAL

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or COLD rooms. Total white blood cells and the percentage of neutrophils, as well as neutrophil-to-lymphocyte ratio were all influenced ($P < 0.05$) by temperature but there was no effect of light or interaction with temperature on other immune cells or measures. These results indicate that temperatures in the range of 15 to 30 °C or light intensity at 11 to 433 lx do not impact reproduction during the follicular phase and into early gestation for mature gilts housed in gestation crates. However, room temperature does impact physiological, behavioral, and immune responses of mature gilts and should be considered as a potential factor that may influence gilt well-being during the first 30-d post-breeding.
Keywords: Fertility, Gestation crate, Gilts, Lighting, Temperature, Well-being

10.2 Introduction

Unexplained reproductive failure is common in swine breeding herds and could be related to variation in environmental factors such as temperature and light intensity. High temperatures have been shown to affect puberty (Flowers et al., 1989), litter size, pregnancy rate (Edwards et al., 1968; Omtvedt et al., 1971), feed intake, body temperature, plasma cortisol concentration, and immune function (Abilay et al., 1975; Oki and Atkinson, 2004). Light intensity has also been shown to affect age at puberty (Diekman and Grieger, 1988) and cortisol (Leproult et al., 2001). However, the relationship between temperature and light intensity and interactive effects on fertility and physiology of female swine remains unknown.

A temperature range of 13 to 29°C with a minimum light intensity of 162 lx is the current recommendation for housing breeding swine in confinement (Midwest Plan Service, 2001) but new EU requirements set 40 lx as the minimum lighting level. Despite the ability of modern confinement systems to regulate temperature and lighting, there is considerable variation within

an individual animal housing system. For example, variation has been reported for the temperature of sows in different crate locations within a building (Verstegen and Curtis, 1988). Unpublished data from our laboratory also indicates variation within breeding and gestation facilities with individual crate temperature varying ± 13 °C (range:12 to 26 °C) and light intensity varying by ± 233 lx (range: 10 to 243 lx). With this potential variation in temperature and light intensity for individually housed breeding swine, it is important to ascertain whether the microenvironment for breeding females housed in crates is related to unexplained failures in reproduction and reduced well-being. This is especially important because most breeding females in North America are located on large farms (PigCHAMP, 2010; USDA-NASS, 2010) with approximately 70% of sows housed in gestation crates. In crates, females have only limited ability to react to thermal or lighting stressors (Verstegen and Curtis, 1988).

The objectives of this study were to evaluate the impact of different temperature and light intensities on reproductive fertility, and behavioral and immune responses for mature gilts housed in crates from the initiation of the follicular phase through breeding and early gestation.

10.3 Materials and methods

The use of animals for this experiment was approved by the institutional animal care and use committee of the University of Illinois.

Experimental Design

This experiment was performed in eight replicates between September 2008 and February 2010. The gilts used (n = 198) were PIC C-22 (replicate 1, n = 36), PIC C-29 (replicates 2 to 6, n = 18/replicate) and Genetiporc Fertilis 25 (replicates 7 to 8, n = 36/replicate).

At approximately 160 d of age, gilts were moved from a finishing barn to a breeding and gestation facility where they were maintained in pens during the selection process. Animals with known estrous events were then moved into individual crates located within the gestation barn and estrus synchronized by feeding 15 mg/gilt/day a synthetic progestagen as a top dress on a small amount of feed for 14 consecutive days (Matrix, Merck Animal Health, Summit, NJ). Three days prior to last feeding of Matrix (**LFM**), BW and rectal temperature were obtained as well as blood samples for baseline cortisol and immune measures. At 0700 h following LFM, gilts were randomly assigned to treatment by BW and moved into individual crates within their assigned treatment room. Gilts were assigned using a 3 x 2 factorial arrangement of treatments to 1 of 3 temperature levels (**COLD**: 15 ˚C; **NEUTRAL**: 21 ˚C; and **HOT**: 30˚C) and 1 of 2 light treatments (**DIM**: 11 lx; **BRIGHT**: 433 lx). Each treatment room was set at the desired temperature and each room (within temperature treatment) was divided in half for the different light treatments. Lights were automatically set to turn on at 0600 h and turn off at 1800 h to provide a 12/12 h lighting regimen. Gilts were individually fed 2.73 kg of a standard gestation diet once daily at 0600 h and provided *ad libitum* access to water.

After LFM, gilts were checked for estrus once daily at 1500 h using 2 min fence-line exposure to a mature boar with application of the back-pressure test. To assess follicle growth, transrectal real-time ultrasonography was performed at 1400 h every other day from LFM until onset of estrus using an Aloka SSD-500 (Tokyo, Japan) with a 7.5 MHz linear transducer (Knox and Althouse, 1999). At the onset of estrus, ultrasonography was performed daily at 1400 h to assess number and size of follicles and determine time of ovulation. Gilts were checked for estrus once daily at 1500 h and AI performed at 0 and 24 h after onset of estrus. Gilts were inseminated using pooled semen (ejaculates from 3 to 5 boars) from PIC or Genetiporc

commercial boar studs with each dose containing approximately 3.0 x 10^9 motile sperm/dose. Any gilt not detected in estrus by d 8 after LFM was considered anestrual. All animals were maintained in their crate in the treatment rooms for approximately 5 weeks. On d 14 following detection of estrus, gilts were nose-snared in their crate for ≤ 2 min while a blood sample was collected by jugular veni-puncture into a Vacutainer (Becton Dickinson, Franklin Lakes, NJ) tube. Blood samples were allowed to clot at room temperature for 1 h and were stored at 4 °C for 12 h before centrifugation at 400 x g at 4°C for 15 min. Serum was transferred into polypropylene tubes for storage at -20 °C until analysis for progesterone concentration. Pregnancy status was assessed on d 26 using transabdominal ultrasonography. Gilts were sent to a local abbatoir at approximately d 30 of gestation and reproductive tracts were collected for determining ovarian and pregnancy status, litter size and fetal measures. Day 30 was chosen as the end point for this study to determine whether any reproductive abnormalities could be associated with the most commonly reported reproductive failures of anestrus, regular and irregular returns, early pregnancy failure or embryo loss (Teague et al. 1968; Omtvedt et al., 1971; Griffith and Minton, 1992a; Love et al., 1993; Dalin et al., 1997; Koketsu et al., 1997; Knox et al., 2012). Ovaries and associated structures were analyzed for number of corpora lutea and abnormalities. These abnormalities could include bursal cysts on the oviduct, thin walled follicular cysts > 12 mm on the ovaries, or luteal cysts which were corpora lutea ≥ 12 mm that had thick luteinized walls with a fluid-filled cavity. Uteri were also analyzed for structural malformations or infection. Healthy fetuses were removed from pregnant uteri and counted and weighed (± 0.01 g), and crown-rump length measured using a caliper (± 1.0 mm). Degenerating fetuses were also counted, weighed and measured when possible and were identified by smaller size and weight (≤ 1 SD below the average healthy fetal litter weight and size) and darker color.

Animal housing and climate control

The experiment was conducted in a specifically designed single structure research facility subdivided into 3 identical rooms and located within an environmentally controlled swine gestation facility at the University of Illinois Swine Research Center. Figure 1 illustrates the layout of the experimental facility. The rooms were identical in layout, air control, curtains, flooring, space, equipment, lighting and wall insulation ($r > 11$). The rooms shared a common pit and air supply system. Due to practical considerations for temperature control throughout all seasons of the year and system design limitations, it was necessary to choose the north room as HOT, the center room as NEUTRAL, and the south room as COLD. This allowed consistent temperature control with and without animals in the rooms for the 7 wk experimental periods. Each room was 6.71 x 6.40 m and constructed over a partially slatted concrete floor. A common drainage pit spanned the entire length of the facility, but airflow between the different rooms was prevented using vinyl curtains that were submerged 60 cm into the liquid in the pit at the interface between the rooms. The pit liquid level was maintained to reach the desired level of the pit curtain for all replicates. The pit was not emptied or charged during a replicate and most of the fluid below the pit curtain was free to flow along the length of the building. Each room contained 12 crates with each crate measuring 0.61 x 2.13 m. The front of the crate was mounted on solid concrete flooring while the rear of the crate was positioned over concrete slats. A black polyethylene and nylon curtain (0.48 kg/m^2) was used to divide each treatment room in half for application of DIM or BRIGHT light intensities. Air temperature for the 3 rooms was provided by a direct expansion refrigeration system with a single air cooled condensing unit located

outside the barn. This unit varied the cooling capacity to match the demands from the individual rooms. Each room contained a ceiling mounted forced air system with cooling coils. Each room had its own thermostatic control system, valves, electric reheat, exhaust fans, dampers and aluminum fresh air ducting. The heating and cooling system was calibrated to turn on or off when air temperature at the sensor located on the ceiling deviated >0.55 °C from the treatment set temperature. The ceiling mounted temperature control unit was installed in the center of the room with symmetric orientation to allow the curtain to separate the room in half to allow equal airflow on both sides of the curtain. The system was designed to provide a minimum 0.94 m^3/s of circulating air and a minimum 0.094 m^3/s of fresh air, introduced into the discharge airstream of each room to mix with conditioned air. The fresh air provided with a single fan blower unit was tempered with proportional electric heat at entering temperatures below 15°C to minimize cold air drafts and air stratification within the rooms. The supply air was balanced with dampers for equal distribution between the 3 rooms. During a pre-trial test period with animals in the rooms, 12 air quality measures were obtained from each treatment (temperature x lx) location. We obtained a mean 20.4 ± 0.3% O_2, 2.1 ± 1.4% CO, and 16.8 ± 2.3% NH_3. Based on these data we did not measure these parameters throughout the study. During the experiment, there was no evidence of poor air quality with pigs showing signs of watery eyes, ocular discharges, respiratory distress or poor health. There was also no evidence of a strong smell of ammonia detected by the investigators or farm personnel. Fluorescent light fixtures were installed over all alleyways to provide uniform lighting at each pig space. Supplemental fluorescent light fixtures were installed above the front of the crates to achieve the higher lighting intensity (433 lx) at the eye level of the pig.

