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Genetic Factors Associated with Thermal Tolerance in Grow-finish Pigs as Measured by Feeding Behavior

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GENETIC FACTORS ASSOCIATED WITH THERMAL TOLERANCE IN GROW-FINISH PIGS AS MEASURED BY FEEDING BEHAVIOR

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This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy in Animal Science degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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TABLE OF CONTENTS

LIST OF TABLES

Chapter 4

LIST OF FIGURES

Chapter 2

Chapter 3

Chapter 4

ABSTRACT

GENETIC FACTORS ASSOCIATED WITH THERMAL TOLERANCE IN GROW-FINISH PIGS AS MEASURED BY FEEDING BEHAVIOR

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The objectives of this study were: one, use electronic monitoring to determine feeding behavior patterns of grow-finish pigs throughout the year and to identify changes that occurred during heat stress events, and second, identify genetic markers associated with changes in feeding behavior due to heat stress. Pigs were placed in a grow-finish barn at approximately eight to ten weeks of age in 6 pens of 40 animals and monitored for 4-months. Gilts and barrows were from three different sire breeds, Duroc, Landrace, and Yorkshire. Each pen had one feeder, designed to feed 5 animals at a time. Feeders were fitted with an antenna and a multiplexer. Data were collected from antennas every 20 seconds. Outside temperature and humidity were obtained from a National Weather Station and used to calculate temperature humidity index (THI). Days in the study were partitioned into groups based on their maximum temperature humidity index (THI), where a THI less than 23.33°C was classified as "Normal", a THI between 23.33°C and 26.11°C was classified as "Alert", a THI between 26.11°C and 28.88°C was classified as "Danger", and a THI greater than 28.88°C was classified as "Emergency". Feeding behavioral differences among breeds and sex were observed across all THI categories. Landrace-sired pigs had fewer feeder visits compared to Duroc- and Yorkshire-sired pigs. Gilts had fewer feeder visits than barrows in all THI categories. A genome-wide association study for an animal's change in feeding behavior between different THI

categories was also conducted. Heritabilities for the difference in a pig's feeder visits between each of the THI categories were low to moderate (0.136 to 0.406). Greater than 71% of genetic variation was explained by regions within eight chromosomes in the comparison between Danger and Emergency THI. Biological processes related to sensory perception and detection of chemical stimuli were over-represented in the set of genes located in these regions. Differences in feeding behavior patterns between THI categories demonstrate that heat stress affects sire breeds and sexes differently. Also genetic markers identified in this study may facilitate genetic selection for improved grow-finish performance during elevated ambient temperatures.

CHAPTER 1

REVIEW OF LITERATURE

INTRODUCTION

Pork is a widely consumed protein source throughout the world. In order for pork to remain competitive, production efficiency must improve. Certain types of stressors encountered throughout production negatively impact production efficiency. Heat stress is a major stressor affecting pork production. Although there have been advances in nutrition, production management, and barn cooling systems, production efficiency continues to suffer during summer months. Heat stress is an economic concern as well as an animal welfare concern. Little is known about thermal neutral zones for different breeds of pigs during the finishing phase or the impact heat stress has on those different breeds. In order to predict thermal neutral zones, it is important to understand feeding behavioral patterns throughout the year. Therefore, the areas that will be covered in this literature review are feeding behavior patterns and factors affected by heat stress.

FEEDING BEHAVIOR

Several different systems are available to study feeding behavior through measuring feed intake, but most of these systems only allow one pig to feed at a time (Brown-Brandl et al., 2013). While these systems provide important information on feed intake and behavior, it is not a true representation of grow-finish commercial production, where animals eat from group feeders. A system that records meal length, meal interval, number of meals per day, and total time spent eating for pigs in a grow-finish commercial setting was created by Brown-Brandl and Eigenberg (2011). Moderate heritabilities have been estimated for feeding behavior in individually housed pigs (Chen et al., 2010). Rohrer et al. (2013) reported heritabilities for feeding behavior ranging from 0.157 to

0.604 in group-housed pigs using the system developed by Brown-Brandl and Eigenberg (2011).

Several factors influence feeding behavior in pigs. These factors include but are not limited to age, sex, growth rate, type of housing (group versus individual), and type of feeder. As pigs increase in age they will need to spend more time at feeders to consume enough feed to meet growth requirements. For example, average time spent at the feeder on day 65 of age was 24 minutes per day and increased to 76.7 minutes per day at 107 days of age (Brown-Brandl et al., 2013). Time spent eating increased roughly 1.28 minutes per day (Brown-Brandl et al., 2013). In contrast, several studies have reported no change in time spent at the feeder, but rate of feed intake increased (Labroue et al., 1994; Quiniou et al., 2000) as age increased. Hyun et al. (1997) reported an average time of 75 min per day spent at the feeder. Rohrer et al. (2013) reported a slightly lower average feeder time of 68 minutes per day for grow-finish pigs, with an average meal length of 360 seconds.

Heritability estimates for meal characteristics were moderate to highly heritable (0.315 to 0.604) (Rohrer et al., 2013). A heritability of 0.38 (± 0.08) for daily feeding time has been reported (Chen et al., 2010). Rohrer et al. (2013) reported a similar heritability for daily feeding time (0.37 ± 0.08). However, de Haer and de Vries (1993) reported a lower daily feeding time heritability of 0.24 (± 0.20). A quantitative trait locus located on chromosome 6 has been discovered for daily feeding time (Houston et al., 2005). Zhang et al. (2009) reported 2 quantitative trait loci, located on chromosomes 7 and 9, for number of meals per day. Time spent at the feeder eating and length of meal were both positively correlated, genetically, to weight and fat at 154 days of age (Rohrer

et al., 2013). Feeding behavior is moderate to highly heritable and correlated to performance traits.

In a feeding behavior study, Brown-Brandl et al. (2013) reported barrows spent more time at feeders than gilts between 91 and 158 days of age. Barrows spent approximately 85 minutes per day at feeders, while gilts only spent around 71 minutes per day at feeders (Brown-Brandl et al., 2013). In contrast, Hyun et al. (1997) reported no difference between sex and time spent at feeders. If barrows and gilts are in mixed pens, barrows could spend more time at feeders than gilts, increasing competition between gilts and barrows.

Animals that have a higher growth rate spend more time at feeders. High gaining pigs had the highest average time spent at the feeder (79.1 ± 0.45 min d^{-1}) and low gaining pigs had the lowest average time spent at the feeder (63.6 ± 0.35 min d^{-1}) (Brown-Brandl et al., 2013). Separation between the high and low gain groups occurred by day 7 (Brown-Brandl et al., 2013). Once high gain pigs were removed from pens, low gain pigs increased their time at the feeder (Brown-Brandl et al., 2013) demonstrating how group housing imposes a stress of competition for feed among pigs.

Stressors impact behavior in pigs. Several methods are used to determine a pig's ability to handle stressful situations. To test how a pig copes with a perceived stressful situation researchers have used the backtest (Hessing et al., 1993; Cassady, 2007; Velie et al., 2009). Heritabilities for total time spent struggling was 0.49 and total attempts to struggle was 0.53 (Velie et al., 2009). Rohrer et al. (2013) reported smaller heritabilities for total time spent struggling (0.154) and total attempts to struggle (0.155). Higher heritabilities reported by Velie et al. (2009) could be due to performing the backtest on

each pig twice during the suckling period, therefore reducing environmental variance. Age was also a difference between the two studies. Rohrer et al. (2013) performed the backtest 2 days after weaning, while Velie et al. (2009) performed the backtest between 7 and 14 days of age. Performing the backtest after the pigs were removed from the sow eliminates environmental effects of the sow's reaction suggesting that behavior is both a genetic and environmental trait.

Another mechanism to examine how a pig handles a stressful situation is to look at temperament scores while in the chute (Holl et al., 2010). Animals are scored on a scale of 1 to 5, with a score of 1 representing calm and little movement and 5 representing continuous movement, vocalizations, and attempts to escape (Holl et al., 2010). Of the pigs studied, 58.1% of the pigs were in category 1, 28.5% in category 2, 8.9% in category 3, 4.0% in category 4, and only 0.5% of the pigs were in category 5 (Holl et al., 2010). Females had lower activity scores than males (Holl et al., 2010). Activity score had a heritability of 0.23 (Holl et al., 2010). When using a threshold model, reported heritability of activity score increased to 0.30 (Holl et al., 2010). Animals with greater activity in the chute had decreased feed intake (Nkurmah et al., 2007).

Behavioral traits are heritable and are correlated with performance traits (van Erp-van der Kooij et al., 2000). Velie et al. (2009) reported a negative phenotypic correlation between time spent struggling and number of struggle events with growth in the farrowing house. Total number of struggle attempts was positively correlated (phenotypic) to backfat (Velie et al., 2009). Therefore, animals that were less responsive to the backtest were leaner. Similar results were reported by Cassady (2007). Activity score is genetically correlated with 154-day weight (Holl et al., 2010). As activity score

increased, growth became slower, but backfat increased (Holl et al., 2010). Decreasing activity score and time spent struggling, is expected to result in more docile and less stressed animals, therefore, maintaining leanness and increasing growth performance.

Backtest and feeding behavior are genetically correlated (Rohrer et al., 2013). Time until first struggle on the backtest had a positive genetic correlation with number of meals per day and a negative genetic correlation with average meal length (Rohrer et al., 2013). Therefore, as time until first struggle increased, number of meals increased and average meal length decreased. Pigs that spent more time struggling had longer average meal lengths, and spent less time at the feeder each day (Rohrer et al., 2013). Animals that had more struggle attempts and spent more time struggling preferred to consume meals on the gate-side of the feeder (Rohrer et al., 2013).

Animals which had a more reactive response to stressors consumed fewer meals of longer length and ate when fewer animals were at the feeders (Rohrer et al., 2013). This correlation could be due to these higher stress animals avoiding other animals while eating. In order to improve animal performance, a decrease in number of stressful events needs to occur. Selection on an animal's ability to cope with stressful events would also increase performance.

HEAT STRESS

Stress is the reaction to stimuli disrupting physiological equilibrium or homeostasis (Khansari et al., 1990). Heat stress is an environmental stressor imposed on animals during warm months. As temperature increases, animals need to remove heat from their bodies. Pigs experience heat stress when they produce more heat than they can

dissipate. Heat production in pigs comes from feed consumption, maintenance, and physical activity (Kerr et al., 2003). Unlike other animals, pigs have a limited capacity to use water evaporation to lose heat (Ingram, 1965). In order to adapt to warm environments pigs decrease heat production by reducing the amount of heat that needs to be eliminated (Nienaber and Hahn, 1982; Collin et al., 2001b; Quiniou et al., 2001).

As global climate continues to change ambient temperature continues to rise, and complications due to heat stress will increase in pork production (Renaudearu et al., 2011). Heat stress occurs in tropical regions for extended periods of time as well as temperate regions during warmer summer months (Collin et al., 2002). Large commercial production units with advanced production management and barn cooling systems as well as smaller production systems without advanced cooling systems are all impacted negatively by heat stress. Although most commercial swine production has barns with engineered ventilation systems, variations in air temperature still occur (Bond et al., 1967). These variations in air temperature can be caused by poor temperature control, poor building design, changes in animal heat loss, and outside temperatures (Bond et al., 1967).

Pigs have a zone of thermal comfort in which they are most productive. This thermal comfort zone is dependent on several different factors, including genetics, physiological status, relative humidity, and velocity of ambient air (NRC, 1981; Nyachoti et al., 2004). High temperatures and humidity negatively impact all stages of pork production. Decreased industry production includes, but is not limited to reduced growth, poor sow performance, increased morbidity and mortality, inconsistent market weights, altered carcass composition, increased days to slaughter, and increased

production costs (Collin et al., 2002; Brown-Brandl et al., 2004; Baumgard and Rhoads, 2013, Gabler and Pearce, 2015).

Heat stress has a large impact on production loss for the swine industry. Economic losses, due to heat stress, for the United States pork industry are estimated at $300 million a year (St-Pierre et al., 2003). This loss is from all aspects of production from farrow to finish. Heat stress decreased dry matter intake, milk yield, and increased body weight loss during lactation in sows as well as decreases growth of market pigs (McGlone et al., 1988; Johnston et al., 1999; Collin et al., 2001a; Renaudeau and Noblet, 2001; Renaudeau et al., 2001). Of this estimated economic loss, a majority of it ($202 million per year) occurs during the grow-finish phase (St-Pierre et al., 2003).

IMPACT OF HEAT STRESS

Respiration Rate

A pig's initial response to heat stress begins with increased respiration rate (Huynh, 2005). Increasing respiration rate allows the pig to try to remove some of the excess heat from its body. Respiration rate increased from 49 ± 2 breaths per minute in thermoneutral (21.5°C) pigs to 94 ± 2 breaths per minute in heat stressed (34.1°C) pigs during the growing phase (Johnson et al., 2015a). Another study, conducted during the finishing phase, reported respiration rates increased from 58 ± 2 breaths per minute to 92 ± 2 breaths per minute in thermoneutral versus heat stressed pigs (Johnson et al., 2015b).

It is important to note that respiration rate will change depending on time of day. At night during hot summer months, temperature will cool down, giving pigs a break from heat. Respiration rate for heat stressed pigs in a hot, diurnal environment went from

41.0 breaths per minute in the morning to 69.3 breaths per minute in the afternoon, while respiration rate for thermoneutral pigs went from 28.6 breaths per minute in the morning to 37.6 breaths per minute in the afternoon (Lopez et al., 1991). Increased respiration rate in thermoneutral pigs could be due to an increase in activity level during the afternoon. Overall, there was a larger increase in respiration rate in the heat stressed pigs compared to thermoneutral pigs. Therefore, as temperature increases, respiration rate will increase in order to dissipate heat being produced by the pig.

Voluntary Feed Intake

If ambient temperature continues to increase and pigs are still producing more heat than they can remove from their body, a voluntary decrease in feed intake occurs (Nienaber et al., 1987a; Quiniou et al., 2000; Le Bellego et al., 2002b; Nyachoti et al., 2004; Huynh, 2005). As voluntary feed intake decreases in order to help alleviate heat stress, decreased production efficiency results. In young pigs (20 kg), maximum voluntary feed intake occurs between 19 and 25°C (Collin et al., 2001b). As temperature increases from 25 to 33°C, voluntary feed intake decreases and when temperature is above 33°C a sizeable decrease in feed intake occurs (Collin et al., 2001b). This change suggests that the upper limit of the thermal comfort zone for pigs weighing 20 kg is 25°C. Several studies have reported upper critical limits for voluntary feed intake between 22.9 and 25.5°C (Le Bellego et al., 2002a; Huynh et al., 2005; Quiniou et al., 2001).

As a pig surpasses the upper critical limit of their thermal neutral zone, a reduction of heat production must occur. As temperature increased from 23°C to 33°C, voluntary feed intake was reduced by 30% in young pigs (20 – 30 kg) (Collin et al.,

2001a). Nienaber et al. (1996) found a slightly smaller (26%) reduction in feed intake at 33°C for grow-finish pigs (40 – 100 kg). Feed intake decreases about 2.6 to 3.0% for every one-degree increase in temperature. Quiniou et al. (2000) reported a similar decrease of 3.4% for every one-degree increase in temperature in heavier pigs. Other studies have reported lower (12 – 19%) reductions in feed intake (Stahly et al., 1979; Le Bellego et al., 2002b; Kerr et al., 2003). Several authors have reported the effects of temperature on feed intake are quadratic (Nienaber and Hahn, 1983; Quiniou et al., 2000). Nienaber et al. (1996) reported an increase in temperature above thermoneutrality, causes a nonlinear decrease in feed intake. Therefore, pigs experiencing higher temperatures will have a larger reduction of feed intake.

Pigs exposed to long-term heat stress for 3 weeks had a reduced feed intake of 771 g, which was a decrease of about 32% in feed intake (Renaudeau et al., 2013). In a commercial setting, constant heat stress over a long period of time is not likely, due to decreasing temperatures at night. However, grow-finish pigs experiencing a diurnal pattern of heat stress had reduced average daily gain and 26% feed intake reduction over a one-month period (Song et al., 2011). Even if temperatures are reduced at night, feed intake will still suffer due to heat stress experienced during the day.

Decreasing feed intake causes a decrease in metabolic heat production, allowing pigs to maintain a normal body temperature during hot conditions (Quiniou et al., 2001; Renaudeau et al., 2011; Renaudeau et al., 2013). Heat stressed finishing pigs, with a 13% reduction in feed intake, had lower total heat production than thermoneutral pigs with the same reduction in feed intake (Brown-Brandl et al., 2000). A decrease in voluntary feed

intake from 23°C to 33°C resulted in a 14% lower fasting heat production (Collin et al., 2001a).

Most of the reported studies have measured heat stress on individually housed pigs or on a small scale (Nienaber et al., 1990; Quiniou et al., 2000; Brown-Brandl et al., 2001; Collin et al., 2001a). Little is known on the impact of heat stress on voluntary feed intake in a commercial setting (Nyachoti et al., 2004). When animals are in commercial settings, barn temperature will fluctuate with outside temperature and humidity. Voluntary feed intake was significantly reduced in grow-finish pigs when temperature was 28°C and humidity went from 65 to 75% (Massabie et al., 1997). Even when a thermoneutral temperature of 24°C was maintained and humidity increased from 45 to 90%, a significant reduction in voluntary feed intake occurred (Massabie et al., 1997). A 32 g d^{-1} decrease in feed intake occurred following a 10% increase in humidity while holding temperature constant at 33°C (Morrison et al., 1969). Feed intake reduction was magnified as relative humidity exceeded 80% (Morrison et al., 1968). Therefore, in a commercial setting, humidity will affect the impact of heat stress on pigs.

Differences in feed intake reduction could be due to body weight, genotype, sex, diet composition, housing, humidity, and temperature. Higher temperatures will affect feed intake in heavier pigs more so than lighter pigs. Interactions between temperature and body weight on feed intake occur (Quiniou et al., 2000). Renaudeau et al. (2011) reported constant feed intake levels below 23.6°C, but once temperature increased over 23.6°C feed intake was reduced by 25 g d^{-1} $°C^{-1}$ in 50 kg pigs. Voluntary feed intake decreased by 9 g d^{-1} $°C^{-1}$, 32 g d^{-1} $°C^{-1}$, and 55 g d^{-1} $°C^{-1}$ for animals weighing 25 kg, 50 kg, and 75 kg respectively as temperature increased from 20 to 30°C (Renaudeau et al.,

2011). This difference could be due to heavier pigs having an increased metabolic rate at thermoneutrality (Renaudeau et al., 2011). Heavier pigs also have a lower ratio of surface area to mass and have more insulation than smaller pigs (Bruce and Clark, 1979), thus decreasing their ability to dissipate heat. Since heavier pigs cannot dissipate heat well, they must decrease feed intake at a higher rate than smaller animals in order to decrease total heat production.

A reduction of feed intake, due to heat stress, could also be caused by certain feed ingredients. Some feed ingredients have higher heat increments. Fibrous feedstuffs have higher heat increments than fat sources (Just, 1982). Diets high in fiber increase the impact of heat stress on pigs, while diets higher in fat decrease the impact of heat stress (Schoenherr et al., 1989). Protein has a heat increment of 36%, carbohydrates have a heat increment of 22%, and fats have a heat increment of 15% (Brown-Brandl et al., 2004). Different diets could be the cause of variation seen in decreased feed intake during heat stress. Diets high in fiber or protein would more than likely cause a higher reduction in feed intake than diets higher in fat.

Although a reduction in voluntary feed intake decreases heat production, it also has a negative impact on growth performance. Body weight gain decreased from 987 g d^{-1} to 621 g d^{-1} as temperature increased from 23°C to 33°C (Collin et al., 2001a), resulting in a 37 g d^{-1} decrease in body weight for every one-degree Celsius increase in temperature. This decrease in body weight is slightly higher than other studies reported (Sugahara et al., 1970; Rinaldo and Le Dividich, 1991). Differences in the decrease in body weight could be due to different initial body weights or different housing systems (group versus individual housing).

Exposure to high ambient temperature does not have to be prolonged (2 – 24 hours) before pigs will start losing significant body weight (Pearce et al., 2013; Pearce et al., 2014). Body weight reduction was almost 3 kg after only 24 hours of heat stress in grow-finish pigs (Pearce et al., 2013). In contrast, Collin et al. (2001a) reported a 1 kg loss of body weight over a 6-day period of heat stress (33°C) in young pigs (20 – 30 kg). Even during diurnal heat stress, a 16.3% decrease in body weight gain was observed over pigs housed in thermoneutral conditions (Lopez et al., 1991).

