

Effects of gestational heat stress on the lactational performance of gilts and growth performance and carcass characteristics of second-generation offspring

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ABSTRACT

Pigs exposed to chronic intrauterine hyperthermia (gHS) experience greater fat deposition during life and yield carcasses with greater fat:lean content at slaughter compared to pigs gestated under thermoneutral conditions (gTN). The objectives of this study were to 1) determine whether gHS impacts the lactational performance of affected gilts (F1 generation), and 2) determine whether these effects of gHS are also evident in the next generation (F2 generation). Twenty-four gilts were bred and exposed to thermoneutral or heat stressed conditions for the entirety of gestation, and F1 female offspring were retained. At puberty, gHS and gTN gilts were bred to farrow in either spring (March / April) or summer (July / August). Colostrum and milk samples were collected at farrowing and on d 7, 14, and 21 of lactation. At weaning, four offspring (two male, two female) were retained and grown to market weight in mixed-pens under identical management conditions. Carcass characteristics were analyzed at slaughter. Milk nutrient analysis indicated that gHS gilts produced less lactose, and tended to produce greater protein, than did gTN gilts. There was no difference in the growth rate of F2 offspring, but pigs born of gHS dams did have a tendency for greater backfat thickness. The patterns of altered milk nutrient content observed in F1 gilts reflects a metabolic profile consistent with previous gHS research, and the greater backfat of F2 pigs at slaughter indicates the adipose-promoting effects of gHS may be diluted, but still evident, in the second generation.

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Dedication

For my mother, Elaine F. Wiegert.

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List of Major Abbreviations

Abbrev.	Definition
HS	Heat stress
TN	Thermoneutral
gHS	Gestational heat stress; pigs developed during HS pregnancies (F1)
gTN	Gestational thermoneutral; pigs developed during TN pregnancies (F1)
OgHS	Offspring of gestational heat stress; pigs born to gHS dams (F2)
OgTN	Offspring of gestational thermoneutral; pigs born to gTN dams (F2)
SUM	Summer; refers to season of farrowing
SPR	Spring; refers to season of farrowing
LAC	Percent milk lactose content
PRO	Percent milk protein content
SNF	Percent milk solids-non-fat content
LW	Liveweight at slaughter
HCW	Hot carcass weight at slaughter
BF	Backfat thickness
LEA	Loin eye area
CRC L	Carcass length
DRESS %	Dressing percentage
LEAN %	Lean percentage
h	Hours
d	Days

Chapter I. Literature Review

Part I: Swine Heat Stress

Introduction

Pigs growing under periods of chronic heat stress experience reduced feed intake and average daily gain and, because of this, require an increased number of days on feed to reach target market weights (Brown-Brandl et al., 1998; Hicks et al., 1998). Additionally, breeding females subjected to the combined effects of long-day photoperiod and heat stress during the late-summer and early-autumn months experience a decrease in reproductive efficiency commonly referred to in the production industry as seasonal infertility (Love, 1978). Collectively, the negative effects of seasonal heat stress on the growing and breeding swine herds was estimated in 2003 to cost US swine producers over \$300 million annually (St-Pierre et al., 2003).

In the past, many producers have been content to “wait out” the heat stress imposed in their herds during the hot summer months (Jones et al., 1983). However, greater research in the field has shown that the pig’s physiological responses to heat stress are more severe than previously thought, and the true annual cost of heat stress may be well above the previously estimated \$300 million. Better understanding of the consequences of exposure to long-term hyperthermia in the growing, breeding, gestating, and lactating pig are required to dictate the appropriate management and heat abatement strategies necessary on the production swine farm.

Effects of Heat Stress on Swine and Swine Production

The threshold for thermal stress in swine is low, and pigs may become heat stressed at temperatures that seem comfortable to humans. Pigs lack effective sweat glands (Guthrie, 2012), and therefore are reliant on panting, wallowing, and radiant heat dissipation to ameliorate their thermal load. This decreases the range of the pig's thermal comfort zone, and makes pigs more susceptible to high environmental temperatures. The homeostatic thermal comfort zone is the range of temperatures between the lower critical temperature and the upper critical temperature (Black et al., 1993). The lower critical temperature is the temperature at which the animal must increase heat production through metabolic or shivering thermogenesis; the upper critical temperature is the temperature at which the pig must dissipate excess heat, usually through altered behavior, increased lung evaporation, or decreased voluntary feed intake (Black et al., 1993). The upper and lower critical temperatures (roughly 12°C and 22°C for sows and 30°C and 37°C for piglets) are not definite, but rather are influenced by factors including humidity, wind speed, air quality, pen stocking density, and pig health and size, among others (Black et al., 1993; Huynh et al., 2005; White et al., 2008; Renaudeau, 2009).