For each replicate, weekly room temperature, humidity and light intensity measurements were obtained for each room during the 5 wk treatment period. Measurements were obtained at pig level (approximately 0.6 m from the floor) at each individual crate location. Humidity and temperature readings within each room were obtained using an Omegaette® reader (Omega Engineering, Inc., Stamford, CT) with an accuracy ±0.7 °C for temperature and ±2.5% for relative humidity. Averaged over all replicates, the temperatures (mean ± SE) within each of the treatment the rooms were 29.8 ± 0.1°C for HOT (CV = 1.5%), 20.3 ± 0.2°C for NEUTRAL (CV = 3.1%), and 13.9 ± 0.2°C for COLD (CV = 3.9%). Humidity measurements averaged 78.3 ± 0.4% for HOT (CV = 5.9%), 80.3 ± 0.3% for NEUTRAL (CV = 5.2%), and 75.6 ± 0.5% for COLD (CV = 8.3%). Light intensity was measured using a Lux Meter-Foot Candle Meter (Sper Scientific, Scottsdale, AZ) with an operational detection limit of 0 to 400,000 lx (accuracy range: ± 0.5 to 3.5%). Throughout the study, light intensity for DIM rooms averaged 10.9 ± 0.3 lx (CV = 32.6%) and the light intensity for BRIGHT rooms averaged 432.7 ± 3.8 lx (CV = 11.4%).

Physiological measurements

All gilts were weighed 3 d before to treatment allotment and once again before slaughter to determine starting and ending BW and total BW gain. Rectal temperature was measured using a veterinary digital thermometer (accurate to ± 0.2°C, Model TM99A, Cooper Instruments Corp., CT) while the gilt was un-restrained and standing. Body temperature was recorded before the experimental period and then on d 0, 6, 13, 20, 27, and 34 during the treatment period. Average daily feed intake was estimated by weight of feed delivered and residual feed 24 h later on d 6, 13, 20, 27 and 34. Plasma cortisol was measured on a random subset of gilts (12 gilts/treatment) on d -2, 5, 12, 19, 26 and 33 posttreatment. Blood samples (4 mL) were collected as previously

described using heparin-coated Vacutainer tubes (Becton Dickinson). Heparin tubes were then centrifuged at 400 x g for 15 min at 4 °C to collect plasma for storage at -20°C until analysis.

Behavioral measures

Postural changes were obtained on the same subset of gilts (n = 12 gilts/treatment) used for cortisol measures. Our choice for behavioral measures to assess for gilt behavior related to housing and stress was based on selected measures from previously published studies (Bergeron et al., 1996; Anil et al., 2002; Boyle et al., 2002; McGlone et al., 2004). Observations related to postural changes were initiated at 0630 h and continued until 1800 h on each Monday and Tuesday during the 5 wk treatment period. Two video cameras were used for each temperature and lighting treatment location with cameras placed in the corners at ceiling height to allow all the animals to be viewed at the same time. Cameras for each room recorded onto a single tape using a multiplexer. The video tapes were played and used to visually record the occurrence of postures and the associated time on the video tape. Data were then copied into a spreadsheet and calculations performed to determine the duration and proportion of recorded time in a particular posture. All observation and duration measures for postures were standardized to 24 h to account for differences when the actual recording time or useable video was less than the 24 h session. Position changes were determined for the number and times an individual pig changed positions between standing, sitting and lying. Within the lying position, animals were further classified by occurrence and time lying in the ventral or lateral position and by the type and duration of contact they had with a neighboring lying pig. Time of lying was classified as either lateral or ventral and further classified by contact with neighboring gilts as 1) Full (majority of dorsal surface in contact with adjacent gilt), 2) Moderate (some dorsal contact with adjacent gilt, but lying in an angled position), and 3) None (no dorsal contact with adjacent gilt).

Immune cell function

The same subset of gilts used for behavioral and cortisol measures (n = 12/treatment) was used for immune cell measures. Gilts were nose snared and blood samples (6 mL) collected via jugular venipuncture using EDTA tubes on d -2, 5, 12, 19, 26 and 33 of treatment. Samples were kept on ice until processing. Total white blood cell counts were made electronically using a Coulter Z1 particle counter (Beckman Coulter, Inc., Miami, FL) by adding 10 μL of whole blood to Isoflow (10 mL; Beckman Coulter), and red blood cells were lysed with Zap-o-globin (Beckman Coulter). Whole blood smears were made, fixed in methanol, stained with Hema-3 staining system (Fisher Scientific, Houston, TX) and viewed under a light microscope to determine leukocyte differential counts.

Whole blood was diluted with Roswell Park Memorial Institute (RPMI) medium (Gibco, Carlsbad, CA) layered over Histopaque 1077 (density = 1.077 g/mL; Sigma, Saint Louis, MO) and 1119 (density = 1.119 g/mL; Sigma Aldrich) and centrifuged at 700 x g for 30 min at 25 °C. Lymphocytes were removed from the 1077 layer and neutrophils were removed from the 1119 layer. Isolated cells were washed twice in RPMI medium, resuspended and counted. For neutrophils, red blood cells were lysed. Cell concentrations were adjusted with RPMI medium based on respective requirements of the specific immune assays.

Neutrophil chemotaxis was measured using an assay previously described (Salak et al., 1993). Briefly, neutrophils were used at a concentration of 3×10^6 cells/mL. Both recombinant human complement-5a (10^{-7} *M*; Sigma Aldrich) and recombinant human IL-8 (100 μg/mL; Sigma Aldrich) were used as chemoattractants. Neutrophil phagocytosis was measured using a flow cytometry-based assay as previously described (Jolie et al., 1999)

with minor modifications described (Niekamp et al., 2006). Fluorescent beads were pre-incubated 30 min with nonheat-inactivated porcine serum, and then beads were added to samples at a 10:1 (beads:neutrophils) ratio and samples were incubated for 45 min. The percentage of engulfment of beads by cells was evaluated using a flow cytometer.

A mitogen-induced lymphocyte proliferation assay was performed using a CellTiter 96 nonradioactive cell proliferation assay (Promega, Madison, WI) using the manufacturer's protocol with minor modification as previously described by Sutherland et al. (2005). Briefly, porcine lymphocytes were pipetted in triplicate into a sterile 96-well flat-bottom plate at cell concentration of 5×10^6 cells/mL. Concanavalin A (Sigma Aldrich) and lipopolysaccharide (Sigma Aldrich) were used as mitogens (0, 25 and 50 µg/mL) to stimulate T and B cells, respectively. Plates were incubated 68 h at 37 °C in a 5% CO_2 humidified incubator and 20 µL **MTT** (3-[4, 5-dimethylthiazol-2-yl] -2, 5-diphenyl tetrazolium bromide; Sigma Aldrich) were added to each well, and the plates were incubated 4 h. Acidified isopropanol (100 µL 0.1 N HCl in anhydrous isopropanol) was added, and the plates were incubated overnight at 37ºC and then read using a microplate reader (BIO-TEK Instruments, Winooski, VT) at wavelength 550 nm with reference wavelength 690 nm. The results are expressed as a proliferation index: optical density $_{(550/690\ nm)}$ stimulated cells /optical density $_{(550/690\ nm)}$ non-stimulated cells.

Hormone assays

Plasma cortisol was measured using a commercially available RIA cortisol kit (Coat-A-Count; Siemens Diagnostic Products, Los Angeles, CA). Intra- and inter-assay CV were 6.9 and 16.0%, respectively, and the minimal detectable concentration was 2 ng/mL. Progesterone samples were evaluated using an RIA kit and procedure previously described

(Printz et al., 1994). This kit was validated using charcoal-stripped, pooled, porcine serum (Coat-A-Count®; Siemens Diagnostic Products, Los Angeles, CA) with a minimum detectable limit of 31 pg/mL and an intra- and inter-assay CV of 4.5 and 13.3%, respectively.

Statistical analysis

Data were subjected to ANOVA. Continuous response measures were analyzed using the PROC MIXED procedures (SAS Institute, Cary, NC) for significance of the main effects using the *F*-Test and differences between least squares means identified using the *t* test. Binary response measures were analyzed using PROC GENMOD and significant effects of treatment and differences between least square means identified using the χ^2 test. This analysis was performed using a binary distribution and a logit-link. The models for the dependent variables included the main effects of temperature (3 levels), light intensity (2 levels) and their interaction. Replicate was included in all models. Repeated measures analyses were also performed for data obtained from the same animals over weeks using the "repeated" statement with the "ar" classification. Baseline measures were included as a covariate and gilt within treatment and replicate was used as the error term. The assumptions of ANOVA for normal distribution of data were evaluated and tested using PROC UNIVARIATE and for homogeneity of variance using Levene's test. Data that could not meet the assumptions were log or rank transformed for analysis. Differences between means were significant at $P < 0.05$. Trends for significance were identified when $P > 0.05$ but less than or $P = 0.10$).

10.4 Results

There was no temperature x lighting effects for any measures assessed in this study ($P > 0.10$). Therefore, only the main effects of temperature and light intensity on response measures are presented. Of the 198 gilts initially assigned to treatment, 194 were included in the final analysis. Animals were excluded for problems unrelated to treatment. Two gilts were excluded for the presence of corpora lutea as detected by ultrasound after LFM and 2 additional gilts were excluded at approximately 12 d post-breeding following a diagnosis of vaginal discharge and uterine infection.

Reproductive responses to treatment

Results for reproductive measures in response to treatment are shown in Table 1. Expression of estrus within 8 d following LFM averaged 93.3% and the interval from LFM to estrus was 145.8 ± 2.7 h but neither was affected by treatment. Duration of estrus tended to be longer ($P = 0.10$) for gilts in BRIGHT compared to gilts in DIM lighting, but temperature had no effect. Treatment had no effect on the interval from estrus to ovulation (40.6 ± 2.0 h) or progesterone concentrations (31.9 ± 1.7 ng/mL) on d 14 of gestation. Also, number of corpora lutea (18.2 ± 0.6), pregnancy rate (87.0%) and embryo survival (79.1%) were all similar among treatment groups.