Heat stress decreases feed intake and growth performance in pigs. However, the impact of heat stress on feed efficiency is inconsistent. Heat stress is reported to have no effect on feed efficiency (Collin et al., 2001a) or decreased feed efficiency (Nienaber et al., 1987a; Johnson et al., 2015b). Pigs become less efficient under heat stress conditions, due to an increase in the feed conversion ratios (Renaudeau et al., 2011). Housing pigs in 33°C reduced feed efficiency compared to pigs housed at 23°C (Kerr et al., 2003). Johnson et al. (2015b) reported a 9% decrease in feed efficiency for heat stressed pigs compared to thermoneutral pigs during the finishing phase. In contrast, no change in feed efficiency was reported in pigs housed in 22°C versus 29°C environments even though feed intake decreased 15% (Le Bellego et al., 2002b). Nienaber et al. (1987a) reported similar findings, of no difference in feed efficiency, for pigs housed in 20°C versus 25°C environments. These differences could be due to the amount and duration of heat stress experienced by pigs. Mild heat stress slightly decreases feed intake and activity, thus resulting in similar feed efficiency. However, severe heat stress results in a greater decrease of intake, possibly causing weight loss. Also, severely heat stressed pigs will use

energy to actively release heat (increased respiration rate), thus having a greater impact on feed conversion.

Feeding Behavior

As previously stated, feeding behavior is affected by age, sex, growth rate, and housing. Temperature is another variable that affects feeding behavior. When temperatures increased, heat stressed pigs spent less time feeding than thermoneutral pigs (Hicks et al., 1998), demonstrating an association between eating activity and the rise in heat production (Nienaber et al., 1999). As ambient temperature increased, physical activity decreased. Decreasing physical activity is another method pigs use to reduce body heat when exposed to high ambient temperatures (Kerr et al., 2003). Brown-Brandl et al. (2000) reported a decrease in physical activity in heat stressed pigs. Pigs spent more time laying and less time eating during high ambient temperatures (Hicks et al., 1998; Brown-Brandl et al., 2001). Decreasing the number of feeder visits will decrease physical activity and body heat production. In contrast, Nienaber et al. (1996) reported that rate of eating was age and weight dependent, but not temperature dependent. Nienaber et al. (1993) and Quiniou et al. (2000) also reported temperature had no effect on daily number of meals in group-housed pigs.

As discussed in the previous section, heat stress reduces feed intake. Changes in eating behavior, mealtime, and meal size are associated with decreased feed intake (Collin et al., 2001b). Reducing meal size as well as number of meals per day, helps reduce the effect high ambient temperatures have on heat production (Nienaber et al., 1999) by decreasing physical and metabolic activity. Consumption time was shorter at

33°C (Collin et al., 2001a). Pigs in thermoneutral environments spent more time at the feeder eating than pigs in heat stress environments. Consumption time decreased from 5.9 min to 3.9 min when temperature increased to 33°C (Collin et al., 2001a). Quiniou et al. (2000) reported decreased ingestion time (64 versus 46 min d^{-1}) when temperature increased from 19 to 29°C. Although consumption time decreased, Quiniou et al. (2000) reported no change in number of meals per day.

Feeder visits ranged from 9 to 11 meals per day (Nienaber et al., 1996; Quiniou et al., 2000). Collin et al. (2001a) reported a higher number of daily meals (15 meals d^{-1}). A diurnal feeding behavior is seen in pigs, with two-thirds of daily meals consumed during the day (Nienaber et al., 1990; Collin et al., 2001a; Labroue et al., 1994; Quiniou et al., 2000). Nienaber et al. (1990) reported eating activity was greatest between early morning hours (0701 to 0900 h) when 14% of total feed was consumed and late afternoon hours (1301 to 1600 h) when 25% of total feed was consumed. Pigs, under thermal neutral conditions, consume 74.8% of their daily feed between 0900 to 1900 hours (Nienaber et al., 1990). It has been reported temperature does not affect the number of daily meals (Quiniou et al., 2000), which is possible due to the fact that pigs shift meals to the evening or early morning when temperatures have decreased (Xin and DeShazer, 1992; Nienaber et al., 1996; Quiniou et al., 2000). A decrease from 65% of meals to 55% of meals consumed during the day occurred when temperature increased from 19 to 29°C, respectively (Quiniou et al., 2000). Although meals may shift to cooler portions of the day, feed intake compensation is hardly ever reached (Xin and DeShazer, 1992).

Carcass Characteristics and Quality

Prenatal heat stress affects piglet body composition and postnatal growth (Foxcroft et al., 2006; Johnson et al., 2015b). Heat stress during gestation is detrimental to protein synthesis in growing fetuses. Piglets that experienced *in utero* heat stress had a 95% increased lipid to protein accretion rate during the finishing phase (Johnson et al., 2015b). *In utero* heat stress affects future nutrient metabolism independent of postnatal environmental exposure (Johnson et al., 2015b).

Heat stress reduces feed intake, decreasing nutrients available for tissue synthesis, thus reducing growth rates (Le Bellego et al., 2002b; Kerr et al., 2003; Johnson et al., 2015b). In young animals, lipid accretion was reduced by 15%, but protein accretion was not affected in heat stressed pigs compared to thermoneutral pigs (Johnson et al., 2015a). However, heat stress during the finishing phase reduced average daily gain, protein deposition, and fat accretion (Johnson et al., 2015b). Both carcass fat and backfat were reduced in heat stressed pigs (Le Bellego et al., 2002b). Although reduced feed intake occurred in younger pigs, they consumed enough to allow for protein deposition (van Millgen and Noblet, 2003), but the larger pigs were not consuming enough for fat accretion or protein deposition.

Under normal conditions during decreased nutrient intake, muscle growth is favored at the expense of adipose accretion (van Milgen and Noblet, 2003). However, during heat stress, typically adipose tissue is increased in carcasses (Collin et al., 2001b). Conversely, reductions in body fat of heat stressed pigs have been reported (Nienaber et al., 1987b; Renaldo and Le Dividich, 1991). Pigs housed in a heat stress environment that caused a 26% reduction in feed intake had lower backfat at slaughter compared to thermoneutral pigs (Nienaber et al., 1996). Backfat deposits decreased under heat stress,

while leaf-pad fat increased during heat stress (Le Dividich et al., 1987; Rinaldo and Le Dividich, 1991; Katsumata et al., 1996). Depositing more leaf-pad fat instead of backfat allows pigs to dissipate more heat through their skin (Katsumata et al., 1996). Decreased backfat could also be a result of pigs not consuming enough nutrients for lipid accretion.

Differing results have been reported for concentrations of ash, lipid, protein, and water in carcasses from heat stressed pigs versus thermoneutral pigs (Stahly et al., 1979; Nienaber et al., 1987b; Kerr et al., 2003). Heat stressed pigs had higher concentrations of water, lower concentrations of protein, and lower concentrations of ash (Kerr et al., 2003). In contrast, Stahly et al. (1979) reported no difference in concentrations of water, protein, or ash in carcasses from heat stressed pigs versus thermoneutral pigs. Pigs housed in 30°C environments had greater concentrations of water, protein, and ash than thermoneutral pigs (Nienaber et al., 1987b).

Several factors impact carcass quality, including carcass weight, fat (depots and firmness), and weight of primal cuts (White et al., 2008). Changes in carcass quality of heat stressed pigs included changes in meat color, decreased carcass weights, and changes in fat depots (Zeferino et al., 2013). Increasing heat load tended to reduce carcass weight, which was due to heat stress experienced 2 to 3 months prior to slaughter (Zumbach et al., 2008). Of the primal cuts, bellies are in high demand and are of high economic value to packers (Moarcous et al., 2007). Bacon from heat stressed pigs had decreased raw and cooked slice weights, and increased lean percentage (White et al., 2008). Heat stressed pigs also had more collagen in belly fat than thermoneutral pigs (White et al., 2008), thus negatively affecting the quality of bacon. Overall, carcass quality is negatively affected by heat stress.

Visceral Changes

Visceral organs produce a larger amount of metabolic heat than the carcass (Baldwin et al., 1980). Heat production of the digestive tract and liver account for 20 to 25% of total heat production (Yen and Nienaber, 1993). Heat stressed pigs are able to reduce total heat production by reductions in visceral mass (Johnson et al., 2015a) and visceral blood flow (Lambert et al., 2002; Leon and Helwig, 2010). Reductions in visceral mass were caused by heat stress (Rinaldo and Le Dividich, 1991; Gabler and Pearce, 2015) and reduced feed intake (Koong et al., 1982). Reduced feed intake and organ size (including digestive tract and liver) are positively correlated (Koong et al., 1982). Heat stressed pigs have a lower total visceral mass and liver weight compared to thermonerutral pigs during the finishing phase (Rinaldo and Le Dividich, 1991; Johnson et al., 2015b). At slaughter, liver, heart, and spleen weights were less in heat stressed pigs (33°C) compared to thermoneutral pigs (23°C) (Collin et al., 2002). Heat stressed pigs had a 7.7% decrease in total visceral weight compared to thermoneutral pigs (Johnson et al., 2015a).

As discussed earlier, feed efficiency may be decreased in heat stressed pigs (Johnson et al., 2015b). Decreased feed efficiency could be due to decreased viscera (Johnson et al., 2015b), instead of a reduction in the conversion of nutrients to weight gain. Due to the decrease in visceral mass during heat stress, heat production is decreased as well as maintenance costs (Johnson et al., 2015b). Kerr et al. (2003) reported a decreased weight of the large intestine and stomach, which affected reduced feed intake.

Although decreasing blood flow and feed intake decreased total heat production during heat stress, it also compromised the integrity of the intestinal barrier (Pearce et al.,

2013). Alterations in tight junction proteins during heat stress led to increased permeability of the intestines (Gabler and Pearce, 2015). Due to the increase in intestinal permeability, endotoxemia and pathogen loads also increased (Gabler and Pearce, 2015). Increased ambient temperature, decreased blood flow and feed intake, cause a decrease in intestinal integrity, therefore, increasing the potential for illness in heat stressed pigs.

Hormones

During heat stress, biological systems are altered. Decreasing feed intake and energy metabolism impacts hormones (Rinaldo and Le Dividich, 1991; Collin et al., 2002). Hypothalamic and neuropeptide hormone changes appear to have an effect on the reduction of feed intake (Pearce et al., 2014). Thyroid weight was not affected by ambient temperature (Collin et al., 2002); however, a reduction in circulating thyroid hormone concentrations has been reported in heat stressed animals (Prunier et al., 1997). Concentrations of T_3 and T_4 were significantly lower in heat stressed pigs (Collin et al., 2002). Thyroid hormones are thermogenic (Collin et al., 2002); therefore, decreased heat production is consistent with decreasing thyroid hormone concentrations at high ambient temperatures (Macari et al., 1986; Rinaldo and Le Dividich, 1991). Heat stressed pigs also have decreased concentrations of circulating glucose and insulin (Johnson et al., 2015b). After 12 hours of heat stress, insulin concentrations began to decrease (Gabler and Pearce, 2015). Insulin is a lipogenic and antilipolytic hormone (Vernon, 1992). Decreased concentration of insulin in heat stressed pigs could be the cause of decreased lipid accretion.

Genetics

Over the past decade, genetic selection has increased the amount of lean tissue in pigs, thus increasing basal heat production (Brown-Brandl et al., 2004). Leaner pigs require more metabolic energy than fatter pigs (Tess et al., 1984). From 1984 to 2002, fasting heat production increased 18.1% (Brown-Brandl et al., 2004). Harmon et al. (1997) reported a 33% difference in total heat production in current production versus the standard. During the finishing phase, total heat production is 26% higher (Brown-Brandl et al., 1998) than the standard obtained from ASAE (1999).

Increased total heat production is due to the increase in leanness of pigs. As percent muscle increases, a linear increase in fasting heat production occurs (van Milgen et al., 1998). Increasing lean tissue by 2.1% increased fasting heat production by 18.7% (Tess et al., 1984). Therefore, current selection for increased production reduces heat tolerance in pigs and genetics does impact heat stress. Genetic selection for increased growth has decreased a pig's ability to handle heat stress (Renaudeau et al., 2011).

Heat stress affects faster growing animals more than slower growing animals (Renaudeau et al., 2011). High growth genetic lines are more vulnerable to heat stress than moderate growth genetic lines (Nienaber et al., 1998), as metabolic heat increases, an animal's ability to cope with heat stress decreases. Newer, leaner genetic lines have a critical temperature limit that is 4°C lower than fatter genetic lines (Nienaber et al., 1997). Sire lines that are leaner and faster growing will be more susceptible to heat stress than maternal lines that are slower growing and fatter. In nursery pigs the critical temperatures change depending on breed Duroc sired animals had a critical temperature

of 26.1°C, Landrace sired animals had a critical temperature of 28.8°C, and Yorkshire sired animals had a critical temperature of 27.9°C (Brown-Brandl et al., 2015).

Since leaner genetic lines are more susceptible to heat stress (Nienaber et al., 1998) and heat stress affects growth rates (Collin et al., 2001a), then leaner genetic lines should experience a larger decrease in growth rates compared to conventional lines. Nienaber et al. (1997) studied the interaction of genetics and heat stress on finishing pigs. Environmental temperatures for the two treatments of heat stressed pigs were adjusted daily to cause a voluntary feed intake reduction of 13 or 26% (Nienaber et al., 1997). Each heat stress treatment group contained both high lean growth and moderate growth pigs (Nienaber et al., 1997). High-lean composite growth rates were significantly reduced by 34 and 25% in heat stressed pigs which were representative of the reduced feed intake treatments of 26 and 13% (Nienaber et al., 1997). Meal size decreased as temperature increased for all genetic lines, but the high lean growth line experienced a greater decrease in meal size than any other line (Nienaber et al., 1997). Selection under heat stress could be used to increase heat tolerance.

Increasing ambient temperature decreased protein deposition (nearly 50%) in the high lean composite, whereas protein deposition in moderate growth composites was not affected by temperature (Nienaber et al., 1997). In heat stressed lean pigs, backfat and leaf fat were increased by 10 to 25% (Nienaber et al., 1997). As fat increased, heat production decreased (Tess et al., 1984). Although protein deposition decreased, fat accretion increased in lean pigs exposed to high ambient temperatures.

Under thermoneutral conditions, organs were on average heavier in lean composite pigs than moderate growth pigs, indicative of higher maintenance

requirements (Nienaber et al., 1997). However, under heat stressed conditions, lean pigs experienced a larger decrease in liver, kidney, heart, stomach, and intestine weights than moderate growth composites (Nienaber et al., 1997). It was concluded that heat stress has a larger impact on high lean growth pigs than moderate growth pigs.

CONCLUSION

Stress impacts feeding behavior of pigs, thus affecting optimal growth performance. The backtest, used to determine an animal's ability to handle stressful situations, was correlated with feeding behavior. Calmer pigs are better at handling stressful situations, causing their time until first struggle to increase. Time until first struggle during the backtest was positively genetically correlated with number of meals per day and negatively genetically correlated with average meal length.

Heat stress is a major stressor impacting performance and production efficiency during warm months. As ambient temperature increases, pigs need to decrease total heat production in order to maintain homeostasis. Pigs have limited ability to use evaporation to remove heat. Therefore, in order to remove heat, pigs first increase respiration rate and then as temperatures continue to increase decreased feed intake occurs. Reducing feed intake allows pigs to decrease metabolic heat production, resulting in an overall decrease in total heat production. A reduction in feed intake due to heat stress also caused a change in carcass characteristics. Heat stressed pigs have reduced protein deposition and variable fat accretion, due to their inability to consume enough feed during the grow-finish phase of production.

Changes in feeding behavior occurred along with changes in feed intake. Heat stressed animals spent less time at feeders and more time lying down during the heat of the day. This decrease in feeder visit activity is similar to pigs that were more stressed or reactive to the backtest. Pigs react to stressors, whether the backtest or heat stress, with changes in feeding behavior.

Many breeding programs have focused on increasing lean growth in commercial pigs. Increasing lean growth also increases total heat production. Therefore, heat stress has a greater impact on high growth lines. Heat stress of leaner breeds should be a concern for producers. Before producers can increase production efficiency, a better understanding of feeding behavior during heat stress and breed impact on thermal neutral zones during the finishing phase needs to occur. Therefore, a study on feeding behavioral patterns in grow-finish pigs throughout the year needs to occur in order to determine changes in feeding behavior of different breeds during heat stress.

CHAPTER 2

FEEDING BEHAVIOR OF GROW-FINISH SWINE AND THE IMPACTS OF HEAT STRESS

ABSTRACT

Heat stress has negative impacts on pork production, particularly in the grow-finish phase. During heat stress events, feeding behavior of pigs is altered to reduce heat production. Several different systems have been developed to study feeding behavior. Those systems are not an accurate representation of grow-finish commercial production, as feed intake is monitored for only one pig at a time. The objective of this study was to utilize a feed monitoring system, representative of commercial conditions, to determine feeding behavior patterns of grow-finish pigs throughout the year and to identify changes that occurred during heat stress events. Feeder visit data were collected on barrows and gilts (n = 1653) from 3 different sire breeds, Landrace, Yorkshire, and Duroc, between July 2011 and March 2016. Days in the study were partitioned into groups based on their maximum temperature humidity index (THI), where a THI less than 23.33°C was classified as "Normal", a THI between 23.33°C and 26.11°C was classified as "Alert", a THI between 26.11°C and 28.88°C was classified as "Danger", and a THI greater than 28.88°C was classified as "Emergency". Feeding behavioral differences among breeds and sex were observed across all THI categories. Landrace-sired pigs had fewer feeder visits compared to Duroc- and Yorkshire-sired pigs. Gilts had fewer feeder visits than barrows in all THI categories. Differences in feeding behavior patterns between THI categories demonstrate that heat stress affects sire breeds and sexes differently.

INTRODUCTION

Swine feeding behavior monitoring enables producers and researchers to better understand factors that influence feed intake. Feeding behavior has been studied using

several different systems that measure feed intake, but most of these systems only allow one pig to feed at a time (Brown-Brandl *et al.*, 2013a). This is not an accurate representation of grow-finish commercial production, where animals are fed from group feeders. Brown-Brandl and Eigenberg (2011) created a monitoring system representative of a typical grow-finish commercial setting. This system consists of a 5-slot feeder fitted with a multiplexor and antennas for each feed slot and records meal length, meal interval, number of meals per day, and total time spent eating.

Several factors influence feeding behavior in pigs, including but not limited to breed, gender, season, and stressors. Stressors are stimuli disrupting physiological equilibrium or homeostasis (Khansari *et al.*, 1990). During warm months, pigs are subject to heat stress. Due to their limited capacity to use water evaporation to lose heat (Ingram, 1965), pigs decrease heat production during times of elevated ambient temperature by decreasing activity, decreasing feed consumption, and increasing respiration rate (Nienaber and Hahn, 1982; Nienaber *et al.,* 1999; Collin *et al.,* 2001; Quiniou *et al.*, 2001; Huynh *et al.*, 2005).

Many advances have been made in production management and barn cooling systems; however, production efficiency continues to decline during warm months. Economic losses for the United States pork industry due to heat stress are estimated at $300 million a year (St-Pierre *et al.*, 2003). This loss covers all aspects of the swine industry from farrow to finish, but a majority of these losses occur during the grow-finish phase. In order to gain a better understanding of feeding behavior changes during heat stress events, it is important to understand normal feeding patterns throughout the year. The objective of this study was to determine feeding behavior patterns of different breeds

and sexes of pigs throughout the year and to identify changes that occurred during heat stress events using a feed monitoring system representative of feeding conditions in commercial grow-finish operations.

MATERIAL AND METHODS

All measurements recorded were approved by the U.S. Meat Animal Research Center's Animal Care and Use Committee and conformed to the Guide for Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010). Data were collected on grow-finish pigs (n = 1653) from July 2011 to March 2016 (Table 2.1). At approximately 8 to 10 weeks of age, barrows and gilts were placed in a grow-finish barn equipped with sprinkle cooling. Feeding behavior was monitored over a four-month grow-out period. Grow-finish groups (n = 240) were distributed across six pens with 40 pigs per pen. Each pen had nearly equal representations of each sex and breed. Sire lines included Duroc, Landrace, and Yorkshire. All pigs were produced from Landrace-Yorkshire composite sows. Upon entry, pigs were tagged with electronic identification tags.