Artificial selection pressure in recent decades for increased lean gain has produced a leaner market hog, and this may influence the upper critical temperature at which heat stress occurs. In 2001, pigs used in commercial swine production exhibited greater lean muscle deposition (+1.76 kg) and decreased backfat thickness (-4.8 mm), at slaughter and required a reduced number of days on feed necessary to reach market weight (-3.6 d) than did pigs on commercial farms in 1991 (Brown-Brandl et al., 2004). Using the findings of Tess et al. (1984), that a 2.1% increase in lean percentage accompanies an 18.7% increase in fasting heat production, Brown-Brandl et al. (2004) calculated that pigs in 2001 produced an extra 14.6%

fasting heat than did pigs of similar genetics just ten years prior. Presumably, modern commercial-line pigs have a greater basal thermal load, and may be more susceptible to heat stress, due to their greater metabolic heat production.

In pigs, heat stress increases core body temperature and causes lethargy, anorexia, tachypnea, and tachycardia (Brown-Brandl et al., 1998). In growing pigs selected for high lean growth, respiration rate increases at temperatures greater than 18°C, and continues to rise incrementally as temperature increases; feed intake is reduced at temperatures greater than 24°C; and water consumption increases at temperatures above 32°C (Brown-Brandl et al., 1998). Larger sized pigs are more susceptible to heat stress than smaller pigs due to greater feed intake, less surface area to body mass ratio, and greater percent body fat (Renaudeau et al., 2012).

Heat stressed pigs exhibit decreased overall activity, evident as more time spent in recumbency, and less time spent at the feeder (Hicks et al., 1998). It is interesting to note that, while the amount of time spent at the feeder is reduced, there is no effect of heat stress on the number of meals consumed per day or the rate of feed intake during each meal (Collin et al., 2001). Presumably, reduced feed intake during heat stress is an evolutionary adaptation to decrease the heat increment associated with digestion.

Studies reviewed by Baumgard and Rhoads (2013) indicate that pigs exposed to heat stress deposit greater adipose tissue at the expense of lean muscle accretion. While the mechanisms supporting the increased adipose:lean tissue ratio are not yet completely understood (Baumgard and Rhoads, 2013), the phenomenon has been documented in numerous studies. Additionally, Stahly and Cromwell (1979) reported a positive linear effect of increasing ambient temperature on carcass length. Greater body length of swine grown under heat stress conditions (as well as a reduced body length of swine grown during periods of cold stress; Stahly and

Cromwell, 1979) may represent a mechanism through which the animal alters their surface area to body mass ratio in an effort to conserve or dissipate body heat.

On-Farm Methods to Alleviate Heat Stress

Traditionally (and in some niche market operations today), domesticated pigs were raised outdoors on pasture or in wooded lots. Pigs raised outdoors will wallow – that is, cover themselves in mud or other mud-like viscous substances (Bracke, 2011) – to provide cooling relief and protection from sunburn. Adult pigs begin to show wallowing behavior at an ambient temperature range of 17-21°C (Bracke et al., 2011), and the amount of time per day spent in the wallow is highly correlated with the degree of ambient temperature and the intensity of sunshine, and is negatively correlated with humidity (McGlone, 1999; Olsen et al., 2001). Pigs raised outdoors during summer with access to a wallowing area show improved growth rates and a better feed to gain conversion ratio than do pigs that are denied access (Culver et al., 1960). However, swine wallowing behavior has lost favor and is discouraged in the current production industry due to the significant role it may play in pathogen persistence and disease transmission on the farm (Callaway et al., 2005).

The majority of pigs in the modern swine industry are housed indoors in facilities that offer some degree of environmental control, generally in the form of forced-air ventilation (USDA, 2012). In addition, heat dissipation may be achieved on indoor farms through the use of evaporative (sprinklers, foggers, misters, and water drippers), conductive (cooling pads containing refrigerated water piped through a conductive metal), and convective methods (snout coolers that blow cold air onto the pig's sensitive snout; Jones et al., 1983).