The effects of treatment on litter traits are shown in Table 2. There were no treatment effects on number of healthy fetuses at d 30 (14.3 ± 0.5/litter), number of degenerating fetuses (0.2 ± 0.04/litter), or average fetal weight (11.4 ± 0.3 g) or fetal length (44.9 ± 0.3

mm). On d 30, within litter variation for fetal weight (1.2 ± 0.08 g) and fetal length (2.2 ± 0.1 mm) were also not different among treatment groups.

Gilt BW and rectal temperature

The main effects of temperature and lighting on gilt performance and rectal temperature are shown in Table 3. Final gilt BW and total BW gain were both affected by temperature ($P < 0.0001$) with final BW and total BW gain being greatest for gilts housed in the HOT environment compared with gilts kept in NEUTRAL and COLD environments. Rectal temperature was greater ($P < 0.0001$) for gilts kept in the HOT environment compared with the other treatments and lowest for those gilts in COLD. Light intensity had no effect on any measures (Table 3**)**.

Postural behaviors and position changes

The main effects of temperature and lighting treatments on postural behaviors of gilts are presented in Table 4. Number of postural changes and proportion of time spent standing or lying were affected by temperature. Gilts kept in the HOT environment made more ($P < 0.0001$) postural changes than did gilts in either the NEUTRAL or COLD environments. Percentage of time gilts spent lying was greater ($P < 0.0001$) among gilts in the HOT environment compared with gilts in either NEUTRAL or COLD environments. Lying position of the gilt was also affected by temperature; with those gilts kept in the COLD environment spending a greater proportion of time in the ventral position, whereas gilts in HOT room spent more time lying laterally ($P < 0.0001$). Conversely, gilts kept in COLD environment spent more ($P < 0.0001$) time standing compared with gilts kept at NEUTRAL or HOT temperatures. Gilt body position when in the lying position with full contact with

neighboring gilts tended ($P < 0.10$) to be influenced by temperature and lighting with gilts in COLD and BRIGHT having the least amount of full contact. Time spent with moderate contact with another gilt was not affected by temperature or lighting. When lying, time spent without any contact with neighboring gilts was not influenced by temperature or lighting.

Cortisol and immune measures

The main effects of temperature and lighting on cortisol and immune measures are presented in Table 5. Cortisol tended to be affected by room temperature ($P = 0.10$) but not light intensity with those gilts kept in COLD having greater cortisol than those in the HOT environment. Total white blood cells ($P < 0.05$), percentage of neutrophils ($P < 0.01$), as well as neutrophil-to-lymphocyte ratio ($P = 0.05$) were all affected by temperature, whereas number of lymphocytes showed a trend ($P = 0.06$) for an effect. There were fewer ($P < 0.05$) white blood cells for gilts kept in the HOT environment compared with other temperature treatments. Percentage of neutrophils were greater ($P < 0.05$) for gilts kept in COLD environment compared with those in NEUTRAL or HOT, which resulted in a greater neutrophil-to-lymphocyte ratio for gilts kept in COLD. The only measure affected by light intensity was percentage of eosinophils, with eosinophils (%) being greater ($P < 0.05$) for gilts kept in DIM than for gilts in BRIGHT. All other immune measures presented in Table 5 were similar among gilts kept at various environmental temperatures and light intensities.

10.5 Discussion

This experiment was designed to determine if exposure of mature gilts housed in crates to different levels of temperature and lighting during breeding and early gestation is associated with reproductive failure, changes in performance, behavior or immune function.

There were no effects of temperature within the range of 15 to 30°C or lighting intensity at 11 to 433 lx on any reproductive measures assessed within the present study. This leads us to conclude that reproductive failure associated with problems in follicular development, estrus or ovulation, pregnancy establishment and embryo survival are not associated with constant exposure of gilts to these temperatures or 12 h exposure to this type of lighting. These results could be extrapolated to suggest that similar variation in temperature and lighting within commercial swine breeding facilities may not be major contributing factors associated with early estrus or pregnancy related failures in gilts or sows.

The most common causes of reproductive failure in swine are those associated with failure to detect estrus and pregnancy failure (Dalin et al., 1997; Koketsu et al., 1997; Heinonen et al., 1998; Vargas et al., 2009; Tummaruk et al., 2010). The causes for these failures are largely unknown but it is likely they are multifactorial and vary among farms (Koketsu et al., 1999). Although there are no data to suggest that ambient temperatures near the lower critical temperature of a pig causes reproductive failure, the lower critical temperature can change based on her age and weight and stage of gestation (Verstegen and Curtis, 1988). Conversely, reproductive failure in swine has been attributed to heat stress (Wetteman and Bazer, 1985) and season of the year with effects on estrus and ovulation (Xue et al., 1994), conception rate, litter size, and litter weights reported (Love, 1993). Most modern swine confinement barns are built to operate based on the normal range of outdoor annual temperatures in the United States (4 to 15 °C; National Weather Service) and depending upon location, may have to plan for extremes for heat and cold. Because there are considerable differences in building size, design, air flow, and sensing systems, despite the settings in the air control system to provide comfortable conditions for all animals within the

breeding barns (18 to 24°C) throughout all weather conditions, it is also true that certain locations within these buildings can differ greatly in temperate as well as in the extremes of outdoor cold or heat. As a result, in any swine confinement building, it should be expected that there will be areas within the building that can be hotter or cooler than the set temperatures for the room. Seasonal infertility in gilts and sows is most often observed in summer and early fall and cannot be attributed to high temperature alone (Hurtgen and Leman, 1980; Prunier et al., 1997; Auvigne et al., 2010). Hormonally, gilts subjected to heat stress (30 to 33 °C) exhibit a reduction in FSH and LH during the follicular phase (Flowers and Day, 1990). Others have shown altered concentrations of estrogen prebreeding and reduced concentrations of progesterone postbreeding (Hoagland and Wettemann, 1984). For reproductive performance of gilts, heat stress ranging from 30 to 39 °C was reported to reduce expression of estrus, pregnancy rate, numbers of embryos, or embryo survival (Edwards et al., 1968; Teague et al., 1968; Omtvedt et al., 1971). However, not all heat stress effects are similar and d'Arce et al. (1970) noted that for gilts exposed to high temperatures above 28°C, estrus expression was normal but ovulation rate was reduced. This was especially evident as the duration of exposure increased during the estrous cycle. Edwards et al. (1968) and Omtvedt et al. (1971) also showed that heat stress before breeding had little effect on estrus or ovulation but when applied after breeding and during the first few weeks of gestation, pregnancy rate and numbers of embryos were reduced. The effect of heat stress was not evident in mid-gestation, but appeared in late gestation with sows having fewer pigs born alive (Omtvedt et al., 1971). Tast et al. (2002) noted that in cases of seasonal infertility in the form of early pregnancy failure, progesterone concentrations were reduced. Our data would support this observation as we did not observe an effect of treatment on number,

formation or function of corpora lutea and noted no effects on progesterone concentration or pregnancy rate. Collectively the results from all of these experiments would suggest that temperatures below 30°C would not be associated with reproductive failure but that increases above 30°C are often associated with some form of reproductive failure. However, evidence also points to reproductive failure as a result of the type of stress, the level of the stress, its duration of application, the age and genotype of the animal (Bloemhof et al., 2008) and which could be influenced by the animal housing environment as well as stage of the cycle or reproductive state of the animal at the time of the stress.

We did not observe an effect of light intensity on any measure of gilt reproductive performance but did note a trend for gilts in BRIGHT to have a slightly longer duration of estrus than gilts in DIM. However, because detection occurred only once daily, a difference of 3 h may not be of practical importance. However, considerable variation in light intensity is evident within and among confinement swine breeding barns, despite industry recommendations for a light intensity of 161 to 215 lx (Midwest Plan Service, 2001). Furthermore, seasonal effects on pig reproduction have also been attributed to the photoperiodic changes in animal exposure to light (Love, 1993). A review of the effects of light on reproduction in swine suggests minimal effect of light intensity per se but more so as a result of photoperiodicity (Claus and Weiler, 1985) and perhaps from the type of light supplied (Wheelhouse and Hacker, 1982). However, there is some evidence to suggest that increasing lux level from 50 to 80 lx improved cyclic activity in prepubertal gilts and that born alive was improved when weaned sows received light intensity above 26 lx (PIGI, 2006). Melatonin is known to be involved in regulating the seasonal reproductive responses in mammals, and evidence from humans shows that melatonin release is responsive to both

the intensity and duration of light (Aoki et al., 1998). However, in gilts, the impact of light intensity in the range of 40 to 10,000 lx is reported to have no effect on melatonin release during the scotophase (Tast et al., 2001) or on ACTH or cortisol secretion (Griffith and Minton, 1992a). However, Paterson et al. (1992) noted that the weak seasonal responses in the pig have made association of melatonin with fertility difficult even though clear diurnal effects in response to light are evident. Although the lighting effect and melatonin relationship remains unclear in the pig, an intriguing study recently revealed that the gene for the melatonin receptor in swine is located on the same chromosome as the genes controlling ovulation rate and litter size (Ramírez et al., 2009). Diurnal effects in the pig have been shown as exposure of pigs to approximately 250 lx during either their natural daytime or nighttime phases results in changes in the pulse frequency of LH in response to estradiol (Evans et al., 1994). In late gestation, exposure to short or long photoperiod in the range of 32 to 266 lx was observed to improve the synchrony (Stevenson et al., 1983) and proportion of sows expressing estrus after weaning (Tast et al., 2005) in response to season of year (Prunier et al., 1994).