Pens were fitted with an electronic feeding system to monitor feeding behavior (Brown-Brandl *et al.*, 2011). All feeders had five slots, allowing more than one animal to eat at any given time. A corn-soybean meal diet was provided *ad libitum* to all pigs. Feed was formulated to meet or exceed all nutritional requirements of the growing pigs. Feeders were fitted with a multiplexor and antennas for each feed slot, allowing the animals' low-frequency radio frequency electronic identification (LF-RFID) tags to be read while the animals were at the feeder. Data were collected every 20 seconds from all antennas during the 4-month period.

Outside temperature (°C) and relative humidity (RH) were obtained from the National Weather Station located three miles northwest of the grow-finish barn and were used to calculate temperature humidity index (THI) (NOAA, 1976):

$$THI\ (°C) = T(°C) - [0.55 - (0.0055 \times RH)] \times [T(°C) - 14.5].$$

THI were calculated in one-hour increments because outside temperatures were reported every hour. Feeder visits were averaged over each one-hour time period. Here, feeder visits in time period 1 were recorded between 12:00:00 AM and 12:59:59 AM, those in time period 2 were recorded between 1:00:00 AM and 1:59:59 AM, and so on.

Using the outline of Brown-Brandl *et al.* (2013b) for THI categories, days were partitioned into temperature groups (Table 2.2), according to their maximum observed THI, in to order examine feeding behavior during extreme heat events. A THI less than 23.33°C were classified as Normal, THI between 23.33°C and 26.11°C were classified as Alert, THI between 26.11°C and 28.88°C were classified as Danger, and THI greater than 28.88°C were classified as Emergency. Feeding behavior data were then summarized based on each animal's sex, breed of sire, time of day, and the THI category for the day. Significance was determined using a paired t-test for breed and sex feeder visit activity means.

RESULTS

Of the 1653 pigs, 309 were Duroc sired, 791 were Landrace sired, and 553 were Yorkshire sired. There were 729 barrows and 924 gilts. Grow-finish groups G1 and G3 consisted of only Landrace-sired pigs, group G2 was comprised of only Yorkshire-sired pigs, and groups G4 – G7 contained an equal number of Duroc, Landrace, and Yorkshire-

sired pigs (Table 2.1). Maximum outside daily THI ranged from -8.36 °C to 30.52 °C for groups G4 – G7, while maximum THI for all of the groups ranged from -9.06 °C to 34.50 °C. Groups G1 – G3 experienced more extreme heat than the last four groups. Therefore, we conducted two separate feeding behavior analyses: (1) groups G4 – G7 which consisted of equal numbers of animals from each of the three sire breeds and (2) the Landrace- and Yorkshire-sired pigs from all seven groups.

Analysis I: Groups G4 – G7

Of the 932 grow-finish pigs used in this analysis, 309 were Duroc sired, 312 were Landrace sired, and 311 were Yorkshire sired. There were 459 barrows and 473 gilts. Average daily feeder visit counts for all sire breeds during all THI categories are shown in Table 2.2. Significant differences in feeding behavior were observed among sire breeds. Yorkshire-sired pigs visited the feeder more often throughout the day than both Duroc- and Landrace-sired pigs in all THI categories ($P < 0.001$). Although Yorkshire sired pigs had the highest average feeder visit count, they still decreased in activity from Normal to Alert THI ($P < 0.001$) and then increased activity from Alert to Danger THI ($P < 0.001$). Landrace sired pigs experienced their highest feeder visit counts during Normal THI compared to the other THI categories ($P < 0.001$). A decrease in feeder visit activity was observed as THI increased from Normal to Alert ($P < 0.001$) and Danger to Emergency ($P < 0.001$) for Landrace-sired pigs. Duroc-sired pigs had daily average feeder visits that were between those of Yorkshire and Landrace-sired pigs for all THI categories ($P < 0.001$). As THI increased from Normal to Danger average feeder visit activity increased for Duroc-sired pigs ($P < 0.001$).

Daily feeding behavior patterns for each of the three breeds is shown in Figure 2.1. The profile of the feeding behavior plots for Landrace- and Yorkshire-sired pigs were similar for Normal, Alert, and Emergency THI, but differed in the behavior during Danger THI. A bimodal feeding pattern was observed in Normal and Emergency THI, while the Alert THI exhibited three peaks in feeder visit activity. In Danger THI, Yorkshires had a bimodal pattern, while Landrace had constant activity throughout the morning, an afternoon decrease, and an evening peak. In all THI categories, Landrace- and Yorkshire-sired pigs both experienced a decrease in activity during the afternoon followed by increased activity in the evening. On average, Landrace-sired pigs had the largest afternoon decrease followed by the largest evening increase in feeder visit activity for all THI categories.

In all THI categories, Duroc-sired pigs' feeding behavior differed from that of Landrace- and Yorkshire-sired pigs. They did not have the same pronounced mid-day decrease in feeder visit activity observed in the other two sire breeds. Duroc-sired pigs had an initial increase in activity in the morning and either plateaued and increased in the evening (Normal THI), continued to gradually increase until a maximum was reached in the evening (Alert and Danger THI), or varied throughout the day until a maximum was reached in the evening (Emergency THI).

As mentioned, the maximal daily peak in activity was always observed in the evening. For Duroc-sired pigs, this maximum typically occurred at time period 18, except during Emergency THI where it was high for the time periods 17 and 19, but dipped during time period 18. Yorkshire- and Landrace-sired pigs both showed similar patterns

as THI increased, where their peak activity was shifted two hours later in the day when THI exceeded Normal.

Throughout all THI categories, the feeder visit profiles of barrows and gilts were quite similar (Figure 2.2), although barrows on average had higher feeder visit counts than gilts ($P < 0.001$, Table 2.3). As THI increased above normal, the barrows' feeder activity remained nearly constant while feeder activity of gilts decreased. A bimodal behavior pattern was observed during most THI categories for both barrows and gilts. When THI reached Emergency range, both sexes had dramatically fewer feeder visit counts between time periods 1 and 6. During Emergency THI, a sharp increase in feeder visits was observed in the barrows during the morning hours, while feeder visits of the gilts gradually increased over a longer period of time. Both sexes had an afternoon decrease in activity.

Analysis II: Landrace- and Yorkshire-sired pigs from all groups

Of the 1344 grow-finish pigs analysis 2: 791 were Landrace sired, 553 were Yorkshire sired, 574 were barrows, and 770 were gilts. Average daily feeder visit counts for Landrace- and Yorkshire-sired pigs by THI category are shown in Table 2.4. Significant differences were observed between the two breeds. Yorkshire-sired pigs had fewer feeder visits than Landrace-sired pigs in Normal THI ($P < 0.001$), but exceeded Landrace-sired pigs in feeder activity in all other THI categories ($P < 0.001$). Yorkshire-sired pigs increased in feeder visit count for each increase in THI category ($P < 0.001$). Landrace-sired pigs decreased feeder visit count for each increase in THI category ($P <$

0.001). Landrace-sired pigs reached their lowest feeder visit count, while Yorkshire-sired pigs reached their highest feeder visit count during Emergency THI ($P < 0.001$).

Feeding behavior profiles of the two breeds were similar for Alert and Danger THI, but differed during Normal and Emergency THI (Figure 2.3). In Alert THI, a 3-peak increase in feeder visit activity occurred and in Danger THI a bimodal pattern was observed. During Alert and Danger THI, both Landrace- and Yorkshire-sired pigs had an afternoon decrease in feeder visit activity, followed by an evening increase in activity. This observation is consistent with what was observed in the first analysis. Landrace-sired pigs had a bimodal pattern during Normal THI, while Yorkshire feeder visits increased during an early time period and plateaued until the evening decrease was observed. In Emergency THI Yorkshire pigs displayed a bimodal feeding behavior. In contrast, Landrace feeder visits increased in the morning, plateaued during the day, and then increased slightly in the evening. In general, during Normal THI Landrace-sired pigs were more active than Yorkshire-sired pigs. The opposite was observed during Emergency THI, with Yorkshire-sired pigs being more active than Landrace-sired pigs.

The observed peaks in daily feeding behavior in this data set were quite similar to those from the first analysis, with maximum activity occurring in the evening hours. The maximum activity for Yorkshire-sired pigs was at time period 18 for Normal conditions, time period 19 for Alert THI, and time period 20 for Danger and Emergency conditions. Landrace-sired pigs peaked later in the day at time period 20 for Normal conditions, time period 22 for Alert and Danger THI, and at time period 21 for Emergency conditions.

DISCUSSION

Environmental temperatures are known to affect swine feeding behavior. Ideally, barn temperatures would have been used in our analyses. Barn temperatures were collected during a portion of our study, using one thermometer at the north end and one at the south end of the barn. However, barn temperatures were only measured for the last four groups. THI was used to approximate the thermal conditions inside the barn due to its strong statistical relationship with barn temperature ($R^2 = 0.848$; Figure 2.4)

Landrace-sired pigs had fewer average daily feeder visit counts compared to the Yorkshire- and Duroc-sired pigs ($P < 0.001$) in Analysis I for all THI categories (Table 2.2). However, in Analysis II, Landrace-sired pigs had higher feeder visit counts than Yorkshire-sired pigs during Normal THI ($P < 0.001$), but then dropped below the Yorkshire-sired pigs for the remainder of the THI categories ($P< 0.001$, Table 2.4). Compared to Analysis I, Landrace-sired pigs had the lowest feeder visit counts during Emergency THI in Analysis II ($P < 0.001$). This drastic decrease in feeder visit activity was more than likely due to Landrace-sired pigs experiencing more consecutive days of extreme heat. Feed intake has been shown to be reduced in pigs that experience long-term heat stress (Song et al., 2011; Renaudeau et al., 2013). Increasing consecutive days of extreme heat has a more drastic impact on feeding behavior than when pigs experience one day of heat stress followed by a cooler day to recuperate.

Daily maximum feeder visits for Landrace- and Yorkshire-sired animals was observed earlier in the day for Normal THI compared to higher THI categories. The same pattern was also observed in the analysis of gilts and barrows. Although this shift was not observed in Duroc sired pigs for all higher THI categories, they did have a shift of

maximum feeder visit activity to later in the day during Emergency THI. Switching the maximum peak of feeder visit activity to later in the evening could be a coping mechanism that pigs implement to avoid heat stress.

Heat production in pigs comes from feed consumption, maintenance, and physical activity (Kerr *et al.,* 2003). In order to decrease heat production from physical activity and consumption of feed as THI increased, Landrace- and Yorkshire-sired pigs decreased feeder visit activity during the heat of the day and increased activity once THI decreased later in the afternoon and early evening. Pigs decrease physical activity in order to reduce body heat when exposed to high temperatures (Kerr *et al.,* 2003), and therefore pigs would spend more time laying down and less time eating during high ambient temperatures (Hicks *et al.,* 1998; Brown-Brandl *et al.,* 2001). This shift in feeder visit activity would allow pigs to adapt to warm environments by decreasing heat production in order to reduce the amount of heat that needs to be eliminated (Nienaber and Hahn, 1982; Collin *et al.,* 2001; Quiniou *et al.,* 2001).

As THI increased, a shift to early morning feeder visit activity was observed. Yorkshire-sired pigs' feeder visit activity between Danger and Emergency shifted to an earlier time period. This shift was also observed in Duroc sired pigs between Alert to Danger THI. Increased feeder visit activity in the early morning could be a coping mechanism to avoid visiting feeders during the heat of the day. Quiniou *et al.* (2000) reported no effect on number of daily meals due to temperature, which could be due to pigs shifting meals to the evening or early morning when temperatures are lower (Xin and DeShazer, 1992; Nienaber *et al.*, 1996; Quiniou *et al.*, 2000).

A mid-day decrease in feeder visit activity was observed for both Landrace and Yorkshire sired pigs in the first analysis in all THI categories. This mid-day decrease may be due to feeder visit competition with Duroc-sired pigs or it may be a normal feeding behavior pattern for the two breeds, as a mid-day decrease was also observed in the second analysis for both breeds during most THI categories.

Sex differences were also observed. On average barrows had higher feeder visit activity than gilts. However, during Normal THI Duroc-sired gilts visited the feeder more than Duroc-sired barrows ($P < 0.001$). Brown-Brandl *et al.* (2013a) reported that barrows spent more time at the feeders than gilts. However, Hyun *et al.* (1997) reported no difference between sex and time spent at feeders. These conflicting results may be due to differences in feed monitoring systems. Hyun *et al.* (1997) used an electronic feeding system that allowed only one pig to eat, while Brown-Brandl *et al.* (2013a) used an electronic feeding system consisting of one feeder with five feeding spaces.

Differences were observed among sire breeds; however, gilts and barrows followed similar feeding behavior patterns. Feeder visit activity for breed by sex is shown in Figures 2.5 – 2.8. Duroc-sired barrows, Yorkshire-sired barrows, and Landrace-sired barrows were similar to the feeding behavior profile for that of all barrows. Yorkshire-sired gilts and Landrace-sired gilts had similar profiles as that of all gilts. However, the feeding activity profile of the Duroc-sired gilts was more similar to the profile of all Duroc-sired pigs, where feeder visit activity increased steadily throughout the day during Alert and Danger THI. During Normal and Emergency THI, Duroc sired gilts feeding activity profile was similar to that of all gilts.

General differences observed in feeder visit activity between the two sexes and three sire breeds could be a result of normal feeding behavior or due to competition. Gilts followed the same feeding behavior profile as barrows, but had fewer numbers of feeder visits. This difference in number of feeder visits may be because gilts do not visit the feeder as frequently as barrows or because there was competition at the feeder. In this same manner, Landrace- and Yorkshire-sired pigs had similar feeding patterns, but Landrace-sired pigs, in general, visited the feeder less frequently than Yorkshire sired pigs. Also, during times that the feeder visits of Duroc-sired pigs were increasing to their peak, activity of Landrace- and Yorkshire-sired pigs was decreasing to their afternoon low. Again this observation could be normal feeding behavior, where Duroc-sired pigs eat throughout the day or it could be due to competition, leading Duroc-sired pigs to have increased feeder visit activity when the Landrace- and Yorkshire-sired pigs are having their mid-afternoon decrease. In addition to heat stress, competition could also affect how pigs from different breeds and different sexes displayed their feeding behavior patterns. Future studies will focus on altering factors associated with competition at the feeder in barrows and gilts sired by commercial Duroc, Landrace, and Yorkshire boars.

Table 2.1: Barn entry and exit dates and sire breeds used for each grow-finish group

Group	Barn Entry	Barn Exit	Sire Breed
G1	July 2011	December 2011	Landrace
G2	March 2012	July 2012	Yorkshire
G3	October 2013	March 2014	Landrace
G4	May 2014	October 2014	Landrace, Yorkshire, and Duroc
G5	December 2014	April 2015	Landrace, Yorkshire, and Duroc
G6	June 2015	October 2015	Landrace, Yorkshire, and Duroc
G7	December 2015	May 2016	Landrace, Yorkshire, and Duroc

Table 2.2: Average daily feeder visit counts (mean ± standard error) for Analysis I (Groups G4 – G7) by sire breed and sire breed-sex at each Temperature-Humidity Index category

		THI Category			
Sire Breed	Sex	Normal $x < 23.33$ °C	Alert $23.33 \leq x <$ 26.11 °C	Danger $26.11 \leq x <$ 28.88 °C	Emergency $x \geq 28.88$ °C
Duroc		14.9 ± 0.031	15.2 ± 0.046	15.4 ± 0.058	15.2 ± 0.14
	Barrow	14.8 ± 0.041	16.2 ± 0.069	16.5 ± 0.087	16.4 ± 0.21
	Gilt	15.1 ± 0.041	14.2 ± 0.061	14.4 ± 0.077	14.0 ± 0.19
Yorkshire		16.5 ± 0.029	16.0 ± 0.051	16.3 ± 0.063	16.3 ± 0.16
	Barrow	17.0 ± 0.046	16.7 ± 0.076	17.1 ± 0.095	17.3 ± 0.24
	Gilt	16.1 ± 0.042	15.2 ± 0.067	15.5 ± 0.083	15.4 ± 0.22
Landrace		12.8 ± 0.028	12.2 ± 0.042	12.1 ± 0.051	11.5 ± 0.12
	Barrow	13.5 ± 0.042	12.7 ± 0.063	12.6 ± 0.077	12.1 ± 0.18
	Gilt	12.0 ± 0.038	11.7 ± 0.056	11.6 ± 0.067	10.9 ± 0.16

Table 2.3: Average daily feeder visit counts (mean ± standard error) for Analysis I (Groups G4 – G7) by sex for each Temperature-Humidity Index category

	THI Category			
Sex	Normal	Alert	Danger	Emergency
	$x < 23.33$ °C	$23.33 \leq x < 26.11$ °C	$26.11 \leq x < 28.88$ °C	$x \geq 28.88$ °C
Barrows	15.1 ± 0.025	15.2 ± 0.041	15.4 ± 0.051	15.3 ± 0.13
Gilts	14.4 ± 0.023	13.7 ± 0.036	13.8 ± 0.044	13.4 ± 0.11

Table 2.4: Average daily feeder visit counts (mean ± standard error) for Analysis II by sire breed for each Temperature-Humidity Index category

	THI Category			
Sire Breed	Normal	Alert	Danger	Emergency
	$x < 23.33$ °C	$23.33 \leq x < 26.11$ °C	$26.11 \leq x < 28.88$ °C	$x \geq 28.88$ °C
Yorkshire	13.6 ± 0.020	14.6 ± 0.038	15.0 ± 0.049	15.5 ± 0.15
Landrace	14.8 ± 0.018	11.3 ± 0.029	9.7 ± 0.030	6.3 ± 0.036

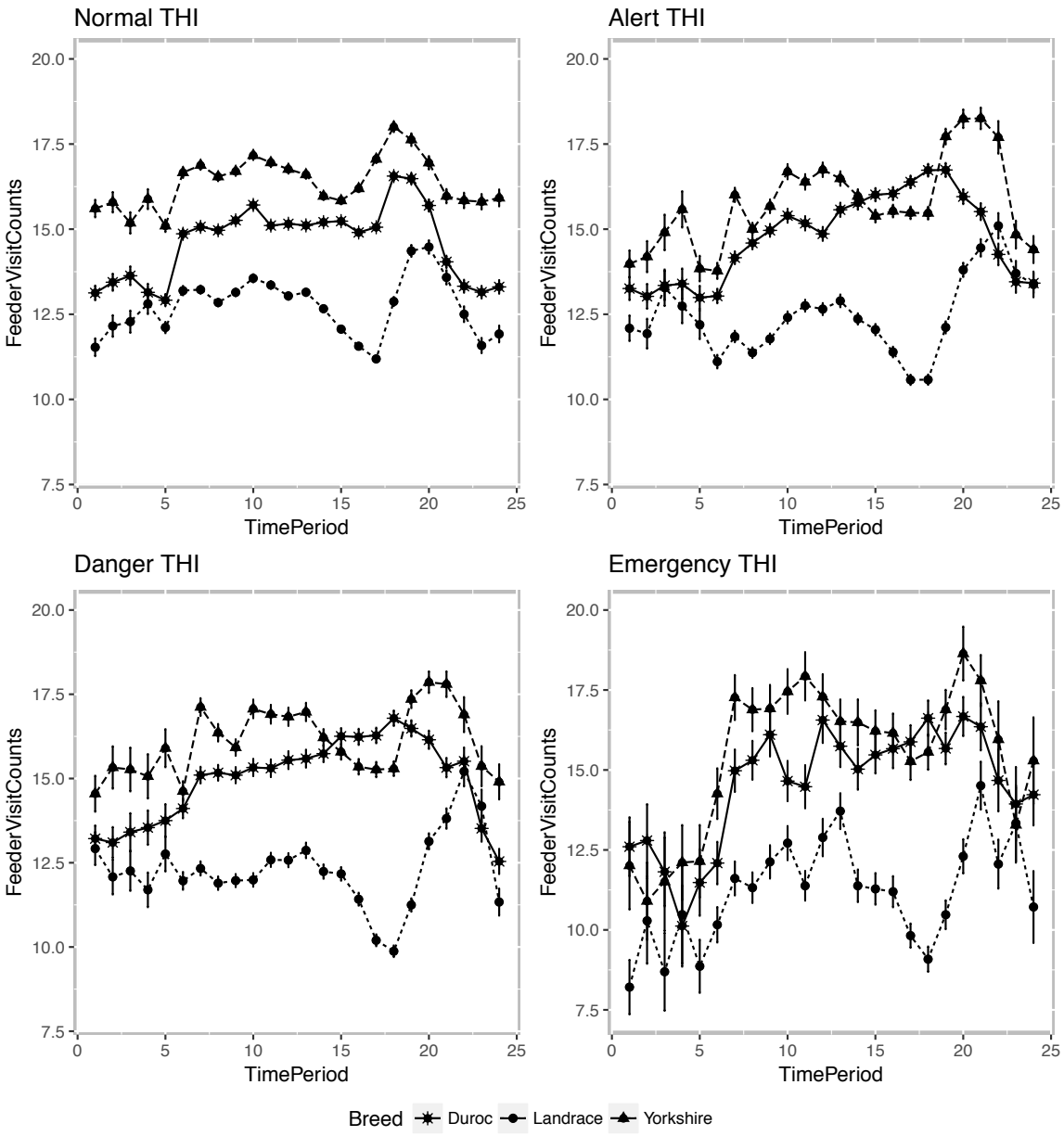


Figure 2.1: Feeding behavior patterns for Normal (x < 23.33°C) THI, Alert (23.33 ≤ x < 26.11°C), Danger (26.11 ≤ x < 28.88°C), and Emergency (x ≥ 28.88°C) in Analysis I (Groups G4 – G7) by sire breed.