Conductive cooling pads have been identified as the preferred cooling system, as indicated in one study by the preference of heat stressed gilts to lie on the cooling pad (49.4% of the heat stress period) as opposed to lying under a water dripper (31.8% of the heat stress period) or in front of a snout cooler (18.8% of the heat stress period; Bull et al., 1997). In the same study, conductive cooling pads also provided the greatest relief to the gilt's rectal temperature ($39.4 \pm 0.4^{\circ}\text{C}$, $39.8 \pm 7.0^{\circ}\text{C}$, and $39.7 \pm .08^{\circ}\text{C}$ for cooling pads, snout coolers, and water drippers, respectively) and respiration rate in breaths per minute (72.7 ± 3.7 bpm, 114.2 ± 7.0 bpm, and 102.7 ± 5.0 bpm for cooling pads, snout coolers, and water drippers, respectively; Bull et al., 1997). However, cooling pads are inherently difficult to construct and maintain on a swine farm, and as such, many operations utilize evaporative cooling methods over any other form of heat stress remediation (Jones et al., 1983).

Metabolic and Endocrine Responses to Heat Stress

Metabolic and endocrine adaptations are necessary to maintain homeostasis during heat stress. Many of these adaptations arise as result of the negative energy balance (NEBAL) state spurred by reduced feed intake (Baumgard and Rhoads, 2013), which in turn complicates analysis of the true effects of heat stress on animal physiology. To overcome this, pair-fed feeding strategies are used to avoid artifact in experiments in which the effect of treatment also impacts feed intake. In pair-fed heat stress trials, the voluntary feed intake of heat stressed animals is recorded, and this same feed amount is provided to thermoneutral animals. A second group of thermoneutral animals is allotted *ad libitum* feed access. In this way, the effects of heat stress may be analyzed independent of the confounding caused by reduced feed intake (Baumgard and Rhoads, 2013).

Independent of feed intake, heat stress reduces concentrations of the catabolic hormones cortisol, triiodothyronine (T₃), and thyroxine (T₄), and increases the anabolic hormones somatotropin and insulin. In addition to the endocrine responses, heat stress also decreases lipolysis and induces an increase in the expression of lipoprotein lipase (Sanders et al., 2009), which among other functions, assists in hydrolyzing circulating triglycerides, promotes storage of free fatty acids, and decreases the abundance of circulating non-esterified fatty acids (NEFA; Pearce et al., 2013).

Interestingly, heightened adipose conservation is the opposite of what would be expected in pigs maintained under reduced feed intake at thermoneutral conditions (Vernon, 1992). Likely, this occurs as a means of lessening the additional thermal load produced through metabolic pathways, but may just as well be an evolutionary strategy to prepare the animal for future conditions wherein feed availability or feed intake is limiting (Baumgard and Rhoads, 2013). Collectively, this mechanism prevents mobilization of body energy reserves and contributes to greater carcass adiposity of pigs raised under heat stress (Baumgard and Rhoads, 2013).

In light of the heightened adipose conservation, the body increases preferential carbohydrate usage, as evidenced by an increase in hepatic gluconeogenesis and glycogenolysis (Collins et al., 1980; Febbraio, 2001), as well as tissue glucose utilization (Wheelock et al., 2010). However, the rate of glucose disappearance exceeds the rate of hepatic production, and shortly precipitates a hypoglycemic state (Baumgard and Rhoads, 2013). Heat stressed animals emphasize lactate utilization in place of glucose. As explained by Baumgard and Rhoads (2013), circulating lactate is increased during heat stress, although the source of this lactate is as of yet unknown. The additional lactate is later converted into pyruvate, which is then utilized in the

tricarboxylic acid (TCA) cycle to produce a large volume of ATP. Pyruvate entrance into the TCA cycle is controlled by pyruvate carboxylase, a rate-limiting enzyme that is upregulated during heat stress. The ATP produced in this process is made available to sensitive tissues, such as the central nervous system, that are otherwise deprived of glucose and NEFA during heat stress.

Characterization of Seasonal Infertility

Hot ambient temperature, high relative humidity, and long-day photoperiod act in concert to decrease the reproductive efficiency of the swine breeding herd (Love, 1978). These environmental conditions are therefore responsible for a period of subfertility – commonly referred to as seasonal infertility – during the late-summer and early-autumn months (Love, 1978). Although not a true seasonal anestrus, seasonal infertility in gilts and sows may cause delayed puberty attainment, lengthened or irregular estrous cycles, increased early embryonic death loss, early disruption/abortion of pregnancy, decreased farrowing rate, delayed wean-to-estrus interval, reduced litter size and number born alive, and increased number of non-productive breeding days (Love, 1978; Love et al., 1993; Peltoniemi et al., 1999; Tast et al., 2002).