We measured gilt body temperature and performance in response to temperature and light intensity with body temperature and weight gain being affected. Changes in pig body temperature in response to their environment have been previously reported (Edwards et al., 1968; d'Arce et al., 1970; Omtvedt et al., 1971). In our study, gilts were restricted fed, and we noted no effects of room temperature on intake, but did record changes in BW gain and body temperature. Gilts in HOT rooms had the greatest body temperatures and BW gains whereas gilts in COLD rooms had the least. These data indicate that for these individually housed gilts, room temperature evoked thermoregulatory responses in their attempt to

maintain a thermoneutral state. Based on lack of BW gain among gilts in COLD it appears that more energy was needed for heat generation to maintain body temperature instead of growth. This effect of decrease in BW gain has been observed previously among gilts exposed to cold temperatures, but reproductive measures were not assessed (Verstegen and Curtis, 1988). For sows housed at temperatures above their thermal comfort zone, it has been shown that these animals have a decreased feed intake, increased water consumption, increased respiration rate (Brown-Brandl, 1998), and as gestation progresses may show increased weight loss (Heitman et al., 1951). Animal behavior is an important assessment as an indicator of adaptation to environmental challenges. Although no changes in behavior were noted in response to light intensity, room temperature evoked various thermoregulatory behaviors for gilts kept in either COLD or HOT environments. Postural changes have been noted in periparturient sows in response to thermal environment (Malmkvist et al., 2009). In the present study, gilts changed postural positions between standing and lying and spent more time in a particular lying position in response to room temperature in attempt to increase (HOT) or decrease (COLD) body surface area. Gilts changed positions more often from a standing to lying position in the HOT room and spent more of their time lying in a lateral position. The greater frequency of position changes and more time in the lateral lying position is likely a behavioral thermoregulatory process used to maximize heat dissipation via surface conduction to the concrete floor. Conversely, gilts in the COLD spent the least amount of time in the lying position, and when lying spent the most amount of time in the ventral position to minimize heat loss by body surface contact with the concrete floor. Collectively, these behavioral changes suggest that gilts in each of the environments were behaviorally adapting to certain levels of thermal stress. Moreover, it appears that the

gilts in HOT environment were better able to behaviorally regulate the loss of heat to their environment without compromising BW gain. However, gilts in COLD may have had some difficulty coping as indicated by their reduced BW gain, which could be related to energy expenditure associated with standing to avoid contact with the cold concrete floor. Another concern is that increased duration of COLD exposure beyond day 30 of gestation would prolong the physiological stress resulting in increased cortisol to aid in mobilizing body energy reserves away from growth and BW gain in order to sustain pregnancy and fetal growth. It was also evident that the exposure to COLD altered some of the immune cell measures and functional tests which could also explain a diversion of energy from away from BW gain.

The effects of temperature were evident on cortisol concentration and a few measures of immune response, while light intensity affected only 1 measure of immune function. Cortisol can be used as an indicator of stress in pigs (Becker et al., 1985), but much of the data suggests limited involvement of acute stress and cortisol on reproductive failure (Turner et al., 2005). However, in the present study, gilts in the COLD temperature showed the greatest cortisol concentrations and without any detrimental effect on reproduction. Temperatures higher or lower than the thermal comfort zone of an animal can cause stress and, if animals are unable to adapt can eventually be challenging to health and well-being (Webster et al., 1993). Data from the present experiment indicated that gilts in the COLD temperature were most likely stressed. Hyun et al. (2005) reported that an increased neutrophil:lymphocyte (**N:L**) ratio is an indicator of a stress response. Based on the behavioral changes, cortisol and N:L ratio it is plausible that those gilts kept in COLD rooms were experiencing stress and were having difficulty coping with their environment which

resulted in reduced BW gain. Furthermore, the gilts in the HOT room, although also under some stress, were able to make postural changes to adapt without evoking changes in cortisol or immune status.

The results of the present study indicate that variation in air temperature in the range of 15 to 30°C and light intensity variation from 11 to 433 lx would not be expected to be associated with failure in estrus expression, ovulation, embryo or pregnancy loss for breeding females housed in crates. However, it would appear that if breeding swine are exposed to cold temperatures for an extended duration during housing in confinement, the stress response could alter use of body reserves and energy intake for thermoregulatory rather than growth processes. As a result, this could ultimately lead to physiological changes that could affect well-being, productivity and longevity. With considerable differences among farms in building temperature regulation, herd genotype and within farm variation in herd parity structure, there would seem to be great potential for effects of animal microenvironment on individual health status and well-being. Therefore, to minimize potential problems in animal health and well-being, evaluation of different locations within barns for temperature variation, especially in seasonal extremes, could prove helpful for managing at risk animals. Furthermore, in certain locations within barns, there may be some advantages in providing supplemental heat, cooling, or provision of additional feed where problems occur. Producers may be able to place less mature, lighter or leaner animals into warmer areas and leave cooler areas for older, larger sows. Lastly, because there was no impact of light intensity on any measures of reproduction and limited effect on well-being, producers may be able to reduce lighting level to reduce energy needs without effects on reproductive performance.

10.6 Tables and figures

Table 1. Least squares means for the reproductive responses of mature, synchronized gilts in response to the main effects of housing in crates in COLD, NEUTRAL or HOT rooms, each with DIM or BRIGHT lighting in the period from the last feeding of MATRIX (LFM) through breeding and early gestation.

	Temperature[1]			**Lux**[2]			
Measure	COLD	NEUTRAL	HOT	BRIGHT	DIM	*P*-value$_{temp}$	*P*-value$_{lx}$
N	65	64	65	97	97		
Estrus expression within 8 d of LFM, %	94.7 ± 3.0	89.5 ± 3.0	95.7 ± 3.0	93.0 ± 3.0	94.5 ± 3.0	0.34	0.69
LFM to estrus, h	148.5 ± 2.7	143.5 ± 2.8	145.4 ± 2.7	144.3 ± 2.2	147.3 ± 2.2	0.41	0.34
Duration of estrus, h	57.3 ± 1.7	56.7 ± 1.7	54.8 ± 1.7	57.8 ± 1.4	54.6 ± 1.4	0.54	0.10
Estrus to ovulation interval, h	40.3 ± 2.0	40.7 ± 2.1	40.7 ± 2.0	42.2 ± 1.7	38.9 ± 1.7	0.99	0.17
Number of corpora lutea at d 30	17.6 ± 0.5	18.9 ± 0.6	18.2 ± 0.6	18.0 ± 0.5	18.4 ± 0.5	0.28	0.59
D 14 Progesterone, ng/mL	31.5 ± 1.7	32.5 ± 1.7	31.6 ± 1.6	32.3 ± 1.4	31.5 ± 1.4	0.91	0.69
Pregnancy rate (d 30), %	86.9 ± 5.0	84.4 ± 5.0	89.8 ± 5.0	85.9 ± 4.0	88.4 ± 4.0	0.63	0.57
Embryo survival (d 30), %	77.4 ± 3.0	77.2 ± 3.0	82.6 ± 3.0	78.5 ± 2.0	79.6 ± 2.0	0.25	0.73

[1]COLD = 13.9 ± 0.2 °C, NEUTRAL = 20.3 ± 0.2 °C, and HOT = 29.8 ± 0.1 °C

[2]DIM = 11 lx, BRIGHT = 433 lx.

Table 2. Least square means for litter traits of mature, synchronized gilts in response to housing in crates in COLD, NEUTRAL or HOT rooms, each with DIM or BRIGHT lighting in the period from last feeding of MATRIX through breeding and early gestation.

	Temperature[1]			Lux[2]			
Measure	COLD	NEUTRAL	HOT	BRIGHT	DIM	P-value$_{temp}$	P-value$_{lx}$
N	65	64	65	97	97		
Number of healthy fetuses	13.9 ± 0.5	14.2 ± 0.5	14.7 ± 0.5	14.1 ± 0.4	14.4 ± 0.4	0.49	0.58
Avg. fetus wt., g	11.4 ± 0.3	11.3 ± 0.3	11.4 ± 0.2	11.5 ± 0.2	11.2 ± 0.2	0.97	0.34
Within litter fetal wt. variation, g[3]	1.2 ± 0.08	1.2 ± 0.08	1.1 ± 0.07	1.2 ± 0.06	1.2 ± 0.06	0.79	0.68
Fetus length, mm	44.9 ± 0.3	44.8 ± 0.3	45.1 ± 0.3	45.0 ± 0.3	44.9 ± 0.3	0.81	0.64
Within litter fetus length variation, mm[3]	2.1 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	2.1 ± 0.1	2.3 ± 0.1	0.37	0.43

[1]COLD = 13.9 ± 0.2 °C, NEUTRAL = 20.3 ± 0.2 °C, and HOT = 29.8 ± 0.1 °C

[2]DIM = 11 lx, BRIGHT = 433 lx.

[3]Within litter measures evaluated at d 30.

Table 3. Least squares means for performance and body temperature measures of mature, synchronized gilts in response to housing in crates in COLD, NEUTRAL or HOT rooms, each with DIM or BRIGHT lighting in the period from last feeding of MATRIX through breeding and early gestation.

	Temperature[1]			Lux[2]			
Measure	COLD	NEUTRAL	HOT	BRIGHT	DIM	P-value$_{temp}$	P-value$_{lx}$
N	65	64	65	97	97		
Initial body weight, kg	145.0 ± 0.5	145.3 ± 0.5	144.7 ± 0.6	145.0 ± 0.5	145.0 ± 0.5	0.76	1.0
Final body weight, kg	162.1 ± 0.8[a]	168.3 ± 0.8[b]	174.0 ± 0.8[c]	168.4 ± 0.7	167.8 ± 0.7	<0.0001	0.51
Body weight gain, kg	16.6 ± 0.8[a]	22.7 ± 0.8[b]	28.4 ± 0.8[c]	22.9 ± 0.7	22.2 ± 0.7	<0.0001	0.51
Daily feed intake, kg[3]	2.7 ± 0.02	2.7 ± 0.02	2.7 ± 0.02	2.7 ± 0.02	2.7 ± 0.01	0.24	0.15
Rectal temperature, °C[3]	37.6 ± 0.04[a]	38.2 ± 0.04[b]	38.6 ± 0.04[c]	38.1 ± 0.03	38.1 ± 0.03	<0.0001	0.32

[1]COLD = 13.9 ± 0.2 °C, NEUTRAL = 20.3 ± 0.2 °C, and HOT = 29.8 ± 0.1 °C

[2]DIM = 11 lx, BRIGHT = 433 lx;

[3]Five week average of measures

[a-c]For each main effect, means with different superscripts within a row are different ($P < 0.05$).