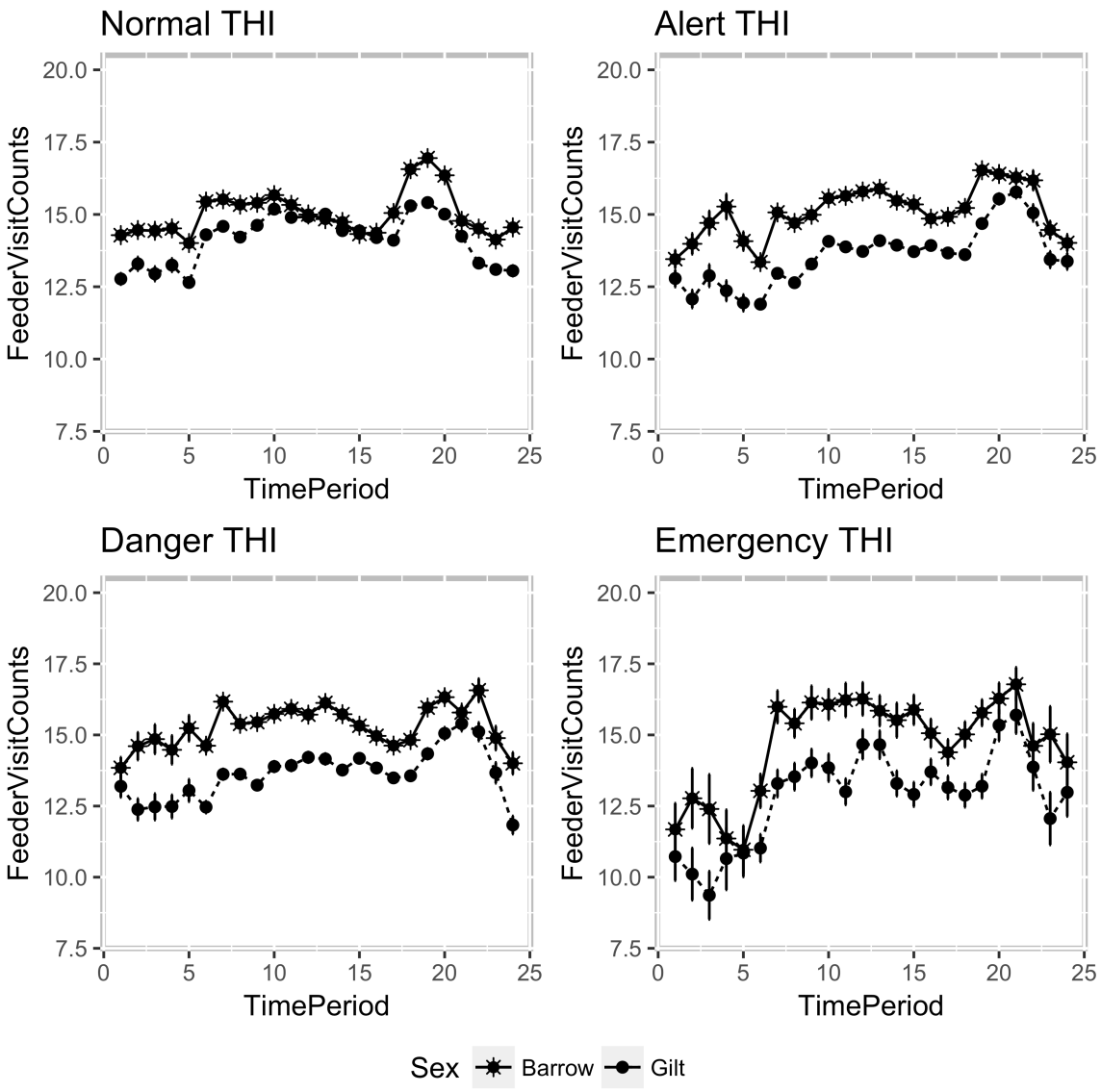


Figure 2.2: Feeding behavior patterns for Normal (x < 23.33°C) THI, Alert (23.33 ≤ x < 26.11°C), Danger (26.11 ≤ x < 28.88°C), and Emergency (x ≥ 28.88°C) in Analysis I (Groups G4 – G7) by sex.

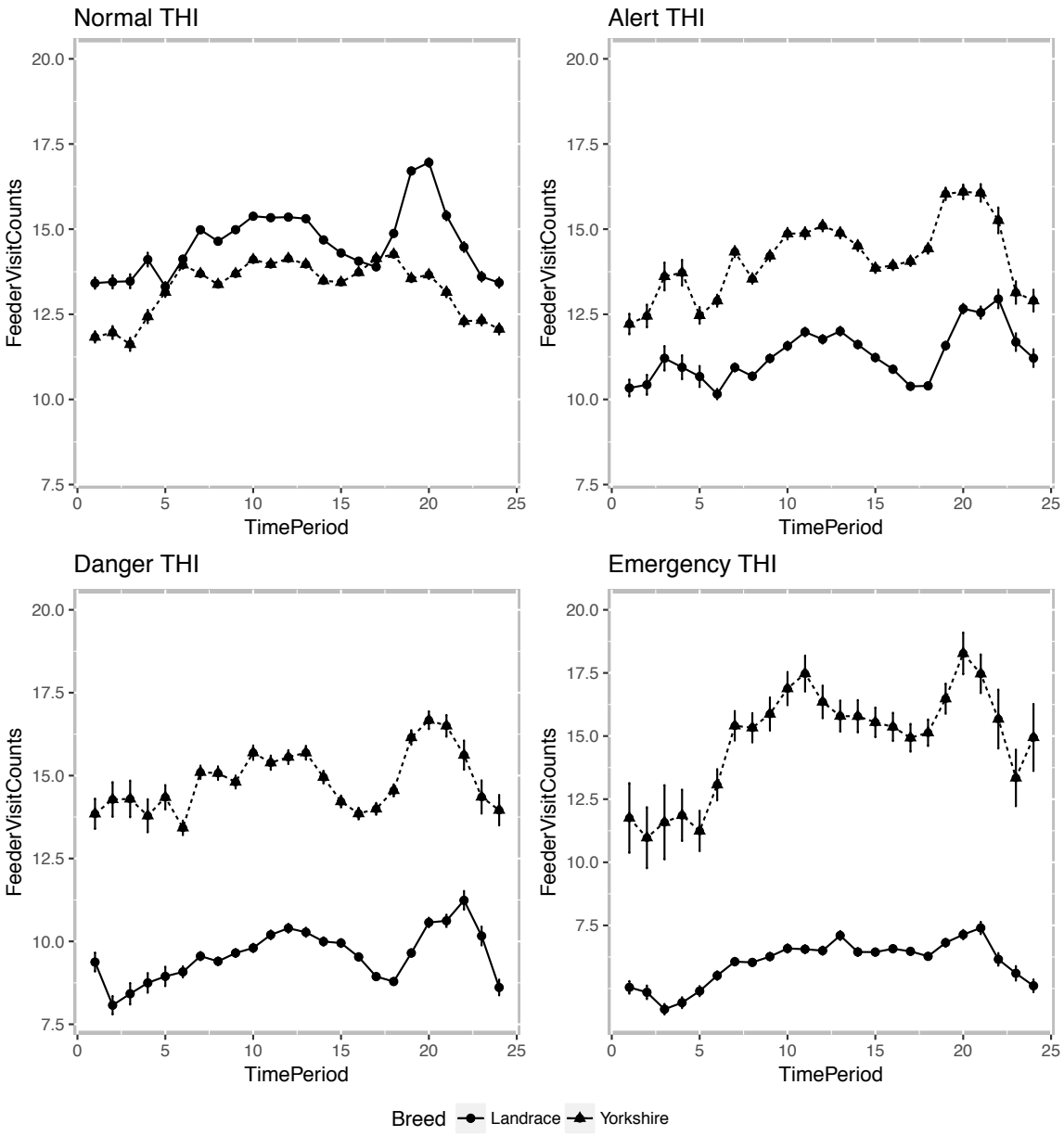


Figure 2.3: Feeding behavior patterns for Normal (x < 23.33°C) THI, Alert (23.33 ≤ x < 26.11°C), Danger (26.11 ≤ x < 28.88°C), and Emergency (x ≥ 28.88°C) in Analysis II (Groups G1 – G7) by sire breed.

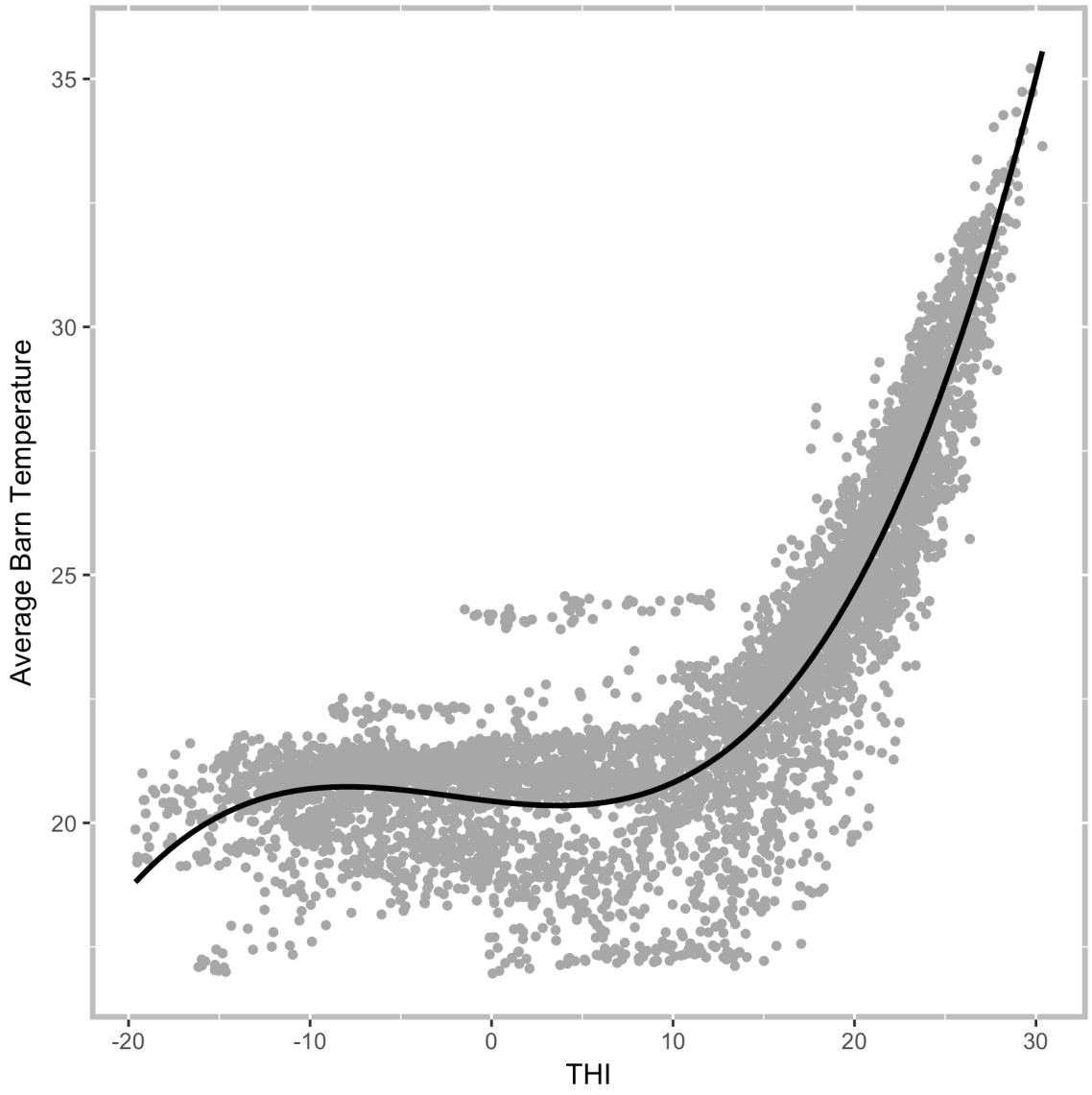


Figure 2.4: THI (°C) versus average barn temperature (°C) 3rd degree polynomial regression.

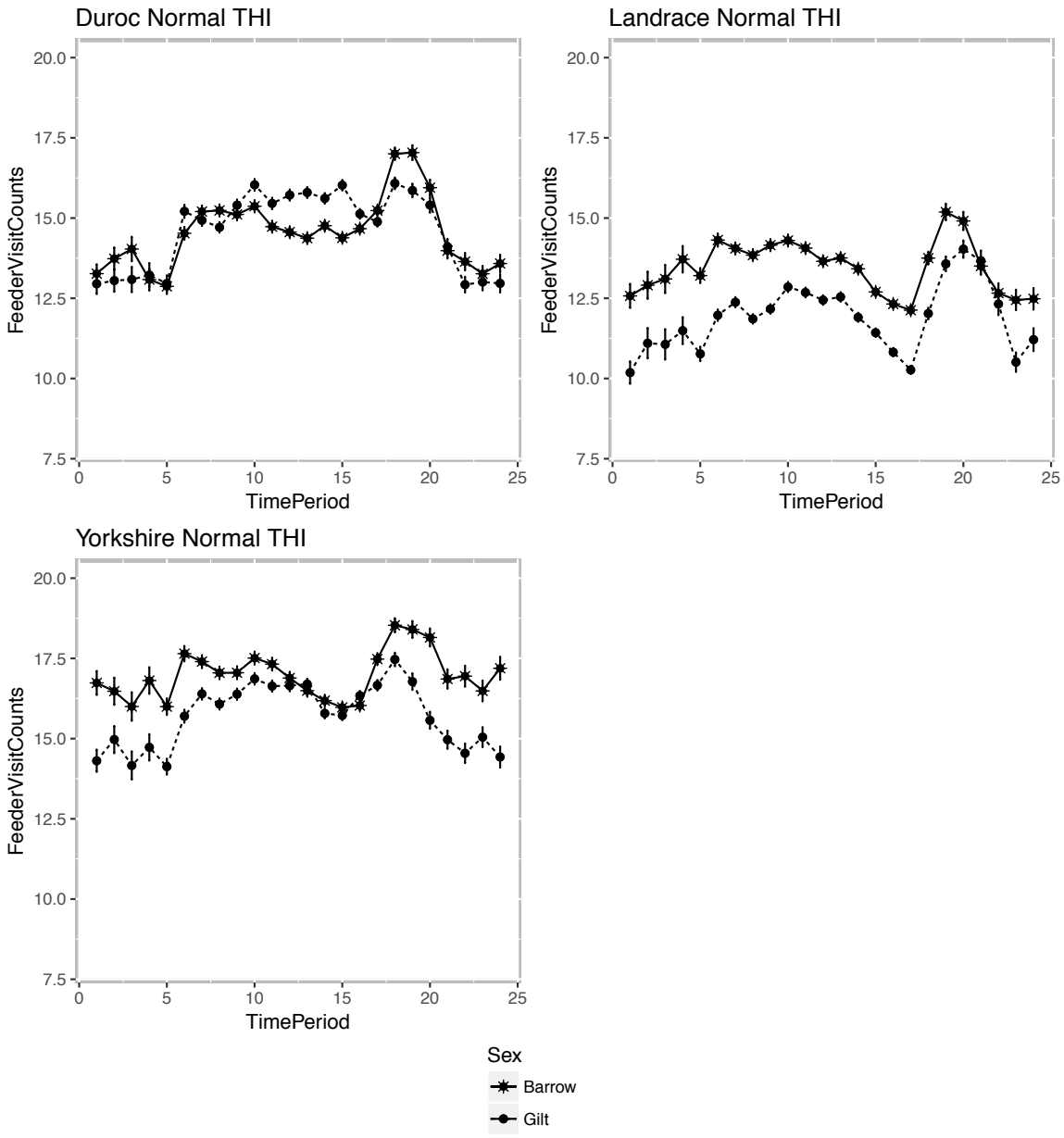


Figure 2.5: Feeder visit activity for breed by sex interaction during Normal THI

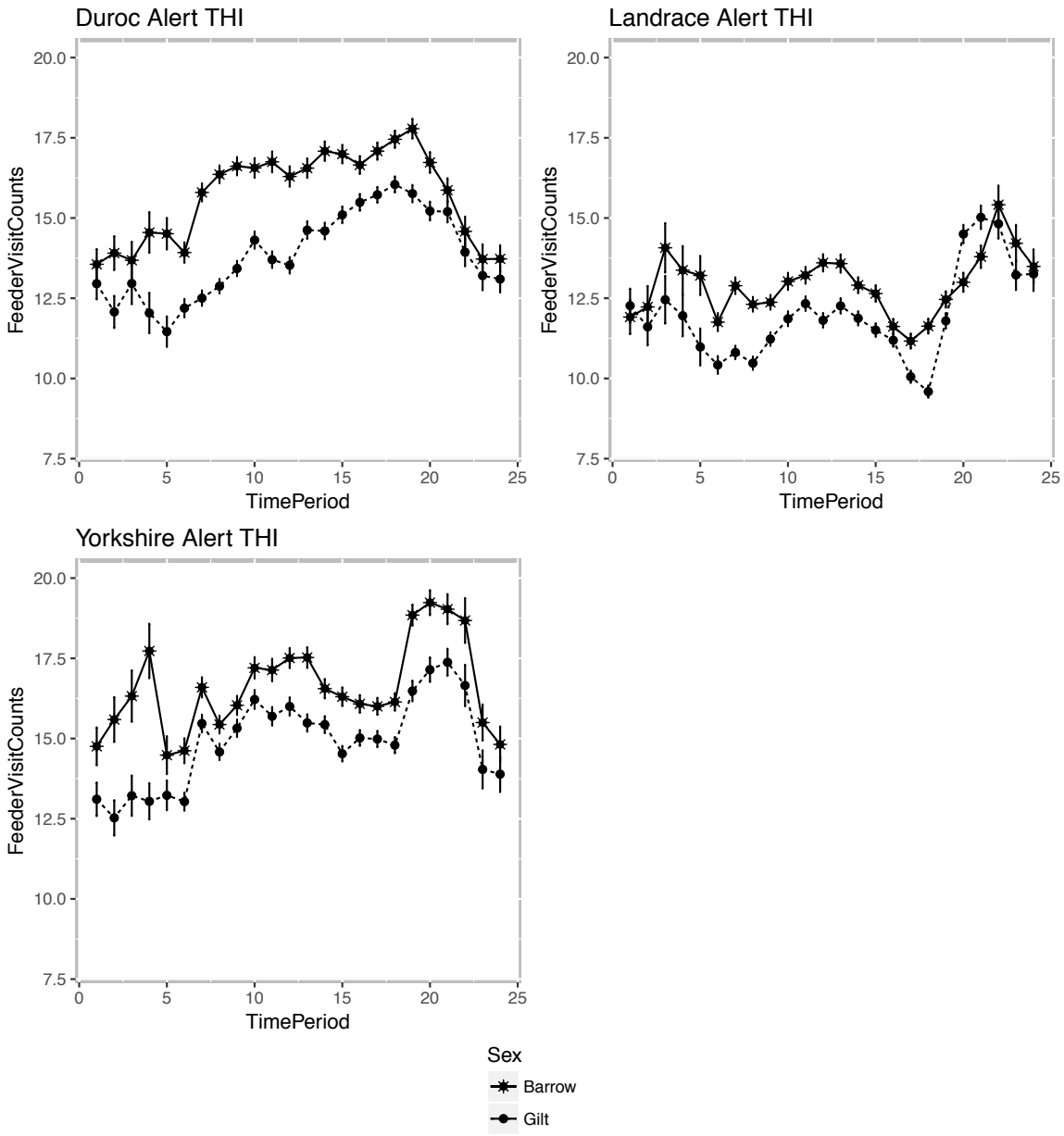


Figure 2.6: Feeder visit activity for breed by sex interaction during Alert THI.