Notably, decreased farrowing rates are of greatest concern to sow farmers (Tast et al., 2002). Farrowing rate is calculated as the proportion of females bred, either through natural service or artificial insemination, that conceive and carry the litter to farrowing (Diah et al., 1992). Data presented in the popular press approximates an industry-wide farrowing rate of 85% (Ketchum and Rix, 2013), while a benchmark farrowing rate of 90% or greater is recommended to achieve maximum sow productivity (Gill, 2007). Decreased farrowing rates of sows bred in

the summer have been documented extensively (Hurtgen and Leman, 1981; Britt et al., 1983; Peltoniemi et al., 1999; Auvigne et al., 2010). Indeed, Peltoniemi et al., (1999) reported an 8.3% average reduction in the farrowing rate of sows bred in summer compared to sows bred in winter. Sows suffering early pregnancy loss typically return to estrus 25 to 35 d post-breeding, or are found to be “not-in-pig” at farrowing (Tast et al., 2002).

Seasonal infertility in the domestic pig (*Sus scrofa domesticus*) coincides with a period of reproductive quiescence in the undomesticated European wild boar (*Sus scrofa scrofa*) (Gethöffer et al., 2007). In the northern hemisphere, wild boars mate in late-autumn and early-winter; farrowing, then, occurs in spring when environmental conditions are favorable and food sources are plentiful (Mauget, 1982). Lactational anestrus prevents re-breeding during the summer (Mauget, 1982). This evidence lends credence to theories that seasonal infertility in the domestic pig is an evolutionary vestige of the ancestral wild boar, and further suggests that pigs are inherent short-day breeders (Love et al., 1993; Guiffra et al., 2000). Modern farm facilities and production practices such as environmentally-controlled housing, appropriately-timed boar contact, high-quality feed ingredients, and abbreviated duration of lactation have allowed the domestic sow to breed regardless of season and produce multiple litters in the same year (Kirkwood, 2003). Data generated from production records of 1.8 million sows indicate a current average of 2.36 litters per mated female per year in the US (Knauer and Hostetler, 2013).

Control of Seasonal Infertility

Photoperiod-dependent seasonal reproduction in mammals is controlled by the indoleamine neurotransmitter melatonin (Arendt, 1998). Retinal response to the duration of darkness is processed by the hypothalamic suprachiasmatic nucleus and induces melatonin

secretion from the pineal gland via sympathetic nervous stimulation (Ebling and Hastings, 1992). Indeed, it is the diurnal scotophase – and not photophase – that induces melatonin release.

The duration of darkness is highly correlated with the duration of melatonin secretion in swine (Tast et al., 2001a). Accordingly, seasonal variation in length of photoperiod yields seasonally appropriate variations in serum melatonin concentrations. In European wild boars, the duration of melatonin secretion is longest in winter and shortest in summer (17 and 6 h, respectively); and in the domestic strain, is longest in autumn and shortest in summer (12 and 8 h, respectively; Tast et al., 2001a), suggesting that, even though seasonal anestrus in the wild boar is lactation-dependent, photoperiodic melatonin is likely a contributing factor to seasonal infertility in the domestic species. Artificial lighting regimens have been used successfully to control melatonin secretion in pigs reared under commercial conditions, provided the pigs have no access to natural light influx (Tast et al., 2001b). Under these conditions, pigs are able to adjust melatonin secretion patterns within one week of an abrupt change in lighting (Tast et al., 2001b).

The exact mechanism by which seasonal melatonin secretion influences seasonal infertility in swine is not yet fully understood (Ikegami and Yoshimura, 2012; Smith, 2012). Results of experiments exposing ewes, a classic short-day breeder, to reproductively prohibitive long-day conditions suggest that melatonin activates thyroid stimulating hormone receptors (TSH-R) in the adenohypophyseal pars tuberalis. Local TSH, then, induces T_4 conversion to the bioactive T_3 within the hypothalamus (Hanon et al., 2008). In turn, T_3 induces a morphological change in the glial endfeet of GnRH nerve terminals, impeding terminal contact with the basal lamina and limiting GnRH secretion into the pericapillary space and the hypothalamic-hypophyseal portal plexus (Hanon et al., 2008). As a result, attenuated GnRH secretion impairs

production of the gonadotrophs LH and FSH. Seasonal variations in the morphology and estrogen-sensitivity of kisspeptin and gonadotropin inhibiting hormone (GnIH) neurons in the hypothalamus have also been proposed to explain seasonal reproduction in sheep (Smith et al., 2008).