Table 4. Least squares means for the postural behaviors of mature, synchronized gilts in response to housing in crates in COLD, NEUTRAL or HOT rooms, each with DIM or BRIGHT lighting in the period from last feeding of MATRIX through breeding and early gestation.

	Temperature[1]			Lux[2]			
Measure[3]	COLD	NEUTRAL	HOT	BRIGHT	DIM	*P*-value$_{temp}$	*P*-value$_{lx}$
n	24	24	24	36	36		
Number of position changes[4]	2.14 ± 0.2^{a}	2.13 ± 0.2^{a}	4.89 ± 0.2^{b}	3.06 ± 0.2	3.05 ± 0.2	<0.0001	0.95
Sitting position, % of recording time	2.66 ± 0.9	4.42 ± 0.8	4.43 ± 0.9	3.21 ± 0.7	4.47 ± 0.7	0.25	0.12
Standing position, % of recording time	43.1 ± 2.3^{a}	27.1 ± 2.2^{b}	14.1 ± 2.3^{c}	26.8 ± 1.9	29.4 ± 1.9	<0.0001	0.31
Lying position, % of recording time	54.0 ± 2.6^{a}	68.8 ± 2.5^{b}	81.6 ± 2.6^{c}	70.0 ± 2.1	66.1 ± 2.1	<0.0001	0.18
Lying Position							
Ventral position, % of lying time	76.7 ± 4.0^{a}	63.0 ± 3.8^{b}	18.0 ± 4.0^{c}	53.4 ± 3.2	53.8 ± 3.2	<0.0001	0.94
Lateral position, % of lying time	18.7 ± 4.0^{a}	36.9 ± 3.9^{b}	82.0 ± 4.0^{c}	46.6 ± 3.3	45.1 ± 3.3	<0.0001	0.75
Position within crate							
Full contact with neighboring gilt, % of lying time[5]	3.62 ± 2.2^{a}	10.5 ± 2.2^{b}	8.96 ± 2.2^{a,b}	5.48 ± 1.8^{b}	9.89 ± 1.8^{a}	0.08	0.085
Moderate contact with neighboring gilt, % of lying time[5]	14.2 ± 3.1	15.1 ± 2.9	17.6 ± 2.97	18.4 ± 2.4	12.9 ± 2.4	0.71	0.12
No contact with neighboring gilt[4], % of lying time	80.4 ± 3.9	74.8 ± 3.7	73.2 ± 3.8	76.1 ± 3.1	76.1 ± 3.1	0.39	1.0

[1]COLD = 13.9 ± 0.2 °C, NEUTRAL = 20.3 ± 0.2 °C, and HOT = 29.8 ± 0.1 °C

[2]DIM = 11 lx, BRIGHT = 433 lx;

[3]Observation period occurred for ~12 h on two consecutive days during the 5 weeks of the experiment

[4]Position changes between sitting, standing, and lying per hour or recording

[5]Relative contact with gilts in adjacent crates, Full= All of back/flank, Moderate= Some contact of back/flank, No= no back/flank contact.

[a-c]For each main effect, means with different superscripts within a row are different ($P < 0.05$).

Table 5. Least squares means for cortisol, white blood cell measures and immune function assays for mature, synchronized gilts housed in crates and assigned to housing in COLD, NEUTRAL or HOT rooms, each with DIM or BRIGHT lighting in the period from last feeding of MATRIX through breeding and early gestation.

	Temperature[1]			Lux[2]			
Measure[3]	COLD	NEUTRAL	HOT	BRIGHT	DIM	*P*-value$_{temp}$	*P*-value$_{lx}$
n	24	24	24	36	36		
Plasma cortisol, ng/mL[3]	25.9 ± 1.8^{a}	22.3 ± 1.9^{ab}	20.9 ± 1.8^{b}	23.8 ± 1.5	22.3 ± 1.6	0.10	0.41
White blood cells x10^{7} cells/ mL	3.3 ± 0.1^{a}	3.5 ± 0.1^{a}	3.1 ± 0.1^{b}	3.2 ± 0.1	3.4 ± 0.1	0.04	0.21
Neutrophils x10^{7} cells/ mL	4.4 ± 0.2	3.79 ± 0.2	3.97 ± 0.2	3.89 ± 0.2	4.22 ± 0.2	0.14	0.21
Lymphocytes x10^{7} cells/ mL	3.9 ± 0.2	4.3 ± 0.2	4.5 ± 0.2	4.2 ± 0.2	4.3 ± 0.2	0.06	0.57
Neutrophils, %	30.0 ± 1.2^{a}	24.4 ± 1.2^{b}	25.2 ± 1.2^{b}	27.0 ± 0.97	26.1 ± 1.0	0.003	0.49
Lymphocytes, %	64.0 ± 4.1	75.4 ± 4.2	68.1 ± 4.2	66.8 ± 3.4	71.6 ± 3.5	0.16	0.31
Neutrophil:Lymphocyte ratio	0.52 ± 0.04^{a}	0.40 ± 0.04^{b}	0.41 ± 0.04^{b}	0.45 ± 0.03	0.43 ± 0.03	0.05	0.64
Monocytes, %	1.3 ± 0.2	1.5 ± 0.2	1.4 ± 0.2	1.3 ± 0.1	1.5 ± 0.1	0.65	0.29
Eosinophils, %	3.3 ± 0.4	4.0 ± 0.4	4.3 ± 0.4	3.4 ± 0.3^{a}	4.3 ± 0.3^{b}	0.23	0.06
C5a Chemotaxis, cells/4 fields	51.9 ± 3.3	51.0 ± 3.5	50.9 ± 4.7	51.2 ± 3.0	51.4 ± 3.3	0.97	0.95
IL-8 Chemotaxis, cells/4 fields	72.7 ± 3.7	62.3 ±4.0	74.8 ± 4.3	69.1 ± 3.2	70.8 ± 3.4	0.06	0.70
Neutrophil phagocytosis, %	61.0 ± 1.7	58.4 ± 1.7	56.9 ± 1.7	58.5 ± 1.3	59.1 ± 1.4	0.22	0.73
ConA proliferation index[4]	1.3 ± 0.4	1.1 ± 0.4	2.0 ± 0.4	1.4 ± 0.3	1.6 ± 0.3	0.19	0.75
LPS-proliferation index[4]	1.7 ± 0.2	1.7 ± 0.2	1.2 ±0.2	1.5 ± 0.2	1.5 ± 0.2	0.24	0.88

[a,b]For each main effect, means with different superscripts within a row are different ($P < 0.05$).

[1]COLD = 13.9 ± 0.2 °C, NEUTRAL = 20.3 ± 0.2 °C, and HOT = 29.8 ± 0.1 °C

[2]DIM = 11 lx; BRIGHT = 433 lx.

[3]N:L = neutrophil-to-lymphocyte ratio; C5a = complement-5a; IL-8 = interleukin-8; ConA=concanavalin A; LPS = lipopolysaccharide.

[4]Proliferation index = optical density of stimulated cells divided by optical density of unstimlulated cells; mitogen concentration 20 nM.

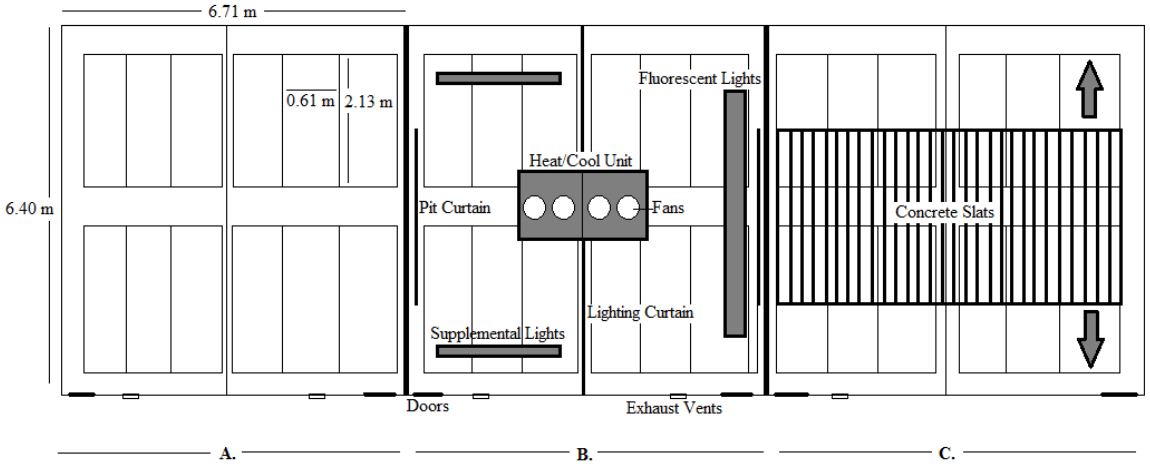

Figure 1: Layout of treatment facility; A. Measurements of room and crate size; B. Placement of regulation devices: Fluorescent lighting- in-built and in all sections, Supplemental lighting- hanging fluorescent light fixtures in BRIGHT treatments only, Pit curtain- minimize air flow between rooms via pit space, Heat/Cool unit- built into ceiling and spans both lighting treatments, Fans- two per lighting treatment, keeps whole room at the same temperature, Exhaust vents- dispel circulated air into gestation wing to maintain correct temperatures, Doors- one per section to avoid mixing lighting treatments; C. Arrows indicate animal orientation within the facility, crates over partially-slatted floors.