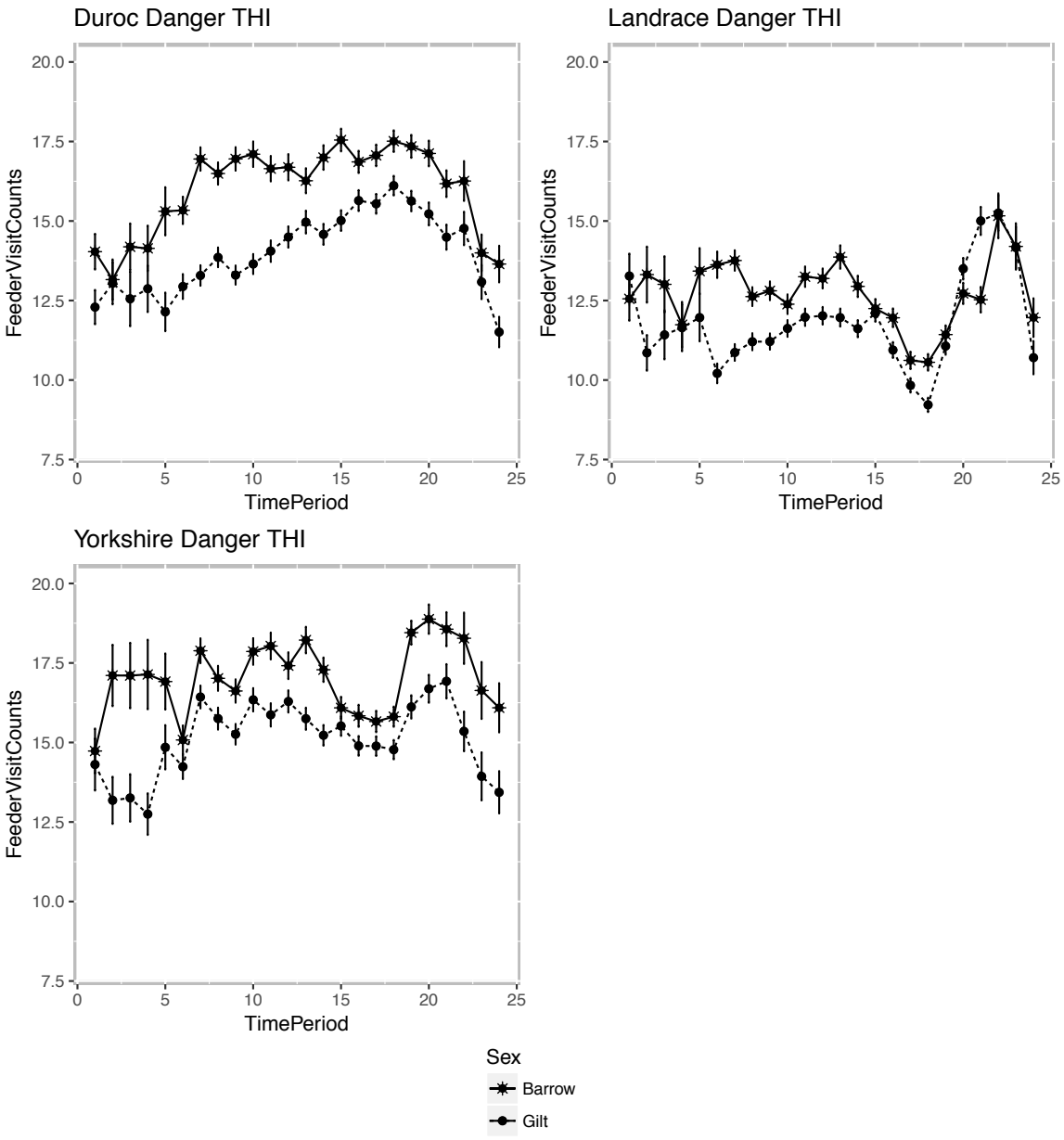


Figure 2.7: Feeder visit activity for breed by sex interaction during Danger THI.

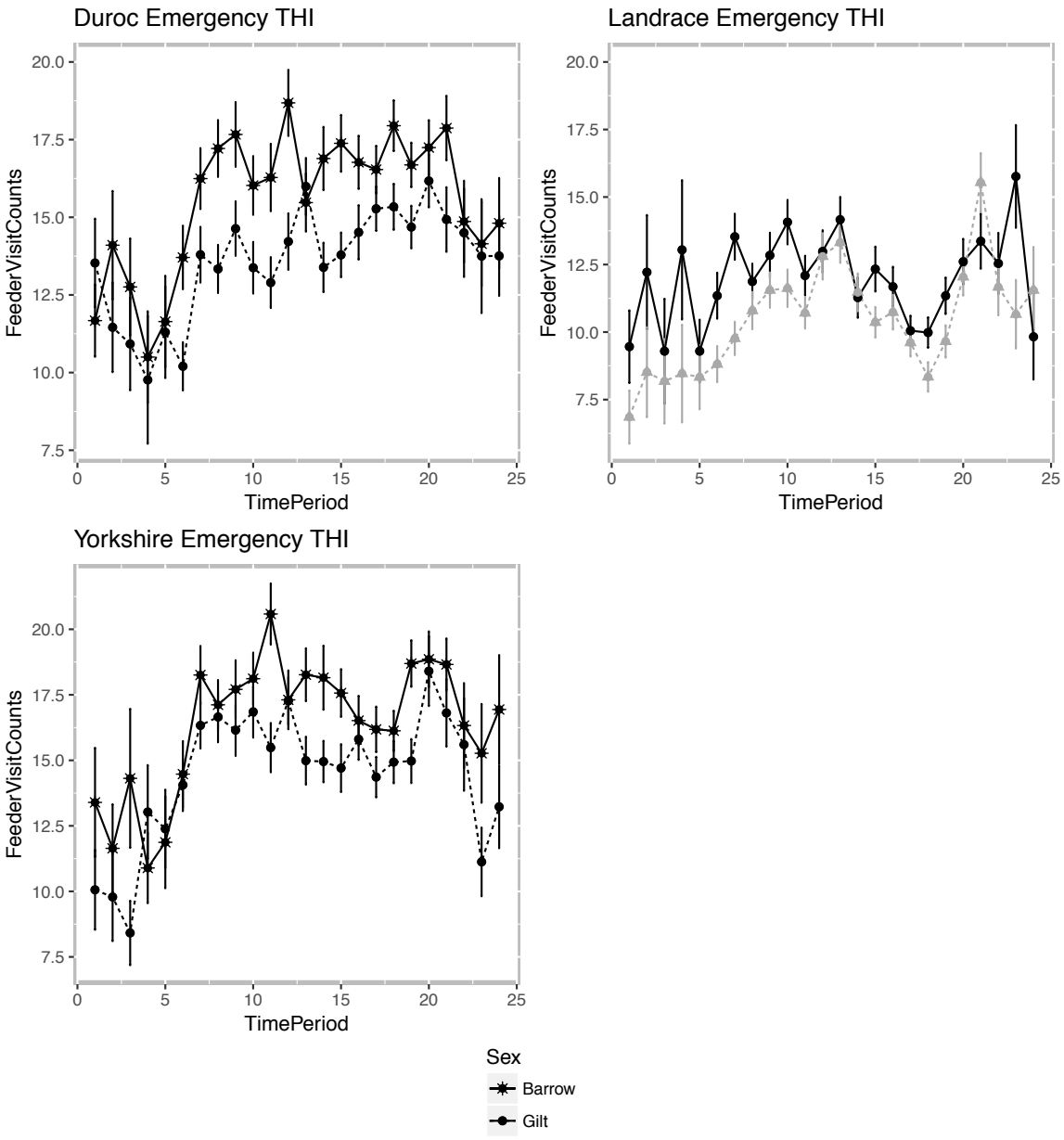


Figure 2.8: Feeder visit activity for breed by sex interaction during Emergency THI.

CHAPTER 3

FEED-FORWARD AND GENERALIZED REGRESSION NEURAL NETWORKS IN MODELING FEEDING BEHAVIOR OF PIGS IN THE GROW-FINISH PHASE

ABSTRACT

Feeding patterns in group-housed, grow-finishing pigs have been investigated for use in management decisions, identifying sick animals, and determining genetic differences within a herd. Development of models to predict swine feeding behavior has been limited due to the large number of potential environmental factors involved and complex relationships between them. Artificial neural networks have been proven to be an effective tool for mapping complicated, nonlinear relationships between inputs and outputs. However, they have not been applied to swine feeding behavior prediction. In this study, we compared the use of feed-forward neural networks and generalized regression neural networks in forecasting feeding behavior of pigs in the grow-finishing phase throughout the year, using time of day and temperature humidity index as inputs. The fruit fly optimization algorithm was applied in order to automatically select optimal parameters for each network. Models were calibrated on electronic feeder visit data from 1,923 pigs, captured using electronic monitoring in a grow-finish facility from 2008 to 2014. These animals consisted of gilts and barrows from three sire breeds: Duroc, Landrace, and Yorkshire. After model calibration, predictive ability of each model was tested using feeder visit data from four additional grow-finish groups reared in the same facility from 2014 to 2016. Based on our findings we concluded that neural networks can be used to predict swine feeding behavior. Feed-forward neural networks trained with the Levenberg-Marquardt and scaled conjugate gradient algorithms were shown to be the most accurate forecasting models.

INTRODUCTION

Feeding behavior of grow-finish pigs can be used to inform producers of both health status and stress level. Many parameters have been studied to better understand swine feeding behavior, including feed intake, meal length, meal interval, number of meals, and total time spent eating (Nienaber et al., 1990; Nienaber et al., 1991; Morgan et al., 2000; Quiniou et al., 2000). Most of these measurements have been obtained from feeding systems that allow only one pig to feed at any given time, which is not representative of commercial production where pigs typically feed in a group setting (Brown-Brandl et al., 2013).

Feeding behavior is dependent on several environmental and genetic factors, including but not limited to temperature, humidity, gender, breed, and time of day. Deviations from normal feeding behavior may indicate that grow-finish pigs are experiencing a stressful event, such as illness, issues with feed quality, or heat related stress. Models of feeding behavior could be used as a management tool to assess stress levels within a population and to identify sick animals.

Several different approaches have been used to analyze and model swine feeding behavior. Linear regression and analysis of variance models have been used extensively (Nienaber et al., 1990; Nienaber et al., 1991; Quiniou et al., 2001; Brown-Brandl et al., 2013). However, application of these methods is limited due to complex, non-linear relationships among multiple input variables (Comrie, 1997). Gaussian models (Morgan et al., 2000), three process random models (Berdoy, 1993), and logistic models (Tolkamp et al., 1999) have also been applied to predict feeding behavior. There are two major drawbacks to these types of models. They tend to be very complex, and they require prior

knowledge of relationships between input variables, i.e. a predefined functional form for the model.

Artificial neural networks (ANN) have emerged as a powerful tool in applications where complexity of relationships between inputs and outputs makes formulating a comprehensive mathematical model nearly impossible (Hecht-Nielsen, 1989). An ANN is a set of computing systems that imitates learning abilities of neurons in the brain. Artificial neural network models have the ability to handle large amounts of noisy data, without requiring prior information of model form. An additional advantage of ANN models over other statistical methods is that they require less training data (Paola & Schowengerdt, 1995). The ability to learn by example makes a neural network a very flexible and powerful tool.

This study focused on application of ANN models for prediction of feeding behavior patterns of pigs during the grow-finish phase. Abilities of feed-forward neural networks (FFNN) and generalized regression neural networks (GRNN) to predict feeding behavior of grow-finish pigs throughout the year, using time of day and temperature humidity index (THI) as inputs, were compared.

MATERIAL AND METHODS

Feed-forward neural network (FFNN)

Feed-forward neural networks are one of the most popular ANN models used in engineering applications. The network architecture and learning algorithm of a FFNN can be viewed as a generalization of the well-known least-mean-square (LMS) algorithm (Haykin, 2007). Figure 3.1 shows the architecture of a typical FFNN. It is comprised of

three layers; an input layer, a hidden layer which is responsible for performing intermediate computations, and an output layer. The input signal propagates through the network layer-by-layer in a feed-forward fashion until it reaches the output layer.

There are three important parameters in a FFNN: the number of hidden layers, the transfer function used in the hidden layers, and the number of neurons in the hidden layers. The number of hidden layers in a FFNN is dependent on the complexity of relationships between inputs and target outputs. Using two or more hidden layers increases the chance of obtaining local minima during the training phase, and therefore it becomes crucial to use many different random initializations to ensure that global optimization is achieved (Svozil et al., 1997). Adding additional hidden layers can also make the gradient more unstable, in turn dramatically slowing training. It has been proven that a FFNN with one hidden layer and non-polynomial transfer function is sufficient to approximate any continuous function to any degree of accuracy (Leshno et al., 1993). Therefore, in this study we chose to use one hidden layer with a sigmoid transfer function in the FFNNs.

Choosing the optimal number of neurons in the hidden layer is the other major component of FFNN design. A general ‘rule of thumb’ for selecting the number of neurons is that the size of the hidden layer should be somewhere between input and output layer sizes (Blum, 1992). Employing too few hidden layer neurons can limit the network’s ability to learn associations between inputs and outputs. On the other hand, using a large number of neurons in the hidden layer often leads to a network that can make successful predictions for the training data, but does not generalize to other data sets (Abraham, 2005). We employed the fruit fly optimization algorithm, described

herein, to determine the optimal number of neurons in the hidden layer of our FFNNs. Each FFNN was generated and trained using the Matlab Neural Network Toolbox (MATLAB 2016a, The MathWorks, Inc., Natick, MA).

Training algorithms for FFNN

Training a neural network refers to the process of finding a set of weighted connections between neurons so that predicted outputs closely match known outputs for a collection of training data. In terms of optimization, training a neural network is equivalent to minimizing a global error function. That is, during the training process the vector of connection weights, w, is iteratively computed and adjusted in order to minimize the mean square error between observed and predicted outputs:

$$E(w) = \sum_{i=1}^{N}(y_i - t_i)^2, \quad (1)$$

where N is the number of input-output pairs in the training data set, y is the vector of predicted outputs from the ANN using weight vector w, and t is the vector of observed outputs. This minimization is a local iterative process that uses the following general strategy:

1) Choose an initial weight vector, w_1, and initialize the iteration count, i.e. set $k = 1$.
2) Determine a search direction d_k and a step size α_k such that $E(w_k + \alpha_k d_k) < E(w_k)$.
3) Update the weight vector: $w_{k+1} = w_k + \alpha_k d_k$.
4) If $E'(w_k) \neq 0$, then set $k = k + 1$ and repeats steps 2-4. Otherwise, return w_{k+1}, the desired minimizer.

Training of FFNN was performed using three distinct algorithms: scaled conjugate gradient (SCG), Levenberg-Marquardt (LM), and Bayesian regularization (BR).

Conjugate gradient methods are a class of optimization algorithms that use the general optimization strategy outlined above, where the positive step size α_k is obtained using a line search, and search direction d_k is generated by the rule:

$$d_{k+1} = r_{k+1} + \beta_k d_k, \quad d_0 = r_0. \tag{2}$$

Here β_k is the conjugate gradient update parameter and $r_k = -\nabla E(w_k)^T$, where the gradient $\nabla E(w_k)$ is a row vector and r_k is a column vector. Different conjugate gradient methods correspond to different choices for the scalar β_k. SCG is a conjugate gradient algorithm, whose major advantage over others is that it avoids the computationally expensive line search to determine step size at each iteration (for details see Moller, 1993).

The LM algorithm (Hagan & Menhaj, 1994) is a modification of the classic Newton algorithm for determining optimal solutions to minimization problems. At each iteration, weights are updated using the following approximation to the Hessian matrix:

$$w_{k+1} = w_k - \left[J_k{}^T J_k + \mu I\right]^{-1} J_k{}^T e_k \tag{3}$$

where J is the Jacobian matrix of output errors, I is the identity matrix, μ is a scalar that controls the learning process, and e is the residual error vector. When $\mu = 0$, Eq. (3) is exactly Newton's method, using the approximate Hessian matrix. If μ is large, the LM method becomes a gradient descent with small step size.

Training using the BR framework takes into account uncertainty in the weight vector by assigning a probability distribution to the weights that represents the relative degrees of confidence for the weight values. After an initial prior distribution has been

set, Bayes' theorem is employed to generate a posterior distribution for the weight probabilities. Optimal network weights can then be found by maximizing the posterior probabilities (MacKay, 1992). Foresee and Hagan (1997) showed that this is equivalent to minimizing a regularized objective function, which combines the conventional sum of the least squares error function with an additional term called "regularization":

$$E_{BR}(w_k) = \gamma_k E(w_k) + \rho_k P(w_k). \quad (4)$$

Here the terms γ_k and ρ_k are regularization parameters, $E(w_k)$ is as defined in Eq. (1), and $P(w_k) = \sum_{i=1}^{n} {w_k}_i^2$ is a penalty term, which penalizes large weight values. In this framework, weights in the network are updated using the same approach as the LM algorithm (Eq. (3)).

Generalized regression neural network (GRNN)

A GRNN is a type of radial basis function network which is based on a statistical technique called kernel regression (Specht, 1991). Figure 3.2 shows the architecture of a GRNN. It is comprised of four layers: an input layer, a pattern layer, a summation layer, and an output layer. The pattern layer is a hidden layer where each neuron is a training pattern, and the output of each neuron, p_i, is a measure of the distance between the input and the stored pattern given by:

$$p_i = \exp\left[-\frac{\left(a - a^i\right)^T \left(a - a^i\right)}{2\sigma^2}\right] \quad (5)$$

where σ is a smoothing parameter, a is the input to the network, and a^i is the pattern vector for neuron i.

Outputs from the pattern layer are passed into the summation layer, which executes two different summations. First, the simple summation S_s computes the sum of pattern layer outputs:

$$S_s = \sum_{i=1}^{N} p_i. \quad (6)$$

Next, the weighted summation S_w computes a weighted sum of the pattern layer outputs:

$$S_w = \sum_{i=1}^{N} w_i p_i, \quad (7)$$

where w_i is the connection weight of pattern neuron i to the neuron in the summation layer.

Outputs from the summation layer are then fed into the output layer, and the output $\boldsymbol{y}$ of the GRNN is computed as follows:

$$y = \frac{S_s}{S_w}. \quad (8)$$

Note that a GRNN has only one parameter, σ, that needs to be determined. This parameter, called the spread parameter in Matlab, was determined using the fruit fly optimization algorithm. Each GRNN was generated and trained using the Matlab Neural Network Toolbox.

Fruit fly optimization algorithm (FOA)

The fruit fly optimization algorithm (FOA), proposed by Pan (2012), is a novel swarm intelligence optimization algorithm based on foraging behaviors of the fruit fly. Fruit flies are able to distinguish various aromas to identify food sources as far as 40 km away. Additionally they use vision to identify food sources based on flocking locations of

other fruit flies. A fruit fly forages as follows. It first locates a food source by smelling through its osphresis organ and then flies toward that location. Once it arrives at its destination, it determines the fitness of that location via tasting. The entire swarm of flies sends and receives information in order to determine a location with optimal fitness. Once this location has been identified, the fly moves to this new location by utilizing its sight to assess the flocking pattern of others in the swarm.

The following steps in the algorithm are carried out for a user-specified number of iterations:

1) *Initialize parameters.* Main parameters that need to be initialized are the number of iterations (maxgen), population size (popsize), initial fruit fly swarm location (X_axis, Y_axis), and forage range. Table 3.1 shows parameter initializations for each of the models tested.
2) *Assign flight direction and distance for each fly.* Each of the *i* fruit flies is assigned a random direction and distance from the swarm by adding a random value from the forage range:

$$X_i = X_{axis} + Random\ Value$$
$$Y_i = Y_{axis} + Random\ Value \quad (9)$$

3) *Population evaluation.* Distance of each fly's position to the origin is computed, and smell concentration judgment value for each fly is assigned to be the reciprocal of the distance:

$$Dist_i = (X_i^2 + Y_i^2)^{1/2} \quad (10)$$

$$S_i = {}^{1}/_{Dist_i}$$

The smell concentration of each fly is computed by evaluating the fitness function at S_i (the parameter being tested), and the fruit fly with the maximal smell concentration is identified. In this study, the ANN with optimal parameters was defined to be that which produced the maximum correlation coefficient between predicted and observed values for the testing data set. Hence, parameter value S_i was used to generate an ANN, outputs were predicted for each inputs in the testing set, and the Pearson correlation coefficient (r) between predicted and observed outputs was computed.