While it is likely that the T_4 , GnIH, and kisspeptin pathways controlling seasonal reproduction are highly conserved across mammals, these physiological mechanisms may not bear the same semblance or significance in species that do not exhibit a true seasonal anestrus, such as the domestic pig (Shinomiya et al., 2014). Indeed, seasonal infertility in the sow is driven by photoperiod, but accelerated by hyperthermia. Results of a five-year field study in northern France show consistently lower pregnancy rates in sows bred in the summer (83.5%) than in the winter (86.4%); however, the year with the highest number of hot days (defined as days when maximum daily temperature exceeded 25°C) also had the lowest sow pregnancy rates (4.5% lower compared to other years; Auvigne et al., 2010). Seasonal infertility, then, should be considered the combined result of long-day photoperiod and hyperthermia.

Reduced GnRH stimulation of the anterior pituitary gland decreases gonadotropin secretion and, thereby, gonadal function, and is believed to be the root cause of heat-induced seasonal infertility (Bertoldo et al., 2012). Specifically, seasonal infertility is the result of diminished oocyte developmental competence, inadequate LH pulsatility, decreased progesterone production, and a failure to illicit the second embryo-derived maternal recognition of pregnancy signal (Bertoldo et al., 2012).

High oocyte developmental competence, generally defined as the ability of a fertilized zygote to achieve blastocyst state, is associated with increased embryo survival (Krisher, 2004). Competence is generally dependent on the oocyte-derived and maternally-provided transcripts

and proteins that assist embryonic development until the time when embryos are able to achieve a self-sustaining state (Verghese et al., 2011). Oocyte developmental competence has been positively linked with follicle size (Lonergan et al., 1994). During heat stress, however, dominant follicle size is decreased in dairy cattle, suggesting that oocyte developmental competence, and thus, embryo survivability, is also reduced (Bandinga et al., 1993; Shehab-El-Deen et al., 2011).

In addition to follicle size, oocyte developmental competence is further influenced by concentrations of intra-follicular steroid hormones. In sows, follicular concentrations of progesterone, androstenedione, and estradiol are greater in large compared to small follicles (Bagg et al., 2007; Bertoldo et al., 2011). Sow ovaries collected during the summer contain lower progesterone concentrations in both large and small follicles compared to winter controls (Bertoldo et al., 2011). When fertilized *in vitro*, oocytes from summer-collected sow ovaries exhibit an impaired ability to develop to the blastocyst stage (Suzuki et al., 2010). Further, even when summer oocytes do go on to form blastocysts following *in vitro* fertilization, they have reduced cell counts in the inner cell mass (Bertoldo et al., 2013). Reduced blastocyst formation of summer collected oocytes has also been observed in other species, including dairy cattle (Al-Katanani et al., 2002). Blastocyst formation rate is important because it is a measurement of oocyte developmental competence (Fair, 2003).

The mechanisms responsible for variations in seasonal follicular progesterone synthesis and feedback are not yet fully understood (Hansen, 2015). Logically, reductions in gonadotropin secretion in response to reduced GnRH will decrease steroid activity in the gonads. Cultures of bovine dominant follicle follicular walls in heat stressed conditions indicate a reduction of estradiol-17 β and androstenedione (Bridges et al., 2005). However, in sow ovaries harvested

post-weaning in summer, the concentration of follicular progesterone is diminished while estradiol-17 β and androstenedione were not impacted (Bertoldo et al., 2011).

Decreased GnRH secretion during summer has also been shown to diminish LH surge amplitude and tonic LH pulsatility in early sow pregnancy (Peltoniemi et al., 1997). A low amplitude LH surge is thought to be responsible for decreased ovulation rate of antral follicles, and correspondingly, a lower number of functioning CL on ovaries collected during summer months (Bertoldo et al., 2011). Additionally, the swine CL exists independently of luteotrophic or antiluteolytic support during early pregnancy (Anderson et al., 1967), but becomes LH-dependent on d 12 post-conception (Peltoniemi et al., 1995). Also occurring at this time is the first maternal recognition of pregnancy signal: embryonic estrogen reroutes normally luteolytic endometrial PGF_{2 α} into the uterine lumen, thereby preserving the CL. A second estrogenic signal is required on d 18 (Pusateri et al., 1996). Bertoldo et al. (2012) theorize that a lower amplitude LH surge would decrease ovulation rate and CL formation, while depressed tonic LH support would decrease CL viability, leading to an overall reduction in the progesterone available to support embryos. This, coupled with reduced oocyte developmental competence, may yield poor quality embryos unable to produce the second maternal recognition of pregnancy signal (Bertoldo et al., 2012). This mechanism is not yet confirmed, but would explain reports of low systemic progesterone in sows that lose pregnancy between the first and second maternal recognition of pregnancy signal and suffer a delay (25 to 35 d) in return to estrus (Tast et al., 2002).