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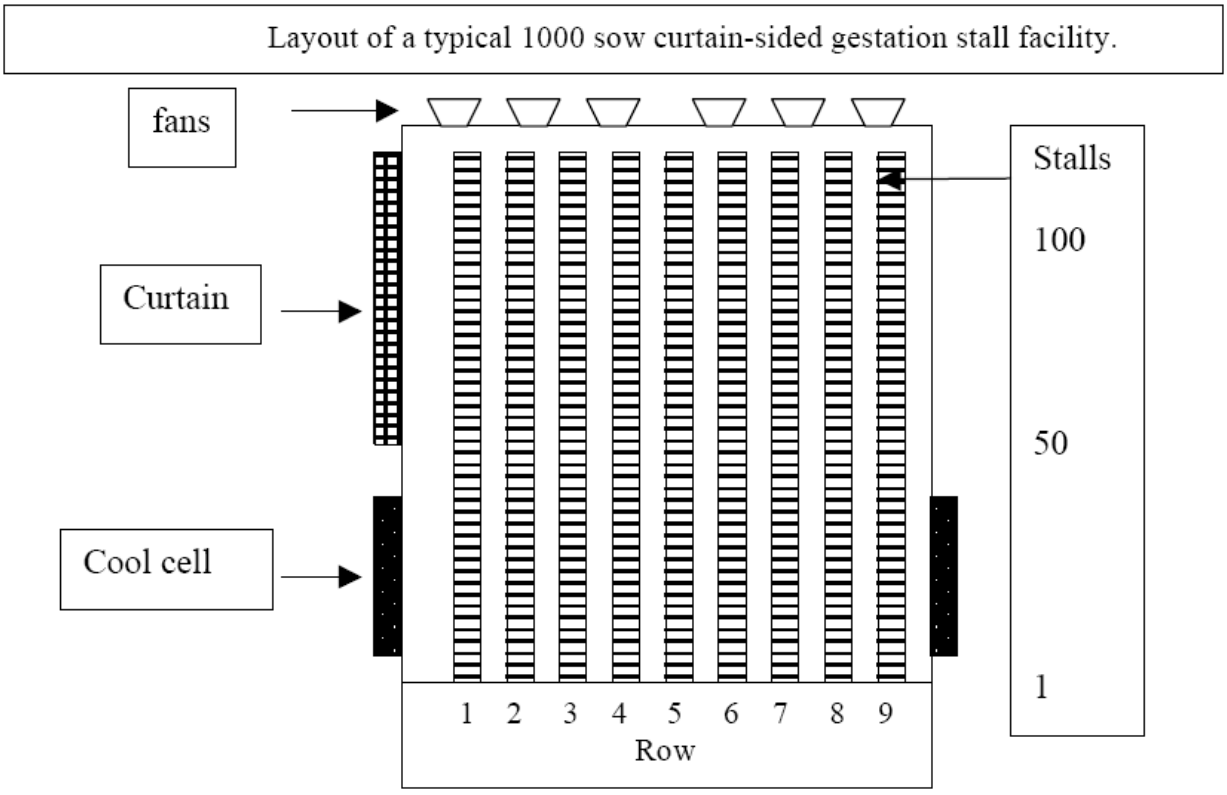
11. Appendix

Housing and Environment Supplemental Figures

Included in this section are pictures outlining the set-up of typical gestation facilities, our experimental facility, and experimental temperature control panel, handheld reader and handheld light intensity reader.

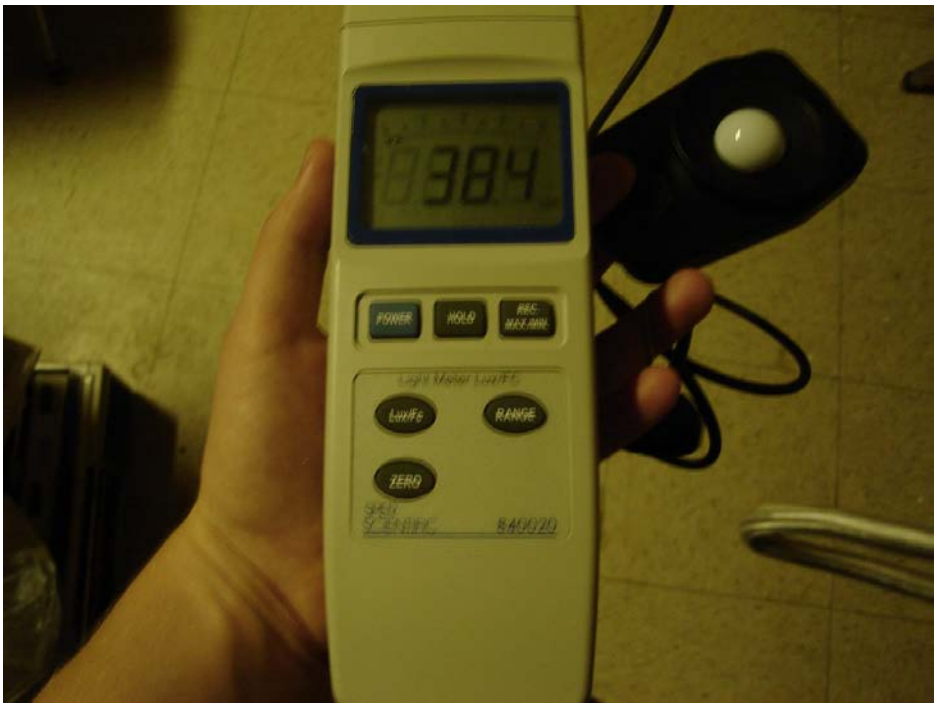


Typical Gestation Facility

Experimental treatment room

Room temperature control panel

Temperature and humidity reader, recorded at crate level weekly



Light intensity reader, recorded at crate level weekly

Reproduction Supplemental Information

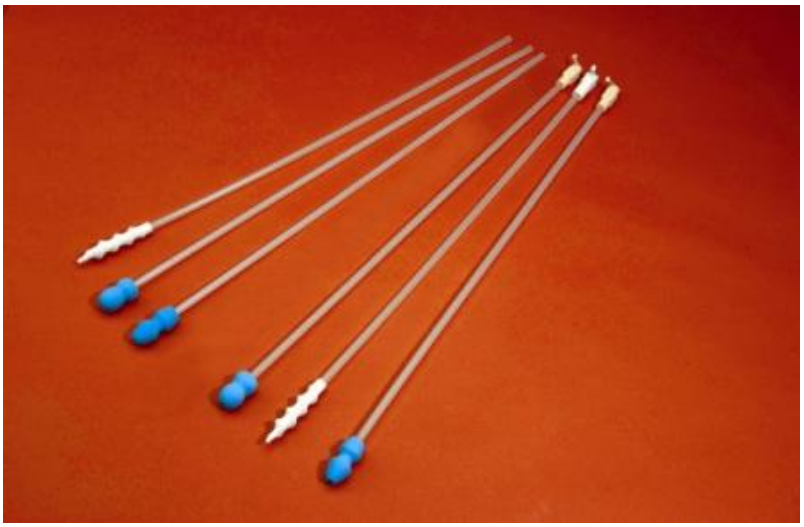
The reproductive procedures in the experiment included ultrasonography, artificial insemination, estrus detection, MATRIX feeding, progesterone concentration radioimmunoassay and slaughter plant tract collection and analysis.

Trans-rectal ultrasonography was utilized on the first day after last MATRIX feeding, beginning with scanning for follicle development on alternate days. Once an animal was determined to be in estrus, ultrasonography was performed daily on that animal until ovulation. This required an Aloka 500 ultrasound with a modified transducer handles in order to achieve the proper position for ovary imaging. Non-medicated lubricant was used to ensure animal safety and prevent injury. Ovaries were scanned with ultrasound to determine

day of ovulation. The same ultrasound machine was used to determine pregnancy status at d 25-28 of gestation using a transabdominal transducer by evaluating amniotic fluid sacs.

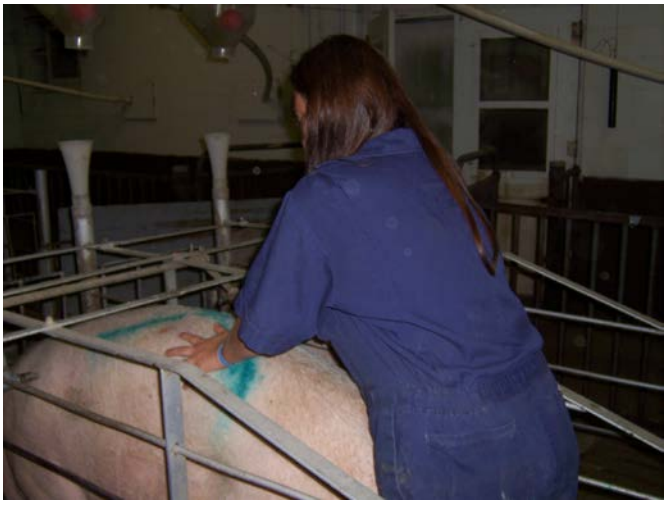
Aloka 500 Ultrasound and transrectal transducer

Artificial insemination was implemented in all experimental trials and involved the use of insemination catheters, pooled semen doses collected from a boar stud farm and non-medicated lubrication. Lubrication was placed on insemination catheters and subsequently inserted into the cervix of the gilts. Semen doses stored in plastic pouches were attached to the end of the catheters and slowly drained through the catheters.



Swine insemination catheters; Copyright of Veterinary Provisions

Estrus detection was utilized every day after the last feeding of MATRIX for one week. It consists of applying back pressure with either hands or body weight and watching for stiffening of voluntary muscles and possible lordosis of the spine and ear standing while in the presence of a mature boar. A gilt should not be able to be moved from their position.