4) *Swarm behavior.* If the maximal smell concentration (i.e. the maximal correlation coefficient) is greater than the previous iteration's maximal smell concentration, the swarm moves to the location that produced this new maximum. Otherwise repeat steps 2-3.

Data collection

All animal protocols conformed to procedures outlined in *Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 2010) and were approved by the U.S. Meat Animal Research Center Institutional Animal Care and Use Committee.

Data were collected on grow-finish barrows and gilts (n = 2,856) from four different sire lines, including purebred Duroc, Landrace, and Yorkshire and a $^{1}/_{2}$ Landrace, ¼ Duroc, and ¼ Yorkshire composite, reared at the U.S. Meat Animal

Research Center from 2008 to 2016. Grow-finish groups (n = 240) were placed in the barn at approximately eight to ten weeks of age and monitored over a 4-month period. Animals were distributed into six pens with 40 pigs per pen, with gilts and barrows comingled.

Each pen was fitted with an electronic system to monitor feeding behavior (Brown-Brandl et al., 2011). Each feeder had five slots, allowing multiple animals to eat at a given time. Pigs were provided a corn-soybean meal diet *ad libitum* designed to meet or exceed nutrient requirements. All feeders were fitted with an antenna at each feed slot and a multiplexer. Upon entry into the barn, animals were tagged with electronic identification tags, which could be read by the antennas while animals were at the feeder. Data were collected from all antennas (n = 30) every 20 seconds during the 4 month period.

Outside temperature (T, °C) and relative humidity (RH, %) were obtained from the National Weather Station located approximately 3 miles northwest of the grow-finish facility and used to calculate the temperature humidity index (THI). Since outside temperatures were only reported every hour, each day was incremented into 1-hour time periods, where time period 1 represents 12:00:00 a.m through 12:59:59 a.m. THI was calculated for each time period as follows (NOAA, 1976):

$$THI(℃) = T(℃) - [0.55 - (0.0055\ x\ RH)]\ x\ [T(℃) - 14.5]. \qquad (11)$$

As mentioned above, feeder visits were recorded by the electronic system every 20 seconds throughout the study. Total feeder visit count for each 1-hour time period in each day was computed. Then total number of feeder visits for each observed time

period-THI pair were calculated by summing across days. These time period-THI pairs served as inputs to the neural network.

Training data

Neural networks were developed to predict number of feeder visits based on THI and time period (time of day). Feeder visit data collected from 9/27/08 to 3/11/14 on 1,923 animals was used to build and train each ANN (Table 3.2). This data set consisted of 22,582 unique time period-THI observations, which was partitioned randomly so that 80% of the data was used for training and 20% used for testing. Testing data points were used in the FOA process to determine optimal parameters for the ANN.

Performance evaluation of the ANN models

Feeder visit data from four additional grow-finish groups, collected from 5/23/14 and 5/11/16, were used to assess performance of each ANN (Table 3.2). Performance was assessed using three statistical measures, root mean square error (RMSE), Pearson correlation coefficient (r), and coefficient of determination (R^2). These are given in Eqs. (12), (13), and (14), respectively.

For a given validation data set, let $\boldsymbol{o}$ denote a vector of observed feeder visits, p a vector of predicted feeder visits, and N the total number of data points. Then

$$\text{RMSE} = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(o_i - p_i)^2} \tag{12}$$

$$r = \frac{N\sum_{i=1}^{N} o_i p_i - \sum_{i=1}^{N} o_i \sum_{i=1}^{N} p_i}{\sqrt{N\sum_{i=1}^{N} o_i{}^2 - (\sum_{i=1}^{N} o_i)^2}\sqrt{N\sum_{i=1}^{N} p_i{}^2 - (\sum_{i=1}^{N} p_i)^2}} \quad (13)$$

$$R^2 = r^2 \quad (14)$$

Root mean square error quantifies how near the line of best fit is to the data points, where the smaller the RMSE the nearer the fit is to the data. The Pearson correlation coefficient takes values in the range [-1,1] and quantifies strength and direction of the linear relationship between predicted and observed values. Values close to 0 indicate weak linear relationships, while values near -1 and 1 indicate strong linear relationships with negative and positive slopes, respectively. Goodness of fit of the model was quantified by R^2, where values close to 0 and 1 indicate poor and strong model fit, respectively.

RESULTS AND DISCUSSION

Neural network training and parameter optimization

Temperature humidity index and time of day (time period) were used as inputs in development of several ANN models to predict feeding behavior of grow-finish pigs. Environmental temperatures are known to affect swine feeding behavior. Ideally, barn temperatures would have been used in the development of our ANN models. However, barn temperature data was only available for part of this study. Thermal conditions inside the barn were approximated by THI due to its strong statistical relationship with barn temperature (R^2 = 0.848; Figure 3.3).

The 22,582 data points in the training data set were randomly partitioned into training (80%) and testing (20%) sets. Training data were used to train each ANN model, while testing data points were used in the FOA process to estimate predictive ability of the model and determine optimal parameters. Four different ANN models were developed: (1) FFNN trained with SCG algorithm (FFNN-SCG), (2) FFNN trained with LM algorithm (FFNN-LM), (3) FFNN trained with BR algorithm (FFNN-BR), and (4) GRNN. Results for model generation and parameter optimization are shown in Table 3.3.

In each of the FFNN models, the optimal number of hidden neurons was dynamically determined using FOA. The number of iterations was set at 100, and swarm size was set to be 40 flies. Figure 3.4 (A)-(C) shows the iterative correlation coefficient trends of the FOA search for the optimal number of hidden neurons in each FFNN. For the FFNN-SCG model, convergence was observed at iteration 5 with correlation coefficient and number of hidden neurons 0.6586 and 7, respectively. Convergence of the FFNN-LM model occurred at iteration 29 with a correlation coefficient of 0.6641, and its optimal number of hidden neurons was 11. In the FFNN-BR model the FOA converged at iteration 10 with correlation coefficient and number of hidden neurons 0.6635 and 16, respectively.

The optimal spread parameter for the GRNN was also identified using FOA. The initial spread parameter was set to be in the range [0, 1], swarm size was set to be 40 flies, and the number of iterations was set to 100. Figure 3.4 (D) shows the correlation coefficient at each iteration of the FOA search for the optimal spread parameter. Convergence occurred at iteration 75 with a correlation coefficient of 0.66417. The optimal spread parameter for the GRNN was 1.033.

Model performance testing

Once the four ANN models had been trained using their optimal parameters, performance of each model was assessed using data from four additional grow-finish groups, Group A, Group B, Group C, and Group D (Table 3.2). The r, R^2, and RMSE statistics for each testing set are presented in Table 3.4. In general, the four models exhibited similar performance across the data sets, with FFNN-LM and FFNN-SCG having consistently higher performance than the other two models. The FFNN-LM model exhibited superior performance in two of the four data sets (Groups A and C), while the FFNN-SCG model performed best in the other two data sets (Groups B and D). However, the difference in correlation coefficients for FFNN-LM and FFNN-SCG is smaller in Groups A and C (0.004 and 0.003, respectively) than in Groups B and D (0.006 and 0.012).

The ANN models tested in this study appear to be robust models for predicting feeding behavior across breeds. Training data was comprised of 62.5% composite animals, 12.5% Yorkshire sired animals, and 25% Landrace sired animals. Duroc sired animals were only present in the four validation sets. Even with these breed differences, the ANN models were able to generate acceptable predictions. As the similarity of training and validation germplasm increases, the models should generate even better predictions.

Large deviations between predicted and observed feeding behaviors in Group A may be due to an outbreak of pneumonia. While Group A was in the grow-finish barn, 22% of the animals were treated at least once for pneumonia. Although a large portion of animals received treatment for pneumonia, it is likely that there were additional pigs in

the barn that experienced mild pneumonia symptoms that were not treated. In the other three groups, the percentage of animals treated for pneumonia was much lower; 7% in Group B, 2% in Group C, and 5% in Group D. This example highlights the potential for ANN models to be used in the automated detection of disease outbreak and other stress events. Future work will focus on the development of ANN model systems that can be used as early predictors of illness and stress events at the individual animal level.

CONCLUSIONS

Artificial neural network models have become increasingly popular in many different fields due their ability to elucidate complex, non-linear relationships between parameters. The focus of this study was to identify an accurate and efficient neural network model for predicting swine feeding behavior based on time of day and THI. Four different ANN models were trained using electronic feeder data from 1,923 grow-finish pigs, and their performance was assessed using data from four additional grow-finish groups reared in the same facility. The results of this study demonstrate that feeding behavior predictions are viable using both the FFNN-LM and FFNN-SCG models. Selection of the number of hidden neurons is an important task in both of these models. In this work we applied FOA to automatically select the appropriate number of hidden neurons to improve feeding behavior forecasting accuracy. In general, neural networks are a useful prediction tool in feeding behavior. Use of machine learning to generate feeding pattern predictions in group-housed grow-finishing pigs could play an important role in management decisions, identifying sick animals, and determining genetic differences within a herd.

Table 3.1 Initial fruit fly optimization algorithm parameters for each of the four artificial neural network models tested in this study.

Model[a]	maxgen[b]	popsize[c]	(X_axis, Y_axis)[d]	Forage Range
FFNN-SCG	40	100	[0, 1]	[-1, 1]
FFNN-LM	40	100	[0, 1]	[-1, 1]
FFNN-BR	40	100	[0, 1]	[-1, 1]
GRNN	40	100	[0, 1]	[-10, 10]

[a] Models tested included feed-forward neural networks trained with scaled conjugate gradient algorithm (FFNN-SCG), Levenberg-Marquardt algorithm (FFNN-LM), Bayesian regularization algorithm (FFNN-BR), and generalized regression neural network (GRNN).

[b] Number of fruit fly optimization algorithm iterations.

[c] Number of flies in the swarm.

[d] Initial range of values for x- and y-axis locations of the fruit fly swarm.

Table 3.2 Grow-finish groups used for model testing and validation of feeding behavior.

	Study Start Date	Study End Date	Number of Animals	Breed[a]	Validation or Training
Group 1	9/27/08	1/25/09	252	C	Training
Group 2	3/19/09	7/19/09	252	C	Training
Group 3	1/20/10	6/1/10	237	C	Training
Group 4	7/8/10	10/14/10	237	C	Training
Group 5	1/6/11	5/10/11	223	C	Training
Group 6	7/14/11	12/21/12	240	L	Training
Group 7	3/12/12	7/8/12	242	Y	Training
Group 8	10/24/13	3/11/14	240	L	Training
Group A	5/23/14	10/22/14	237	D, Y, L	Validation
Group B	12/18/14	4/30/15	232	D, Y, L	Validation
Group C	6/11/15	10/7/15	232	D, Y, L	Validation
Group D	12/23/15	5/11/16	232	D, Y, L	Validation

[a] D = Duroc, Y = Yorkshire, and L = Landrace, C = Composite ($^1/_2$ Landrace, ¼, Duroc, ¼ Yorkshire)

Table 3.3 Optimal parameters for each of the artificial neural network models determined using the fruit fly optimization algorithm (FOA).

Model[a]	# Hidden Neurons	Spread Value	r	# FOA Iterations Until Convergence
FFNN-SCG	7	n/a	0.6586	5
FFNN-LM	11	n/a	0.6641	29
FFNN-BR	16	n/a	0.6635	10
GRNN	n/a	1.0925	0.6641	9

[a] Models tested included feed-forward neural networks trained with scaled conjugate gradient algorithm (FFNN-SCG), Levenberg-Marquardt algorithm (FFNN-LM), Bayesian regularization algorithm (FFNN-BR), and generalized regression neural network (GRNN).

Table 3.4 Statistical performance of artificial neural network models for test groups A-D.

Group/Model[a]	r	R^2	RMSE
Group A			
FFNN-SCG	0.483	0.233	653
FFNN-LM	0.487	0.237	651
FFNN-BR	0.472	0.223	657
GRNN	0.470	0.221	658
Group B			
FFNN-SCG	0.744	0.554	506
FFNN-LM	0.738	0.544	511
FFNN-BR	0.733	0.538	515
GRNN	0.733	0.537	515
Group C			
FFNN-SCG	0.623	0.390	692
FFNN-LM	0.626	0.392	691
FFNN-BR	0.610	0.372	702
GRNN	0.611	0.3734	702
Group D			
FFNN-SCG	0.754	0.569	494
FFNN-LM	0.742	0.550	504
FFNN-BR	0.737	0.543	509
GRNN	0.738	0.545	507

[a] Models tested included feed-forward neural networks trained with scaled conjugate gradient algorithm (FFNN-SCG), Levenberg-Marquardt algorithm (FFNN-LM), Bayesian regularization algorithm (FFNN-BR), and generalized regression neural network (GRNN).

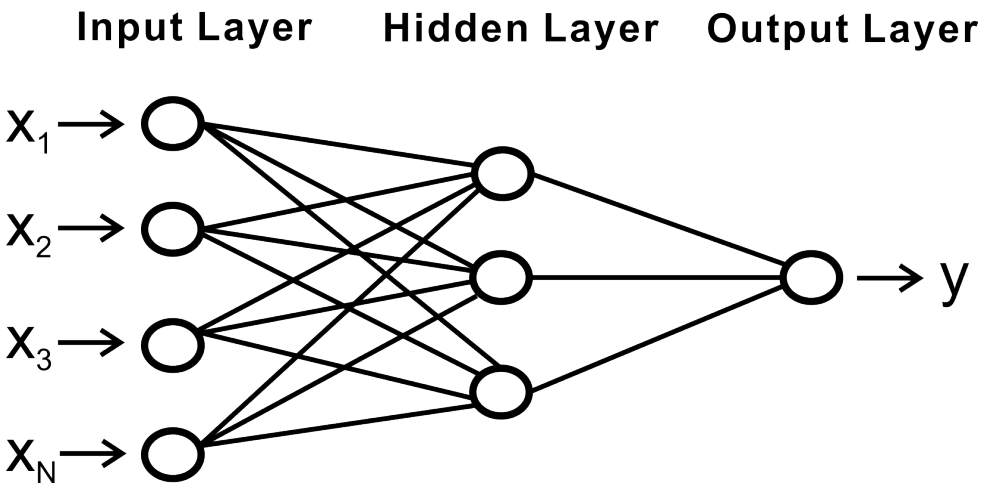


Figure 3.1 Typical feed-forward neural network (FFNN) architecture. The FFNN is comprised of three layers. These layers include an input layer, a hidden layer, and the output layer.

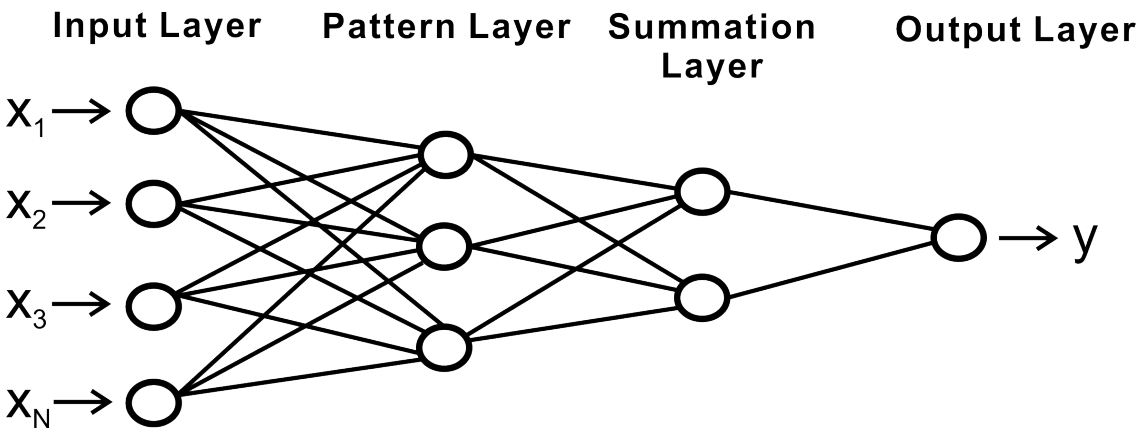


Figure 3.2 Generalized regression neural network (GRNN) architecture. The GRNN is comprised of four layers. These layers include an input layer, a pattern layer, a summation layer, and the output layer.

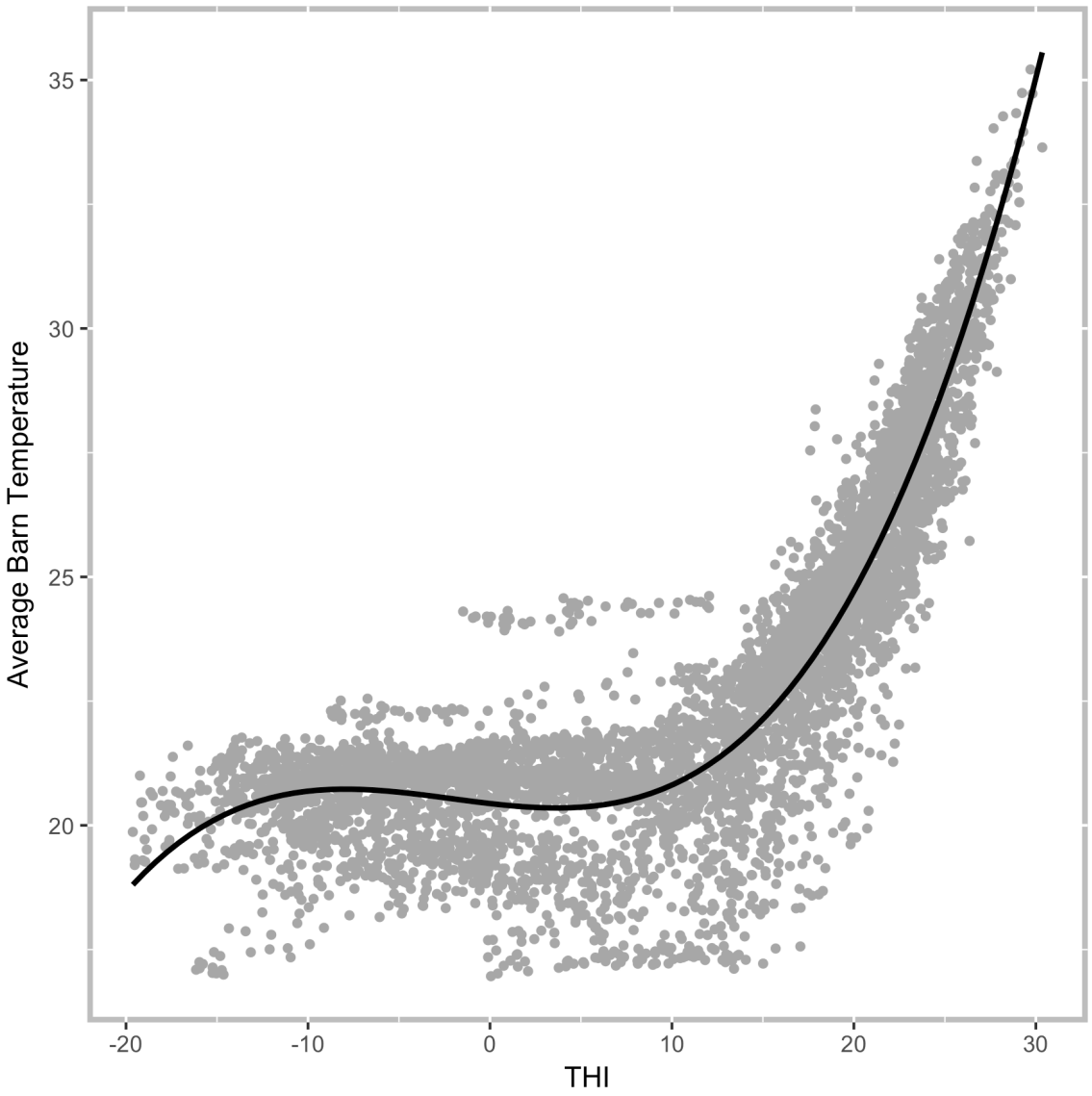
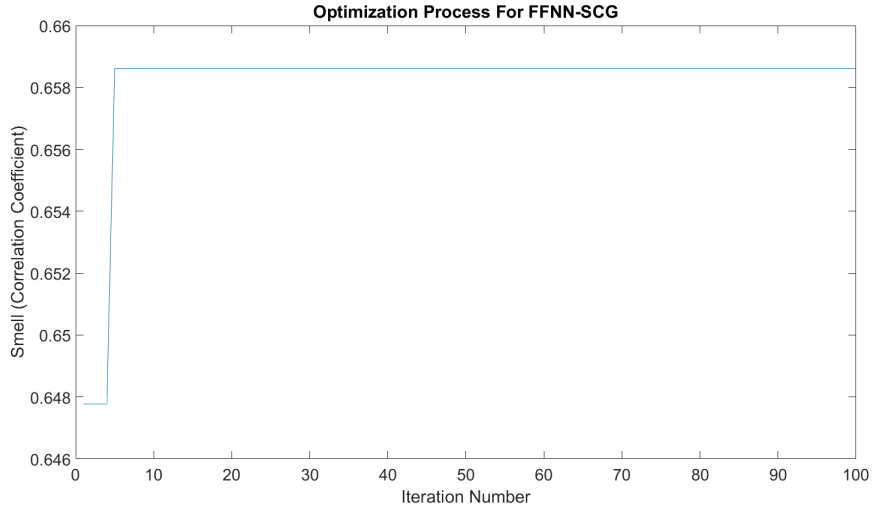
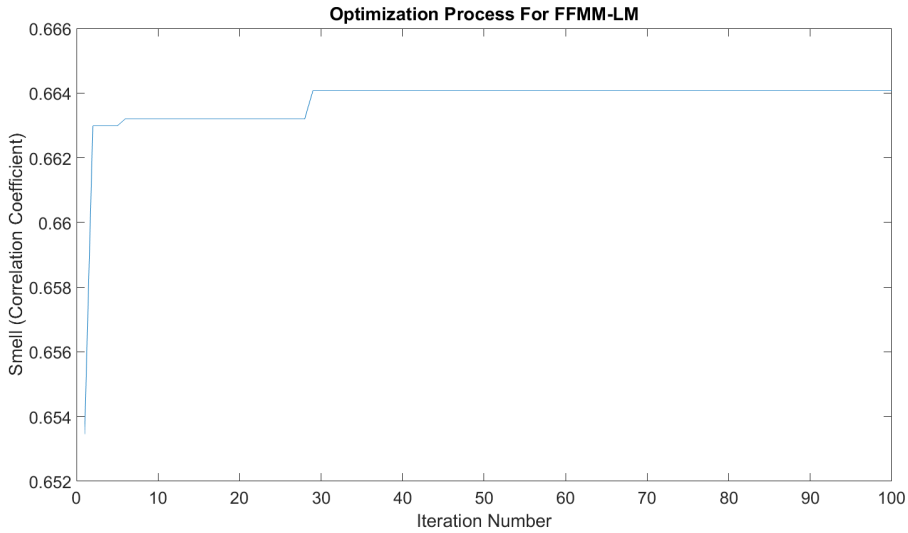