Providing exogenous estrogen injections to maiden gilts at the time of the first, but not second recognition signal extends pseudopregnancy to approximately 30 d (Geisert et al., 1987). The delay in return to estrus in these cases is similar to the irregular returns observed during

times of seasonal infertility, and supports theories that pregnancy is lost between the first and second maternal recognition of pregnancy signal.

In spite of ongoing research, the physiology of seasonal infertility is not completely understood, and has been only minimally investigated in the sow. Several variables may exacerbate the basal effects of photoperiod-based seasonal infertility, and include factors such as management, facility design, presence and type of cooling system, bedding provisions, diets, and most importantly the severity of heat stress (Kirkwood, 2003).

Effects of Heat Stress on Embryonic Development

Peri-conceptual and post-conceptual heat stress insults induce embryonic mortality (Tast et al., 2002). Tompkins et al. (1967) noted a significant decrease in embryo survival when sows were exposed to heat stress for 120 h post-breeding. In a different study, a heat stress commenced on d 2 post-conception increased embryo mortality 28% in gilts (Widlt et al., 1975). In dairy cattle, Ealy et al (1993) found that heat stress decreases embryonic development and survival when applied on d 1, but not on d 3, 5, or 7 post-insemination. Fertility is particularly impacted when heat stress occurs the day after conception. Heat stress on the day of or the day before insemination will decrease conception rate, as well, although to a lesser extent (Gwazdauskas et al., 1973). Embryos become increasingly heat-resistant with age (Sakatani et al., 2012). Indeed, *in vitro* culture of bovine zygotes at 40°C significantly reduced blastocyst attainment, but did not affect the blastocyst attainment when morulas were exposed to the same temperature (Sakatani et al., 2012).

According to Hansen (2015), embryonic mortality due to heat stress is a function of “decreased competence of cleaved embryos to develop rather than competence of the oocyte to

cleave.” In general, embryos are most sensitive when subjected to heat stress at the one- and two-cell stages, although cleavage continues normally until embryonic mortality occurs at the eight-cell stage (Hansen, 2015). Rivera and Hansen (2001) utilized *in vitro* fertilization techniques to determine the developmental sensitivity of bovine embryos cultured at a physiologically relevant heat stress (41°C). This temperature represents the anticipated temperature of the *in vivo* bovine oviduct and uterus during a heat stress challenge. Rate of blastocyst attainment was greatly decreased when heat stress was applied at fertilization and during the one-cell and two-cell stages for 9 or 12 h. No effects were observed when heat stress was applied for only 3 or 6 h. Interestingly, the authors note that fertilization at 40°C tended to increase the proportion of oocytes that formed blastocysts compared with those oocytes fertilized at 38.5°C (Rivera et al., 2001).

Under the same physiologically relevant culture conditions previously described, Rivera et al. (2004a) found that only $25 \pm 7\%$ of heat stressed embryos developed past the eight-cell stage, compared to $67 \pm 7\%$ of normothermic embryos. Further, it was shown that the two- and four-cell stages were not affected, as indicated by the high proportion of these embryos continuing development under heat stress ($98 \pm 15\%$ and $96 \pm 21\%$, respectively; Rivera et al., 2004a).

Embryonic mortality under heat stress is largely the result of structural rather than biochemical mechanisms. Rivera et al. (2003) noted organelle migration away from the periphery and toward the center of the embryo. Additionally, changes in membrane permeability lead to mitochondrial swelling and a decrease in the number of functioning mitochondria (Rivera et al., 2004a). However, even though the number of functioning mitochondria is decreased, there is no change in the oxygen consumption or ATP content of the embryo, and suggests that free

oxygen radicals are not the direct cause of embryonic death in bovine embryos (Rivera et al., 2004a). These changes in embryo structure are caused by reorganization of the microtubule and microfilament cytoskeleton (Rivera et al., 2004b).

These cytoskeletal malformations at the eight-cell stage occur at the moment of bovine embryonic genome activation (Memili and First, 2000) and may suggest a connection between heat stress mortality and failed genome activation (Rivera et al., 2004). In swine, activation of the embryonic genome occurs at the 4-cell stage (Tománek et al., 1989). It is unknown, then, if heat stress induces the same developmental disorders in porcine embryos as observed in bovines.