Detection of estrus in swine

In order to synchronize the display of estrus among mature gilts, producers use a

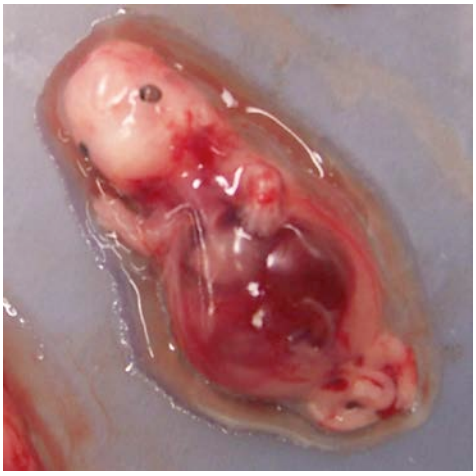
synthetic progesterone for 14 d in the feed of gilts. This product can be sprayed onto feed in increments of 6.8mL per day in order to achieve a synchronized estrus.

Administration of MATRIX product to feed

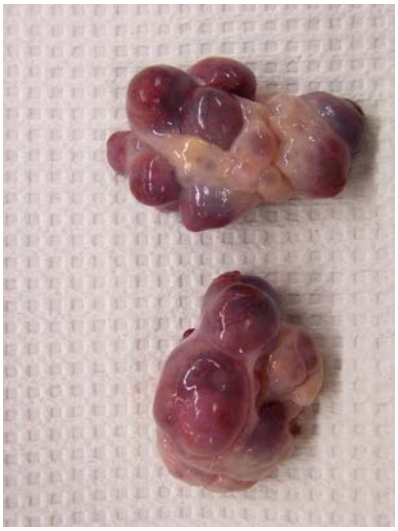
Progesterone concentration was measured in all gilts at approximately d 14 of gestation. This blood collection consisted of nose-snaring the gilt and using venipuncture in the jugular vein with a non-coated vacutainer tube. The blood sat at room temperature for one hour and then was refrigerated overnight for roughly 12 hours. This allowed for clotting factors to be removed and the tubes were centrifuged at 400 x g at 4°C for 15 min. Serum was collected out of the tubes and transferred into polypropylene tubes for storage at -20˚C. Samples were thawed before being used in the Siemens Progesterone RIA kit for determination of concentration and run as described in the kit protocol.

Reproductive tracts were collected from the slaughter plant at the time of slaughter

and placed into plastic bags with the ear tag from the corresponding gilt. Tracts were placed into large dissection tubs after transportation and ovaries were removed and placed in cups with corresponding ear tag numbers to evaluate for CL or follicle numbers and any deformities present. The uteri were evaluated by dissecting out each amiotic sac and placing the fetus into a litter weighing tray. Once all fetuses were removed from the uterus, each was weighed on a scale and measured with calipers.



Example of swine fetus at gestation d 30



Examples of CL on ovaries

Physiological Supplemental Information

Physiological measures with supplemental information included here involve rectal temperature, feed intake measurement, body weights, blood sampling and cortisol RIA.

Rectal temperature was measured using a digital thermometer inserted one inch into the rectum. Enough time was allowed for the thermometer to complete its reading. Gilts were required to be standing at the time of measurement.

Example of rectal thermometer; Copyright RuralKing

Feed intake was measured by giving gilts an exact measure of feed using a marked scooper and collecting the residual feed the next morning using the same scooper. It was uncommon for gilts to not completely finish the amount of feed given, even in the hot environment. Each gilt was fed about 6 lbs of feed daily.

Example of measured feed scoop; Copyright RuralKing

Body weights were measured before gilts were assigned to a treatment using a large livestock scale. The scale was zeroed before each gilt was weighed. At the end of the experiment period, on the day before transport to the slaughter facility, each gilt was weighed again using the same scale.

Example of livestock scale used for body weight measurements; Copyright Direct Industry

Blood sampling for the cortisol assay was performed in a similar fashion as the progesterone assay, however, these samples were taken weekly on a subset of gilts analyzed for immune function, cortisol and behavior. The samples were obtained at the same time every week to prevent differences in the circadian profile of cortisol secretion. Blood samples were collected in heparin-lined vacutainer tubes and chilled on ice until immediate centrifugation at 400 x g for 15 min at 4°C to collect plasma for storage at -20˚C until analysis. These samples were then run in the Siemens cortisol RIA kit as described in the provided protocol.

Behavior Supplemental Information

Because the rooms did not provide enough room at the head of each crate for video recording capabilities, video cameras were placed behind recorded animals at the height of the ceiling. Each camera was linked to a multiplexor on the roof of the experimental building which was in turn connected to three separate VCRs. The camera in each treatment was recorded onto the same VHS tape as the opposite lighting treatment of the same temperature. Video recording occurred on two days consecutive days per week during the lit hours (12:12h). Once the tapes had been collected, analysis for different postural types was performed. A timestamp for each VHS served as the common starting point for all six animals recorded on one tape. The tape was then played or fast forwarded until any of the six pigs included make any position change or increased or decreased its contact with a neighboring gilt. The video was then paused and the timestamp was recorded. After each tape had been analyzed for content, a spreadsheet was constructed to determine the length of time each sow had spent in any of the positions recorded or in differing amounts of contact

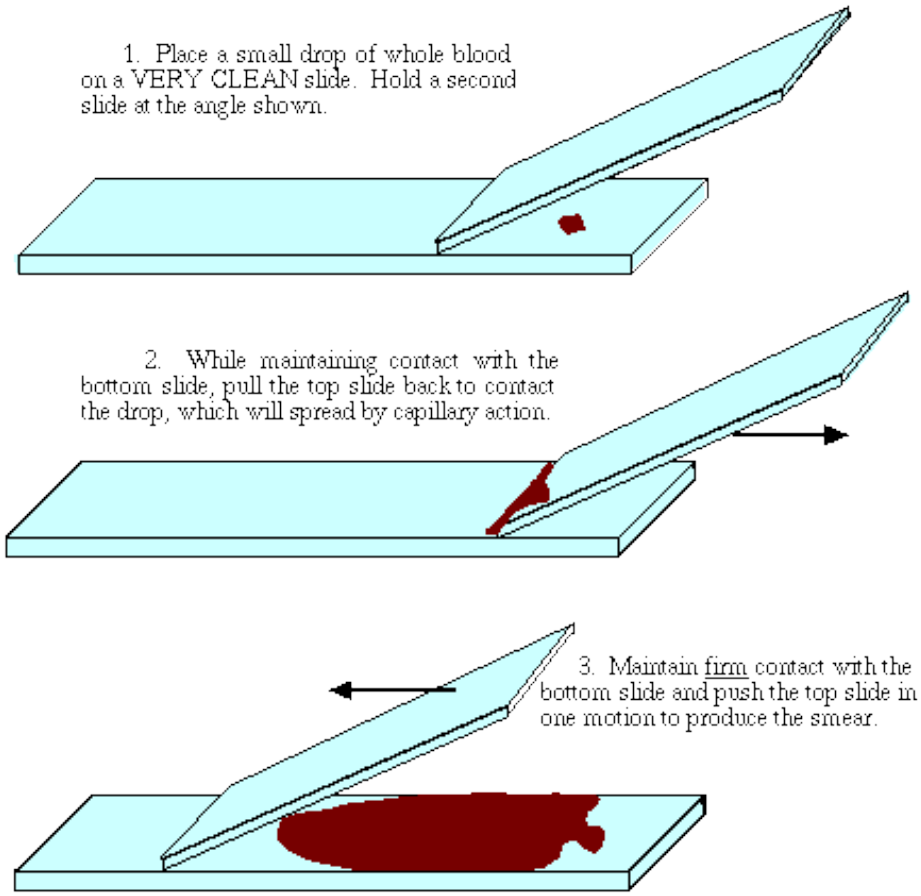
with other gilts. The time was expressed as a percentage due to variability in recording lengths of VHS tapes and unreliability of VCRs.

Camera mounted at ceiling to record three gilts in one temperature/lighting treatment

Immunology Supplemental Information

Immunological measures that are described in this section include white blood cell (WBC) count differentials, WBC, neutrophil and lymphocyte counts using the coulter counter, and neutrophil chemotaxis, neutrophil phagocytosis and lymphocyte proliferation assays.

WBC differentials were performed by utilizing one drop of blood from the heparin-coated cortisol vacutainer tubes and placing it onto a glass slide near the labeling area. Using another clean glass slide, contact is made and quickly draws the drop of blood toward the other end of the slide. Below is an example of the procedure from Rice University:



WBC Differential slide creation procedure; Copyright David Caprette, Rice University

After the smear has been made, the slide is allowed to air dry for about 24 h. It is then dipped in 100% ethanol and allowed to dry for another 24 h. Finally, using differential dyes, the slide is dipped in red and blue dyes and rinsed and allowed to dry again. After it has dried completely, the slide can be read using a light microscope. With a drop of oil and using 100x magnification, one hundred white blood cells need to be counted on the slide, noting the relative amounts of each cell type.

The Beckman Coulter cell counter was utilized to determine the concentration of WBC in whole blood, as well as concentration of isolated neutrophils and lymphocytes. For whole blood WBC concentration, first a small plastic cup of 10mL of isoflow fluid

(Beckman Coulter) is placed onto the stage. The machine needs to be primed by pressing 'function' twice on the machine. After priming, a new cup of isoflow is filled to 10 mL and 10uL of whole blood from the heparin-coated cortisol blood collection tubes is pipetted into the isoflow liquid. Because the blood also contains red blood cells, three drops of Zap Oglobin (a RBC lysing agent) must be added to the cup. The lid should be placed onto the cup and inverted several times. Once mixed, the cap can be taken off the cup and the whole cup is placed in the machine for counting. Each blood sample should be counted two times and averaged.

Beckman Coulter cell counter; Image courtesy of Beckman Coulter, Inc.