Figure 3.3. Results for temperature humidity index (THI) versus barn temperature 3rd degree polynomial regression. Here, $R^2 = 0.848$.

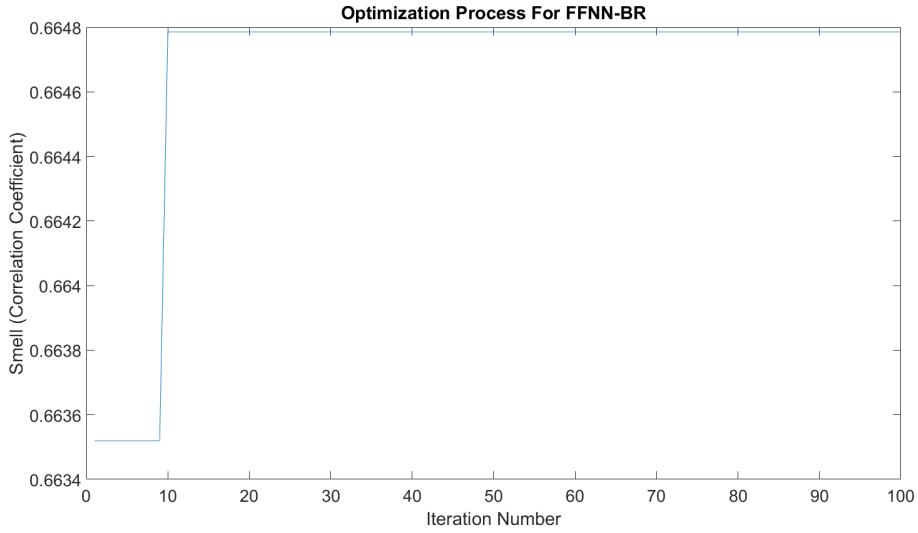
(A)


Optimization Process For FFNN-SCG
Smell (Correlation Coefficient)
Iteration Number
0.66
0.658
0.656
0.654
0.652
0.65
0.648
0.646
0
10
20
30
40
50
60
70
80
90
100

(B)


Optimization Process For FFMM-LM
Smell (Correlation Coefficient)
Iteration Number
0.666
0.664
0.662
0.66
0.658
0.656
0.654
0.652
0
10
20
30
40
50
60
70
80
90
100

(C)



(D)

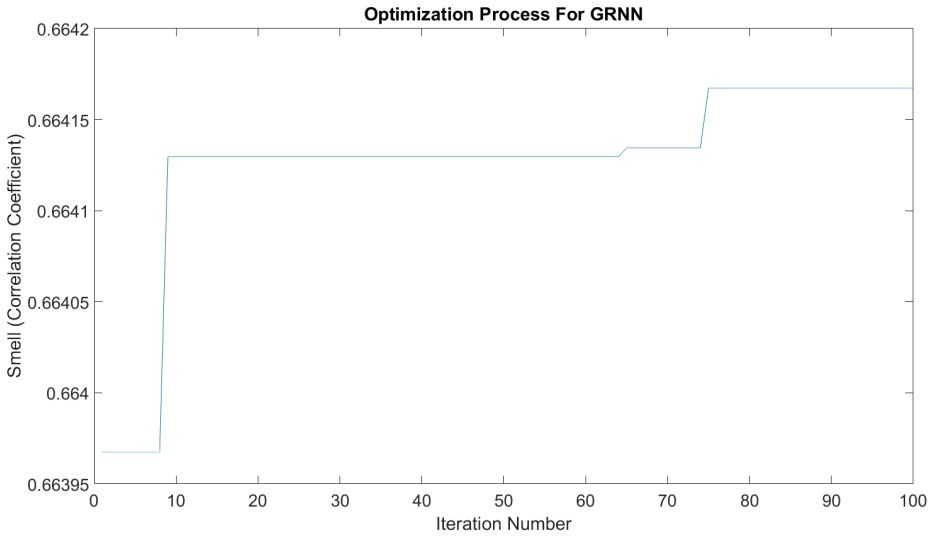


Figure 3.4. Correlation coefficients obtained during the fruit fly optimization algorithm process for (A) feed-forward neural network trained with scaled conjugate gradient algorithm (FFNN-SCG), (B) feed-forward neural network trained with Levenberg-Marquardt algorithm (FFNN-LM), (C) feed-forward neural network trained with Bayesian regularization algorithm (FFNN-BR), and (D) generalized regression neural network (GRNN).

Chapter 4

GENOME WIDE ASSOCIATION OF CHANGES IN SWINE FEEDING BEHAVIOR DUE TO HEAT STRESS

ABSTRACT

Heat stress negatively impacts pork production, particularly during the grow-finish phase. As temperature increases, feeding behavior changes in order for pigs to decrease heat production. The objective of this study was to identify genetic markers associated with changes in feeding behavior due to heat stress. Feeder visit data were collected on 1154 grow-finish pigs using an electronic feeding system from July 2011 to March 2016. Days in the study were partitioned into groups based on temperature humidity index (THI), where a THI less than 23.33°C was classified as "Normal", a THI between 23.33°C and 26.11°C was classified as "Alert", a THI between 26.11°C and 28.88°C was classified as "Danger", and a THI greater than 28.88°C was classified as "Emergency". All animals (n = 1154) were genotyped using Illumina BeadChip products and were imputed to the NeoGen Porcine GGPHD chip. A genome-wide association study (GWAS) for an animal's change in feeding behavior between different THI categories was conducted. Heritabilities for the difference in a pig's feeder visits between each of the THI categories were moderate to high (0.136 to 0.406). Strikingly, more than 71% of genetic variation was explained by regions within eight chromosomes in the comparison between Danger and Emergency THI. Gene ontology (GO) enrichment analysis showed that biological processes related to sensory perception and detection of chemical stimuli were over-represented in the set of genes located in these regions. Genetic markers identified in this study may facilitate genetic selection for improved grow-finish performance during elevated ambient temperatures.

INTRODUCTION

Heat stress is a major economic concern in the swine industry. In the United States, economic losses due to heat stress are estimated at $300 million a year, and a majority of these losses occur during the grow-finish phase (St-Pierre et al., 2003). Production losses due to heat stress may result from decreased growth of market hogs, reduced feed intake, and mortality (Nenaber et al., 1996; Collin et al., 2002; Gabler and Pearce, 2015).

Swine feeding behavioral patterns change as temperatures increase. Pigs spend less time eating and more time lying down during high temperatures (Hicks et al., 1998; Brown-Brandl et al., 2001). Changes in eating behavior, mealtime, and meal size are associated with increased temperatures (Hicks et al., 1998; Collin et al., 2001). A study by Nienaber et al. (1999) showed that reducing meal size as well as number of meals per day can reduce the effects of high temperatures on heat production by decreasing physical and metabolic activity.

Although advances in production management and barn cooling systems have occurred, production efficiency continues to suffer during warm months. Pigs have a thermal comfort zone in which they are most productive. Thermal comfort zone is dependent on several different factors including sex, genetics, relative humidity, and velocity of ambient air (NRC, 1981; Nyachoti et al., 2004). Genetic selection for increased growth has been associated with a decrease in pigs' ability to handle heat stress (Renaudeau et al., 2011). Genetic markers that are associated with heat stress could be used to select and breed for more heat resilient pigs. The objective of this study was to identify genetic markers associated with changes in feeding behavior due to heat stress in grow-finish pigs.

MATERIAL AND METHODS

Data Collection

All animal protocols conformed to procedures outlined in *Guide for Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 2010) and were approved by the USMARC Institutional Animal Care and Use Committee.

Data were collected on grow-finish pigs (n = 1154) reared at the U.S. Meat Animal Research Center from July 2011 to March 2016. Pigs were placed in the barn in grow-finish groups (n = 240) at approximately eight to ten weeks of age. Barrows and gilts were comingled and distributed into six pens with 40 pigs per pen. Three different sire lines, Duroc, Landrace, and Yorkshire, were represented. Animals were tagged with a low-frequency electronic identification tag upon entry into the grow-finish barn.

Pens were fitted with an electronic feeding system that monitored feeding behavior as described by Brown-Brandl et al. (2011). Briefly, each pen had one feeder with five slots, allowing up to five animals to eat at any given time. Pigs were provided *ad libitum* access to a corn-soybean meal diet designed to meet or exceed an animal's nutrient requirements. Each feeder slot was fitted with an antenna and a multiplexer. Data were collected from antennas every 20 seconds over a 4-month period.

Temperature humidity index (THI) was calculated (NOAA, 1976) using outside temperature (°C) and relative humidity (RH) as follows:

$$THI(°C) = T(°C) – [0.55 – (0.0055 \times RH)] \times [T(°C) – 14.5]$$

Outside temperatures were reported every hour; therefore to obtain the THI for a given day, the day was partitioned into 1-hour increments and the maximum THI was used to categorize each day. Days were classified into THI categories as outlined by Brown-

Brandl et al. (2013b). THI categories included "Normal" (< 23.33°C), "Alert" (23.33°C ≤ x < 26.11°C), "Danger" (26.11°C ≤ x < 28.88°C), and "Emergency" (≥ 28.88°C). It should be noted that not all animals experienced every THI category.

For each animal, the total number of feeder visits was computed for each day, and the animal's average number of daily feeder visits was computed for each THI category. Similarly, an average number of daily feeder visits was computed for each breed and sex combination in each THI category. Then for each individual animal, the difference between its observed average number of feeder visits and the appropriate breed-sex average was computed and standardized ($\mu = 0$, $\sigma = 1$) for each THI category. Differences in feeding behavior between two THI categories (e.g. Alert-Normal) were quantified by calculating the difference in standardized feeder visits between the two categories.

Genotyping

Tail samples were collected on pigs and stored at -20°C. Genomic DNA was extracted using a WIZARD genomic DNA purification kit according to the manufacturers protocol (Promega Corp., Madison, WI, USA). Genotyping was conducted using three different platforms: the NeoGen Porcine GGPHD chip (GeneSeek, Lansing, USA), the Illumina Porcine SNP60 V2 chip (Illumina, Inc., San Diego, USA), and the GGP-Porcine chip (GeneSeek, Lansing, USA). As a quality control, genotypes were filtered to include only those with minor allele frequency ≥ 5% and that had a unique map position in the Sscrofa10.2 genome assembly (Groenen et al., 2012). After quality control, 58,096 single nucleotide polymorphisms (SNP) from the GGPHD chip, 38,598 SNP from the Porcine SNP60 V2 chip, and 6,882 SNP from the GGP-Porcine chip were retained for use in

subsequent analyses. In total, 1,118 pigs were genotyped using the GGPHD chip, 2 pigs were genotyped using the SNP60 V2 chip, and 34 pigs were genotyped using the GGP-Porcine chip. Genotypes for animals genotyped on the Porcine SNP60 V2 chip and GGP-Porcine chip were imputed to the NeoGen Porcine GGPHD chip using FImpute v2.2 with a pedigree imputation (Sargolzaei et al., 2014).

Genome-wide association study (GWAS)

Priors for genetic and residual variances and the prior proportion of SNP that are assumed to have no effect on differences in feeding behavior, within an iteration of the Monte Carlo Markov Chain (MCMC) for each trait were obtained by running Bayes-Cpi using GenSel (Fernando and Garrick, 2008). Genomic regions associated with each trait were identified and quantified using a Bayes-C variable selection method using GenSel software (Fernando and Garrick, 2008). Pi values used in Bayes-C analyses are shown in Table 4.1. A chain of 41000 iterations was used with the first 1000 cycles discarded as burn-in. Each trait was analyzed with sex and sire breed as fixed effects, and farrowing group as a contemporary group effect. Effects were sampled every 40 iterations to obtain a posterior distribution for the genetic variance. Genomic regions associated with each trait were identified using 1-Mb genome windows.

Functions of genes in significant genomic regions

Genes located in significant genomic regions were obtained using the NCBI annotation of Sscrofa10.2 (Release 104). The PANTHER classification system (version 11.1; Mi et al., 2016; http://www.pantherdb.org/) was used to determine functions of these genes.

Enrichment analysis of gene function was performed using PANTHER's implementation of binomial test of overrepresentation. Significance of gene ontology (GO) terms was assessed using the default Ensembl *Sus scrofa* GO annotation as background for the enrichment analysis. Data from PANTHER was considered statistically significant at a Bonferroni corrected P-value < 0.05.

RESULTS

Breed-sex feeding behavior patterns

Of the 1154 grow-finish pigs (507 barrows and 647 gilts) used in the study, 305 were Duroc sired, 335 were Landrace sired, and 514 were Yorkshire sired. In all three sire breeds, feeding activity of barrows exceeded that of gilts in all THI categories (Table 4.2). It was also observed that Yorkshire and Duroc sired pigs had greater feeder visit activity than Landrace sired pigs across all THI categories (Table 4.2). Feeder visit activity in Yorkshire and Duroc sired pigs increased as THI increased, while the opposite trend was observed for Landrace sired pigs, which had a drastic decrease in feeder visits as THI increased from Danger to Emergency THI.

GWAS

Heritabilities from GenSel for each of the THI category comparisons are shown in Table 4.3 and detailed results for each window that explained a significant percentage of genetic variation (≥ 1.0%) are shown in Table 4.4. Normal-Alert behavior changes showed a strong heritability of 0.337 and more than 78% of the genetic variance was explained by regions within twelve chromosomes. Two regions on chromosome 15

accounted for 16.14% and chromosome 7 had six regions that accounted for 13.78% of genetic variance.

Feeding behavior changes between Normal and Danger THI were found to be most heritable (heritability = 0.406). Over 45% of genetic variance was explained by regions within ten chromosomes. Four regions on chromosome 7 accounted for 11.31% of the total genetic variance, while chromosome 1 had two significant regions accounting for 7.25%. Heritability for the Normal-Emergency comparison was considerably less (0.268), with approximately 49% of genetic variance explained by regions within twelve chromosomes. Chromosome 7 accounted for the largest percentage of variance (9.43%).

Alert-Danger feeding behavior changes were moderately heritable (0.189) and 60% of genetic variance was explained by regions within nine chromosomes. Chromosome 1 had two regions that explained 29.67% and chromosome 7 had four regions that explained 7.09 % of genetic variance. Heritability for Alert-Emergency THI comparison was 0.178 and 70% of genetic variance was explained by regions within seven chromosomes. Chromosome 13 had two regions that accounted for 36.04% and chromosome 1 had three regions that accounted for 15.57% of genetic variation. Greater than 71% of genetic variation was explained by regions within eight chromosomes for the Danger-Emergency THI comparison. Chromosome 13 explained the most genetic variance (43.19%). The heritability for this comparison, 0.136, was the lowest of the six THI comparisons.

Functions of genes in significant regions

The PANTHER classification system was used to analyze overrepresentation of GO terms for the set of genes located in significant genomic regions from each of the six GWAS. Significant GO term over-representation was identified in gene sets from only two of the GWAS, Alert-Danger and Danger-Emergency THI. Biological process terms detection of chemical stimulus involved sensory perception of smell, detection of chemical stimulus involved in sensory perception, detection of chemical stimulus, detection of stimulus involved in sensory perception, sensory perception of chemical stimulus, detection of stimulus, sensory perception, neurological system process, G-protein coupled receptor signaling pathway, system process, response to chemical, signal transduction, single organism signaling, signaling, cell communication, cellular response to stimulus, and response to stimulus were significantly over-represented in the set of genes comparing changes in feeder visit activity between Danger versus Emergency THI (Table 4.5). In this gene set, molecular function terms olfactory receptor activity, G-protein coupled receptor activity, transmembrane signaling receptor activity, transmembrane receptor activity, signaling receptor activity, receptor activity, molecular transducer activity, and signal transducer activity were significantly over-represented. Additionally, over-represented cellular component terms included plasma membrane, cell periphery, integral component of membrane, intrinsic component of membrane, membrane part, and membrane.

Biological process terms organic substance catabolic process and response to chemical were significantly over-represented in the set of genes comparing changes in feeder visit activity between Alert versus Danger THI (Table 4.6). Molecular function

terms glutathione transferase activity, transferase activity (transferring alkyl or aryl groups), aspartic-type endopeptidase activity, aspartic-type peptidase activity, and odorant binding were significantly over-represented, while synaptic membrane was the only over-represented cellular component term.

DISCUSSION

Environmental temperatures are known to affect swine feeding behavior. In this study, THI was computed using outdoor temperature. Ideally, barn temperatures would have been utilized. During the feed trials, barn temperatures were collected from two thermostats, one located on the north end of the barn and the other on the south end for some of the groups of pigs studied. However, there were many missing data points due to thermometer failure and other technical issues. Temperature humidity index was thus used in our analyses, since it was found to be a good predictor of barn temperature (adjusted $R^2 = 0.848$; Figure 4.1).

In this study, barrows from all three sire breeds had higher average daily feeder visit counts than gilts in each THI category. This is consistent with findings of a previous study, where Brown-Brandl et al. (2013a) reported that barrows spent more time at feeders than gilts. However, a different study Hyun et al. (1997) reported no difference in time spent at feeders between sexes. In the study of Hyun et al. (1997) the electronic feeding system allowed only one pig to eat at a time, while Brown-Brandl et al. (2013a) used an electronic feeding system, like the one in our study, consisting of one feeder with five spaces for pigs to eat at, which is representative of current production systems. This

observed difference in feeder activity between barrows and gilts may be due to space competition or differences in how each sex handles heat stress.