Effects of Heat Stress on Fetal Development

Chronic heat stress during pregnancy affects the physiology of both dam and fetus. Notably, placental growth is severely retarded in dams exposed to chronic gestational heat stress (Bell et al., 1987). Placental insufficiency occurs as consequence of decreased uterine and umbilical blood flow (Bell et al., 1987). In chronically heat stressed cows, uterine and umbilical blood flow is decreased 66% and 82%, respectively, compared to thermoneutral controls (Reynolds et al., 1985). Fetal growth, then, is reduced as a consequence of poor placental development and function (Reynolds et al., 1985).

Reduced blood flow to the pregnant uterus during chronic heat stress is dictated by blood metabolite and endocrine activity. Panting in response to prolonged heat stress induces respiratory alkalosis and arterial hypocapnia, and this has been observed in sheep and other species (Bell et al., 1987). Low blood pCO₂, then, induces vasoconstriction of the maternal micro-vasculature, restricting blood flow to the uterus (Querido and Sheel, 2007). To confirm this, Oakes et al. (1976) provided heat stressed ewes with rebreather masks that maintained

normal blood pCO₂ levels. Uterine blood flow in untreated ewes (i.e., ewes experiencing respiratory alkalosis) decreased 53 ± 3 % compared to the maximal flow rate recorded at thermoneutral conditions, while uterine blood flow in treated ewes decreased only 30 ± 6 % (Oakes et al., 1976). It can be inferred, then, that respiratory alkalosis as a result of increased respiration rate is responsible for approximately 23% of the decrease in uterine blood flow under these conditions.

The neurohypophyseal hormones vasopressin and oxytocin are released during hyperthermia and have also been indicated as possible mediators of uterine blood flow during heat stress (Dreiling et al., 1991). In particular, vasopressin reduces uterine blood flow by increasing myometrial activity and the basal tension of uterine blood vessels (Åkerlund and Andersson, 1976). Dreiling et al. (1991) noted that blood concentrations of vasopressin and oxytocin increased 60% and 100%, respectively, in pregnant ewes exposed to an acute heat stress. During this time, uterine blood flow also decreased 70% (Dreiling et al., 1991).

The fetus normally maintains a higher basal temperature than does the dam. During periods of acute heat stress, fetal temperature is generally well preserved (Faurie et al., 2001). However, the temperature gradient between fetus and mother collapses during periods of sustained heat stress (Bell et al., 1987). It is estimated that 84.5% of fetal heat transfer occurs from fetus to maternal blood during normothermic conditions, while the remainder is diffused through the uterine wall (Gilbert et al., 1985). Reduced maternal blood flow during periods of chronic hyperthermia may impair the ability of the fetus to dissipate the high thermal load.

Chronic heat stress retards fetal growth as a consequence of placental insufficiency, although the reduction in fetal size is not as severe as the reduction in placental size (Reynolds et al., 1985). This is problematic because placental utilization of oxygen and glucose is inhibited in

ovine models of chronic gestational heat stress, and the degree of placental oxygen and glucose utilization is highly correlated with placental and fetal weight (Bell et al., 1987). Thureen et al. (1992) noted that fetal arterial oxygen saturation is reduced in heat stressed compared to thermoneutral fetuses ($37.3 \pm 6.1\%$ vs $56.8 \pm 3.8\%$, respectively). Additionally, when maternal plasma glucose is kept constant, arterial glucose concentration of heat stressed fetuses is significantly less than thermoneutral counterparts (13.3 ± 1.2 mg/dL vs 21.1 ± 0.8 mg/dL, respectively), indicating a large trans-placental glucose difference between maternal and fetal circulation and an inability of the placenta to facilitate glucose transport to the fetus (Thureen et al., 1992). The result of chronic gestational heat stress, then, is a hypoxic and hypoglycemic fetus (Bell et al., 1987). As a result, the duration of heat stress correlates highly with the severity of fetal growth restriction (Galan et al., 1999).

Gestational heat stress results in disproportional fetal growth retardation (Alexander and Williams, 1971; Brown et al., 1977). Developmentally heat stressed lambs exhibit comparatively longer bodies and larger skulls, brains, kidneys, and pituitary and adrenal glands, but smaller livers, thyroids, and bicep muscles (Alexander and Williams, 1971). Brown et al. (1977) noted that disproportional ovine fetal dwarfing occurs only when dams are heat stressed during the last trimester.

Specifically, the liver is the organ most severely affected by heat stress teratogeny (Alexander and Williams, 1971; Reynolds et al., 1985; Bell et al., 1989; Dreiling et al., 1991). When adjusted for day of gestational age, Reynolds et al. (1985) found that the livers from heat stressed bovine fetuses weighed only 61% of the livers from thermoneutral fetuses (173 g vs. 284 g, respectively). Reduced liver weight in pigs as a result of gestational heat stress has also been documented (Johnson et al., 2015a). Although hepatic glycogen content isn't affected (Alexander

and Williams, 1971), livers from heat stressed fetuses have reduced total RNA and protein content, possibly indicating a reduced capacity for protein synthesis throughout life (Reynolds et al., 1985).