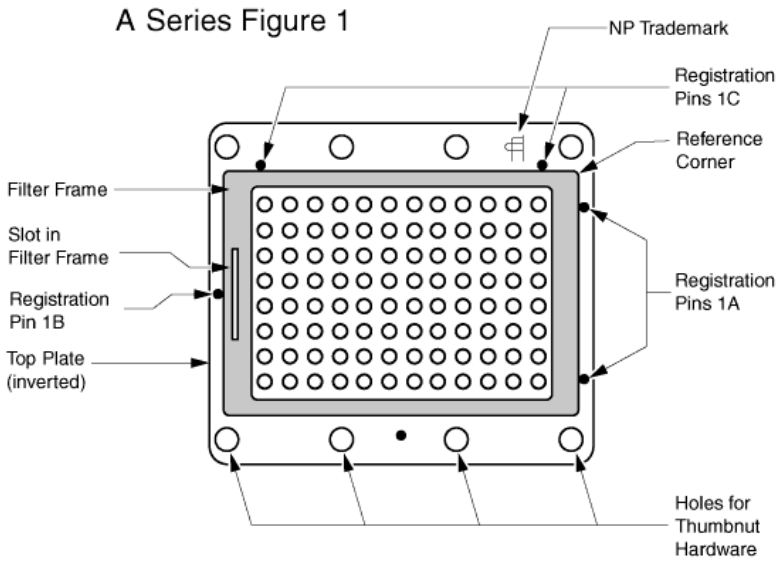
Determining the concentration of neutrophils and lymphocytes uses the same process as above, however, the different cell types must be first isolated from whole blood samples. The blood samples collected for this and the immunological function assays use EDTA-coated vacutainer tubes in same fashion as previously mentioned. These samples are stored on ice until used, preferably within one hour. In a 15 mL conical tube, one per blood sample,

3 mL of Histopaque 1119 is pipetted and carefully layered with 3 mL of Histopaque 1077 on top- the best method is to slightly tip the conical tube containing Histopaque 1119 and slowly pipet out 1077, creating a layering effect of the two different product densities. On top of the two layers of Histopaque, 6 mL of mixed whole blood from the EDTA tubes should be layered next. This can be performed in the same manner as the Histopaque to create three layers. The 15 mL conical tubes should be capped, labeled and centrifuged at 700xg and 18-26℃ for 30 minutes. After the centrifugation is complete, using a 1mL pipet tip, aspirate out the top plasma layer and discard. The next layer, the buffy coat, contains lymphocytes. This should be collected with a new pipet tip and placed in a clean 15 mL conical tube, mixed with RPMI to make the final liquid level 1 mL, and kept on ice until being used. The next layer in the original conical tube should be collected until about two thirds of the way through the red layer of the tube. This contains the neutrophils. It will also contain RBC so this layer should be collected with a clean pipet tip and placed into a new 50 mL conical tube. Once all samples have been placed into individual, labeled 50 mL conical tube, 20 mL of ice cold deionized water is added to the tubes, they are covered and inverted to mix about fifteen times. This cold water can remain in the tube for up to sixty seconds. Once mixed, 5 mL of 5x PBS is added to the 50 mL conical tube and mixed again. These tubes are then centrifuged for 10 minutes at 200xg and 4℃. Once this is complete, the supernatant is decanted and the white pellet of cells at the bottom of the tube is reconstituted with 1 mL of RPMI until a homogenous mixture. This mixture is aspirated into another 15 mL conical tube with enough RPMI to make the total fluid level 10 mL. These tubes are centrifuged again on the same program of 10 minutes at 200xg and 4℃. This washing and spinning cycle is repeated three times and on the last time, the liquid is decanted and the total volume

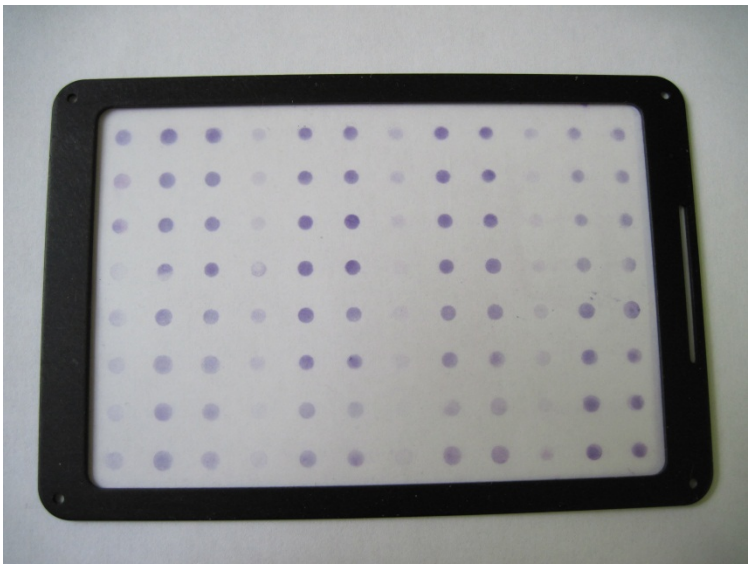
after reconstitution is raised to 1 mL in RPMI. The two cell type, neutrophils and lymphocytes are then ready to be counted by the Beckman Coulter cell counter using the same protocol as described for whole blood. The only difference is that these samples do not need the Zap Oglobin as there are theoretically no RBC in the samples.

For neutrophil chemotaxis, a final cell concentration of $3x10^6$ is needed, therefore, $3x10^6$ should be divided by the concentration of neutrophils counted in the coulter counter for each sample. This number should then be multiplied by 1000 to determine the amount of that particular suspension needed to provide enough cells for the assay. The amount of suspension needed should be subtracted from the total fluid needed (1000uL) in order to determine how much of the RPMI/5% FBS mixture will make up the rest of the solution for a total of 1000uL. The chemoattractants for the assay can be prepared concomitantly, and require 10 uL of IL-8 and 990 uL of RPMI for the IL-8 chambers, placed in a 5 mL tube. C5a will need a dilution- 10 uL is added to 990 uL of RPMI in a 5 mL tube. One hundred microliters from this solution is then added to 900 uL of RPMI in another 5 mL tube. A chemotaxis chamber should be pepared by placing 30 uL of RPMI, C5a and IL-8 alternately in the wells of the chamber. This requires quick but deliberate pipetting into each well in order to create a convex bubble at the top of the well with no bubbles in the media. Once all wells have been filled with the respective chemoattractants, a PVP-free filter needs to be placed carefully across all wells with the shiny side of the filter facing up. After the filter is in place (a new chamber needs to be made if the filter is moved or does not contact all of the wells), the gasket should be screwed onto the top. The screws need to be tightened in a star pattern to prevent uneven pressure distribution. The chamber should be incubated at 37℃, 95% humidity and 5.0% CO_2 for 10 minutes. After incubation, 50 uL of the neutrophil

suspension should be added to each well, taking note to create duplicates of each chemoattractant for each gilt blood sample. The location of each gilt blood sample on the chamber should be recorded. The chamber can then be replaced into the incubator for 1 h in the same settings as previously mentioned. After 1 h of incubation, the chamber should be flipped upside down, screws removed and filter gently peeled off with forceps. The shiny side of the filter (non-cell side) is dipped into a PBS-filled petri dish several times and scraped, with care not to contaminate the other side. The filter is allowed to dry attached to forceps but touching nothing else. Once dried, the non-shiny side (cell side) is dipped into a petri dish containing methanol several times without scraping. After drying, the same side is then dipped into red dye in a petri dish 6-8 times. Immediately following the red dye, the filter is dipped into purple dye 6-8 times and promptly rinsed with water. The filter can be placed on a glass slide and read at the earliest convenience. When reading the slides, the well being examined should be noted and five different areas in each well should be counted at 100x magnification.



Example of chemotaxis chamber (Photo courtesy of NeuroProbe)



Example of chemotaxis filter after staining for neutrophils

Neutrophil phagocytosis uses the same neutrophil isolation technique as described above. Once the neutrophil concentration has been determined, a final concentration of $2x10^6$ is required for the assay in 5 mL tubes. Fluorescent beads at a ratio of 10:1 with inactivated porcine serum should be incubated for 30 min at 37℃. After incubation, 100 uL of the bead solution is added to each sample of neutrophils that are at a concentration of $2x10^6$. These samples with the bead mixture are capped and inverted to mix and incubated and rotated for 45 min at 37℃. The samples are then centrifuged at 1000xg for 5 mins and decanted. The cells at the bottom of the tube should be resuspended in 1 mL of RPMI with 100 uL of 4% formaldehyde to fix the cells. These tubes were analyzed using flow cytometry and control samples of the bead solution.

Lymphocyte proliferation is measured in response to two mitogens in three concentrations and a control. The lymphocytes prepared in the isolation steps need to be converted to a concentration of $5x10^6$ in RPMI with 10% FBS. In a 96 flat-welled plate, 100

uL of the cell solutions should be pipetted in triplicate, completing three columns of the entire plate per sample. The mitogens, Concanavalin A and lipopolysaccharide at concentrations of 0, 25 and 50 µg/mL, were used to stimulate T and B lymphocytes. Each row contained a different mitogen or mitogen concentration, resulting in a plate with each blood sample proliferating in response to each mitogen in three different wells. Plates should then be incubated for 68 h at 37℃ in a 5% CO_2, 95% humidity incubator. Twenty microliters of MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) would then be added to each well and plates are then incubated 4 h. Acidified isopropanol (100 µL 0.1 N HCl in anhydrous isopropanol) is then added, and the plates are allowed to incubate overnight at 37 ºC. Using a microplate reader, the plates are read at wavelength 550 nm with reference wavelength 690 nm. The results are expressed as a proliferation index (PI): Optical density (550/690 nm) stimulated cells ÷ Optical density (550/690 nm) non-stimulated cells.

Example of BioTek plate reader used in lymphocyte proliferation assays; This image is property of BioTek Instruments, Inc. (www.biotek.com)