We found that heat stress appears to impact feeding behavior differently in pigs from different breeds. Feeding activity of Duroc and Yorkshire sired pigs increased as THI increased, while activity of Landrace sired pigs decreased as THI increased. Several different approaches have been used to determine a pig's ability to handle stressful situations. To test how a pig copes with a perceived stressful situation researchers have used the backtest, in which piglets are placed on their backs to determine time until first struggle or time spent struggling (Hessing et al., 1993; Cassady, 2007; Velie et al., 2009; Rohrer et al., 2013). Therefore, as animals are calmer and handle stressful situations better their time until first struggle will increase. Time until first struggle during the backtest has been shown to be positively genetically correlated with number of meals per day and negatively genetically correlated with average meal length (Rohrer et al., 2013). As THI increased, Landrace sired pigs spent less time visiting the feeder, while Yorkshire and Duroc sired pigs increased feeder visit activity. Hence, the decrease in number of feeder visits when temperature increases in Landrace sired pigs may indicate a decreased ability to handle stressful situations, in particular, heat stress.

Genome-wide association study for various THI category comparisons identified similar significant genomic regions. The Normal-Alert THI comparison and the Normal-Danger THI comparison had ten regions on seven different chromosomes that were the same (SSC 2_24, SSC 2_26, SSC 6_15, SSC 7_10, SSC 7_46, SSC 7_53, SSC 10_54, SSC 14_138, SSC 15_19, SSC 18_1). Chromosome 7 had a region, SSC 7_53, that appeared in four of the six analyses (Normal-Alert, Normal-Danger, Normal-Emergency,

and Alert-Danger). Evaluation of this region identified a heat shock protein (*DNAJA4*), located at 53.2 Mb. Heat shock proteins are proteins that protect cells from stressors (Basiricò et al., 2011). In particular, *DNAJA4*, was shown to be expressed at higher levels after heat stress in chicken testes (Wang et al., 2013). This region also contains members of the acetylcholine receptor subunit family. Two of these genes, *CHRNA3* and *CHRNB4*, form a complex that activates *POMC* neurons which stimulate *MC4R* and regulate eating behavior (Online Mendelian Inheritance in Man, 2017). Thus, all three of these genes (*DNAJA4, CHRNA3, and CHRNA4*) warrant further investigation.

A second region on SSC 7, ranging from 44 to 47 Mb, was present in every comparison with Normal THI. This region contains two heat shock proteins, *HPS90AA1* and *HSP90AB1*, located at 45.1 Mb. *HSP90AA1* is an inducible protein that is expressed during times of cellular stress. This gene was also found to be more highly expressed in testes of heat stressed chickens (Wang et al., 2013). Polymorphisms in *HSP90AA1* have been associated with adaptation to thermal conditions in sheep (Marcos-Carcavilla et al., 2010), while polymorphisms in *HSP90AB1* have been associated with heat tolerance in cattle (Charoensook et al., 2012).

Five windows were present in two analyses (SSC 1_228, SSC 9_103, SSC 15_19, SSC 18_1, and SSC X_2). One of these regions SSC18_1, harbored a heat shock protein, *DNAJB6*, and nearby (SSC 18:1.27 Mb) was *PTPRN2* which is involved in ghrelin regulation. Ghrelin plays a role in meal initiation and feed intake. The gene *KIAA1324L*, located in SSC 9_103, may have a role in cellular response to stress.

Three genomic regions were associated with feeding behavior changes for the Alert-Emergency THI and Danger –Emergency THI comparisons (SSC 1_18, SSC

12_58, SSC 13_176). Moreover, these regions explained a large portion of genetic variance in each of the analyses: 48.03% of genetic variance in Alert-Emergency and 49.25% Danger-Emergency.

The most significantly enriched GO terms identified in the PANTHER analysis for Danger-Emergency comparison were sensory to olfactory stimuli, sensory perception, and response to stimulus. One of the genes involved in response to stimulus was TRPV3 (SSC 12_51.5), a member of the transient receptor potential (TRP) protein family. Proteins in this family are cation channels that function in a variety of processes including temperature sensation and vasoregulation (Caterina et al., 1997; Xu et al., 2002). Expression of TRPV3 in mice occurred mainly in skin keratinocytes, but appear also in neurons (Peier et al., 2002; Xu et al., 2002). In TRPV3 knockout mice, sensory reactions remained the same, except for their response to increased temperatures (Moqrich et al, 2005), indicating that it plays a role in thermosensation. Temperatures reported in the study of Moqrich et al. (2005) were greater than temperatures experienced by grow-finish pigs during Danger and Emergency THI. Pigs, unlike most other mammals, have a limited capacity to use water evaporation to lose heat (Ingram, 1965); hence, TRPV3 activation may occur at lower temperatures in pigs compared to other mammals.

Another member of the TRP family, TRPV1, was located in the same genomic window as TRPV3. Activation of TRPV1 can occur due to increased temperature or due to chemical agonists such as capsaicin (Caterina et al., 1997; Caterina, 2007). Once nerves detect increased heat, a signal is sent to the hypothalamus causing warmth-sensitive neurons to trigger a heat-loss reflex, either by vasoconstriction or behavioral

mechanisms (Caterina, 2007). Hence, a change in behavioral mechanisms could be observed through changes in feeder visit activity in grow-finish pigs when exposed to increased temperatures.

Changes in feeder visit activity are indicative of grow-finish pigs' response to heat stress. Candidate genes for heat stress were identified using feeder visit activity differences between THI categories. Genes involved in sensory to olfactory stimuli, sensory perception, and response to stimuli were among those over-represented in the set of genes comparing changes in feeder visit activity between Danger and Emergency THI. Genes involved in response to stimulus included a gene family, TRP, that impacts thermosensation. Candidate genes and genetic markers identified in this work may facilitate genetic selection for improved grow-finish performance during increased temperatures. Selection for heat tolerant grow-finish pigs would lead to increased production efficiency.

Table 4.1: Pi values obtained from Bayes-Cpi analyses and used in Bayes-C analysis in GenSel for each of the Temperature-Humidity Index (THI) category comparisons.

THI category comparison[a]	pi
Normal - Alert	0.999916
Normal - Danger	0.999847
Normal - Emergency	0.999898
Alert - Danger	0.999938
Alert - Emergency	0.999938
Danger - Emergency	0.999942

[a] Normal ($x < 23.33°C$), Alert ($23.33°C \leq x < 26.11°C$), Danger ($26.11°C \leq x < 28.88°C$), and Emergency ($x \geq 28.88°C$)

Table 4.2: Average total number of daily feeder counts (mean ± standard error) by sire breed and sire breed-sex at each Temperature-Humidity Index category.

Breed	Sex	Normal $x < 23.33$ °C	Alert $23.33 \leq x < 26.11$ °C	Danger $26.11 \leq x < 28.88$ °C	Emergency $x \geq 28.88$ °C
Duroc					
	All	153.4 ± 0.5	168.9 ± 0.9	172.7 ± 1.1	182.6 ± 3.0
	Barrow	154.8 ± 0.7	179.0 ± 1.4	183.5 ± 1.7	194.3 ± 4.4
	Gilt	152.0 ± 0.6	159.1 ± 1.2	162.1 ± 1.4	171.1 ± 4.0
Yorkshire					
	All	140.9 ± 0.4	145.3 ± 0.7	150.0 ± 0.9	157.8 ± 3.1
	Barrow	156.5 ± 0.7	160.5 ± 1.3	170.7 ± 1.6	188.4 ± 4.7
	Gilt	130.9 ± 0.5	135.5 ± 0.8	137.5 ± 1.0	137.2 ± 3.6
Landrace					
	All	134.3 ± 0.3	122.5 ± 0.6	108.5 ± 0.7	65.8 ± 1.0
	Barrow	140.3 ± 0.4	131.7 ± 0.9	118.0 ± 1.1	71.2 ± 1.7
	Gilt	129.3 ± 0.3	115.6 ± 0.7	101.7 ± 0.8	62.4 ± 1.1

Table 4.3: Estimates of heritabilities from Bayes-C analyses in GenSel for changes in feeding behavior of each Temperature-Humidity Index (THI) category comparison.

THI category comparison[a]	h^2
Normal - Alert	0.337
Normal - Danger	0.406
Normal - Emergency	0.268
Alert - Danger	0.189
Alert - Emergency	0.178
Danger - Emergency	0.136

[a] Normal ($x < 23.33°C$), Alert ($23.33°C \leq x < 26.11°C$), Danger ($26.11°C \leq x < 28.88°C$), and Emergency ($x \geq 28.88°C$)

Table 4.4: Identified windows from GenSel that explain more than 1.0% of genetic variation for each Temperature-Humidity Index (THI) category comparisons.

THI category comparison[a]	Chromosome	Position[b] (Mb)	% of genetic variance explained	Number of SNPs	Frequency of iterations with (P > 0)
Normal - Alert					
	15	19	14.19	33	0.88
	19	2	7.79	44	0.66
	18	1	5.76	31	0.46
	17	2	4.87	32	0.40
	7	53	4.71	28	0.44
	13	85	4.65	7	0.43
	5	107	4.29	31	0.37
	17	3	3.66	29	0.29
	2	24	3.65	34	0.26
	18	14	2.51	28	0.27
	5	68	2.39	26	0.31
	10	54	2.28	24	0.26
	7	46	2.17	23	0.21
	7	10	2.09	32	0.20
	7	1	2.00	41	0.19
	15	152	1.95	33	0.20
	7	52	1.49	28	0.17
	5	108	1.47	28	0.16
	6	15	1.46	39	0.13
	7	45	1.32	26	0.17
	2	26	1.25	30	0.13
	14	138	1.03	42	0.09
	12	37	1.02	19	0.14
Normal - Danger					
	7	53	6.10	28	0.62
	1	228	6.04	13	0.62
	15	19	3.58	33	0.44
	14	138	3.43	42	0.42
	6	15	2.99	39	0.34
	2	26	2.99	30	0.34
	4	10	2.96	34	0.39
	10	44	2.27	24	0.33
	7	10	1.84	32	0.23
	2	24	1.83	34	0.22
	7	47	1.69	20	0.23
	18	1	1.69	31	0.21

	7	46	1.68	23	0.24
	10	54	1.61	24	0.24
	18	55	1.36	24	0.20
	1	230	1.21	11	0.16
	13	46	1.06	20	0.15
	15	135	1.05	44	0.15
Normal - Emergency					
	7	53	5.99	28	0.30
	9	103	4.89	9	0.25
	5	98	4.25	32	0.23
	12	12	4.15	35	0.25
	19	2	4.06	44	0.27
	5	10	3.17	36	0.20
	11	21	2.81	30	0.21
	1	117	2.40	16	0.18
	13	7	2.33	36	0.16
	7	43	2.1	21	0.15
	17	49	1.81	31	0.15
	11	22	1.77	26	0.14
	5	100	1.71	26	0.11
	4	103	1.43	26	0.12
	2	103	1.38	18	0.10
	7	44	1.29	22	0.09
	2	102	1.27	14	0.09
	6	15	1.15	39	0.08
	19	3	1.10	63	0.09
Alert – Danger					
	1	228	28.66	13	0.95
	17	17	6.23	22	0.31
	4	93	4.05	20	0.24
	7	53	3.54	28	0.20
	18	23	3.44	24	0.18
	18	42	1.89	15	0.11
	2	9	1.43	38	0.07
	2	11	1.28	28	0.08
	7	52	1.27	28	0.09
	15	131	1.21	38	0.09
	4	10	1.20	34	0.09
	7	134	1.19	32	0.08
	7	62	1.09	18	0.06
	2	143	1.07	43	0.06
	8	140	1.04	33	0.07
	14	138	1.04	42	0.07

	1	286	1.01	27	0.07
Alert - Emergency					
	13	176	33.73	16	0.92
	1	16	11.03	38	0.37
	12	58	10.82	34	0.41
	9	103	3.97	9	0.18
	1	18	3.48	26	0.11
	13	197	2.31	32	0.10
	11	22	1.89	26	0.09
	6	68	1.46	35	0.06
	14	13	1.13	27	0.06
	1	210	1.06	9	0.04
Danger - Emergency					
	13	176	43.19	16	0.94
	12	51	5.51	28	0.14
	12	58	5.01	34	0.19
	19	126	4.24	28	0.17
	6	149	3.15	28	0.12
	7	104	3.05	21	0.13
	2	71	3.04	8	0.11
	16	78	1.93	48	0.06
	7	133	1.27	13	0.06
	1	18	1.05	26	0.03

[a] Normal ($x < 23.33°C$), Alert ($23.33°C \leq x < 26.11°C$), Danger ($26.11°C \leq x < 28.88°C$), and Emergency ($x \geq 28.88°C$)
[b] Positions are based on build 10.2 of the swine genome.

Table 4.5. List of ontology terms that were significantly over- and underrepresented in the set of genes located in 1 Mb windows identified for Danger-Emergency Temperature-Humidity Index category comparison.

	Gene Set (n genes)				
Ontology Term	Annotated genes[a] (21398)	Genes[b] (101)	Genes expected	Over (+) or Under (-)	P-value
Biological Process					
Detection of chemical stimulus involved in sensory perception of smell	1039	29	5.05	+	6.13E-11
Sensory perception of smell	1055	29	5.13	+	9.02E-11
Detection of chemical stimulus involved in sensory perception	1062	29	5.16	+	1.07E-10
Detection of chemical stimulus	1079	29	5.24	+	1.59E-10
Detection of stimulus involved in sensory perception	1091	29	5.30	+	2.10E-10
Sensory perception of chemical stimulus	1109	29	5.39	+	3.17E-10
Detection of stimulus	1152	29	5.60	+	8.20E-10
Sensory perception	1308	29	6.36	+	1.87E-08
Neurological system process	1482	30	7.20	+	6.89E-08
G-protein coupled receptor signalling pathway	1625	30	7.90	+	6.56E-07
System process	1788	30	8.69	+	6.42E-06
Response to chemical	2894	37	14.07	+	7.09E-05
Signal transduction	4081	42	19.83	+	2.51E-03
Single organism signalling	4336	43	21.07	+	4.76E-03
Signalling	4339	43	21.09	+	4.85E-03
Cell communication	4394	43	21.36	+	6.90E-03
Cellular response to stimulus	4960	48	24.11	+	1.37E-03
Response to stimulus	5795	54	28.17	+	4.22E-04

Molecular Function					
Olfactory receptor activity	1039	29	5.05	+	1.98E-11
G-protein coupled receptor activity	1427	29	6.94	+	4.93E-08
Transmembrane receptor activity	1777	31	8.64	+	3.84E-07
Transmembrane signalling receptor activity	1745	30	8.48	+	1.17E-06
Signalling receptor activity	1829	31	8.89	+	7.79E-07
Receptor activity	1988	32	9.66	+	1.35E-06
Molecular transducer activity	1988	32	9.66	+	1.35E-06
Signal transducer activity	2084	32	10.13	+	4.33E-06
Cellular Component					
Plasma membrane	3670	42	17.84	+	2.01E-05
Cell periphery	3761	42	18.28	+	4.13E-05
Integral component of membrane	5563	50	27.04	+	1.15E-03
Intrinsic component of membrane	5609	50	27.26	+	1.49E-03
Membrane part	6303	53	30.63	+	3.55E-03
Membrane	7806	59	37.94	+	2.15E-02

[a] Number of genes in the background *Sus scrofa* annotation set with given GO term. Total number of annotated genes is shown in parentheses.

[b] Number of genes with given GO term. Total number of genes with annotations in the background *Sus scrofa* annotation set is shown in parentheses.

Table 4.6. List of ontology terms that were significantly over- and underrepresented in the set of genes located in 1Mb windows identified for Alert-Danger Temperature-Humidity Index category comparison.

	Gene Set (n genes)				
Ontology Term	Annotated genes[a] (21398)	Genes[b] (158)	Genes expected	Over (+) or Under (-)	P-value
Biological Process					
Organic substance catabolic process	980	22	7.42	+	3.33E-02
Response to chemical	2894	45	21.91	+	8.37E-03
Molecular Function					
Glutathione transferase activity	23	6	0.17	+	5.92E-05
Transferase activity, transferring alkyl or aryl groups	48	6	0.36	+	4.18E-03
Aspartic-type endopeptidase activity	39	5	0.30	+	2.68E-02
Aspartic-type peptidase activity	40	5	0.30	+	3.03E-02
Odorant binding	230	15	1.74	+	7.06E-07
Cellular Component					
Synaptic membrane	94	7	0.71	+	9.09E-03

[a] Number of genes in the background *Sus scrofa* annotation set with given GO term. Total number of annotated genes is shown in parentheses.

[b] Number of genes with given GO term. Total number of genes with annotations in the background *Sus scrofa* annotation set is shown in parentheses.

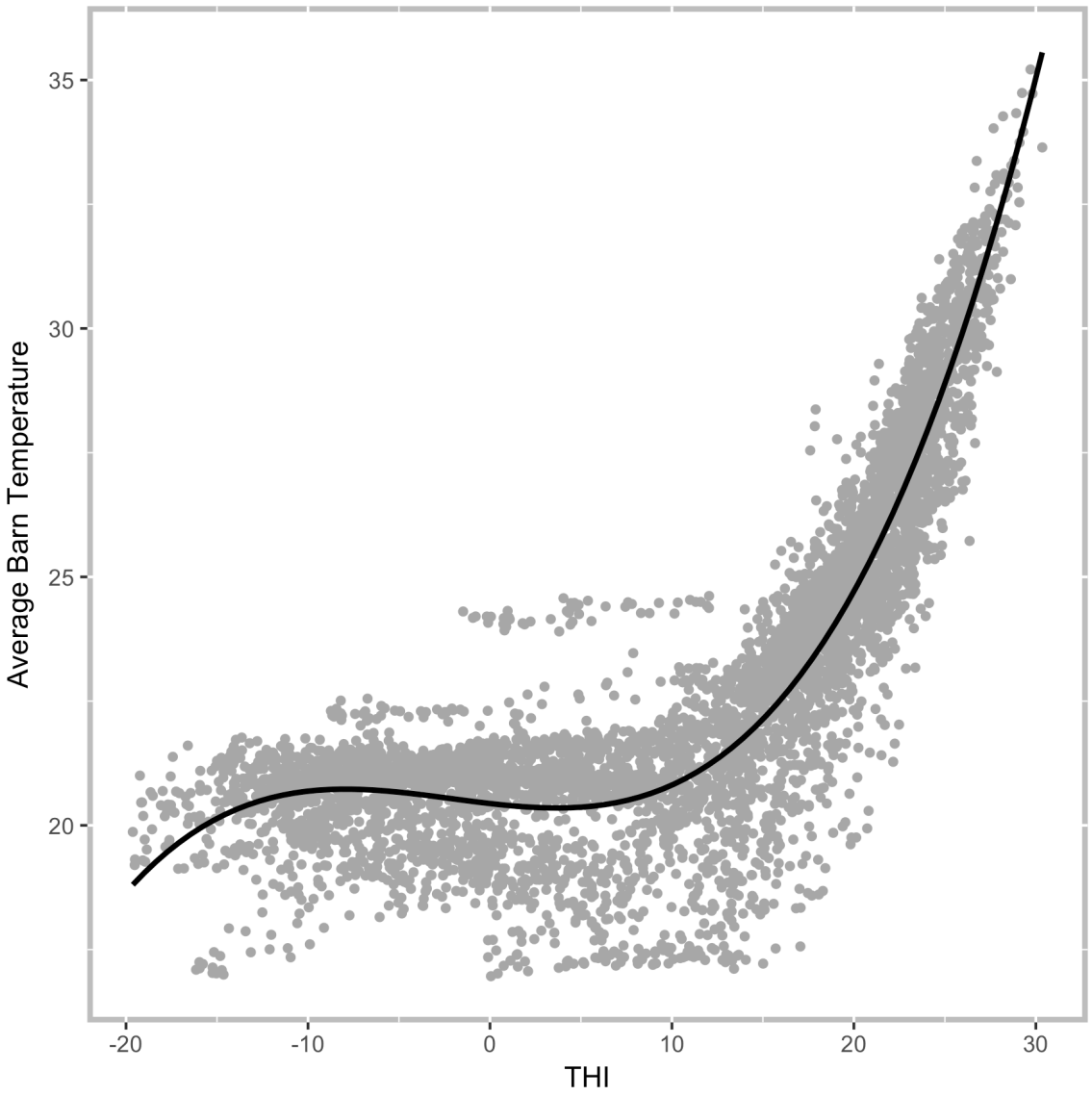


Figure 4.1: Temperature-Humidity index (THI; °C) versus average barn temperature (°C) using a 3rd degree polynomial regression for all time periods when both measures were available.

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