Effects of Gestational Heat Stress on Offspring Performance

Exposure to elevated temperatures *in utero* creates lasting impressions on animal physiology that persist into adulthood. These modifications occur largely as a carry-over effect of heat on maternal physiology and fetal development (Tao and Dahl, 2013). However, the effects of thermal stress on the epigenome of plants and animals are beginning to be understood (Molinier et al., 2006; Seong et al., 2011). These physiological and epigenetic alterations logically exist to instill stress resistance later in life or in subsequent generations (Molinier et al., 2006).

Epigenetics refers to any non-genomic (i.e., environmental, nutritional, biochemical, etc.) modifications to the genotype that produces a phenotype that would not otherwise be expected based on simple Mendelian inheritance (Goldberg et al., 2007). The most widely studied mechanisms of epigenetic action are DNA methylation of cytosine/guanine (CpG) dinucleotide pairs and histone modification. Methylation of CpG pairs induces transcriptional silencing, and effects on areas of the genome with large CpG clusters (known as CpG islands) produce pronounced phenotypic effects (Goldberg et al., 2007). Further, modifications to the histone, which is the chief protein associated with DNA packaging during gene regulation and replication, may induce alterations in the physical properties of chromatin. This modifies histone-DNA interactions and further regulates transcription activity (Goldberg et al., 2007).

Epigenetic modifications prompting altered phenotypes following thermal stress have been elucidated in lower organisms. In *Drosophila*, heat shock during early embryogenesis results in transcription factor phosphorylation and disrupted heterochromatin formation of stress-related genes (Seong et al., 2011). Generational experiments with these flies showed that when heat stress was applied to the F1 generation, similar gene silencing was observed in the F2, but not the F3 through F5 generations. However, when heat stress was applied to both the F1 and F2 generations, partial silencing was observed in the F3 through F5 generations (Seong et al., 2011), indicating that the degree of epigenetic change of some genes may be related to the intensity or duration of stress exposure. Similar epigenetic actions with trans-generational implications following thermal stress have been shown in rodents (Tetievsky and Horowitz, 2010), fish (Takle et al., 2005; Meier et al., 2014), and plants (Molinier et al., 2006). Although it has yet to be proven, it is widely assumed that the response of higher mammals to developmental heat stress is at least partially the result of epigenetic modifications, as well (Johnson, 2014).

Avian offspring exposed to developmental heat stress gain additional thermotolerance later in life. Broiler chickens exposed to increased incubation temperatures showed lower body temperatures and decreased mortality when exposed to a secondary heat stress at marketing age (Piestun et al., 2008). These birds also showed decreased T_3 , T_4 , and cortisol responses to thermal stress compared to controls (Piestun et al., 2008), which would suggest effects on lifetime metabolism.

Conversely, pigs exposed to gestational heat stress have shown an increased rectal temperature compared to gestational thermoneutral pigs when subjected to a secondary thermal stress in adolescence (Johnson et al., 2013). No differences in respiration rate, feed intake, or daily growth were observed in these pigs, although large numerical differences in feed intake

(2.24 kg/d gTN vs 2.07 kg/d gHS), bodyweight gain (0.68 kg/d gTN vs 0.42 kg/d gHS) and gain to feed ratio (0.32 kg/kg gTN vs 0.15 kg/kg gHS), combined with small sample sizes (n = 6 pigs per treatment; Johnson et al., 2013) may indicate that biological effects do in fact exist. In this regard, gestational heat stress may compromise the subsequent thermotolerance and growth performance of affected swine.

Indeed, gestationally heat stressed pigs have been shown to exhibit decreased feed efficiency (evidenced as greater feed intake without a concomitant increase in average daily gain) when compared to gestational thermoneutral controls in the finishing, but not growing phases (Johnson et al., 2015a; 2015b). Carcass analysis at slaughter indicated that, while growth did not differ between pigs from different gestational environments, there was a pronounced difference in body composition. Pigs from heat stressed dams showed a 16% decrease in protein accretion, increased lipid accretion (292 g/d vs 220 g/d) and a 95% increase in the ratio of lipid to protein accretion (Johnson et al., 2015b). Additional studies have noted a numerical but not statistically significant decrease in weight at finishing in barrows exposed to gestational heat stress (Wilmoth et al., 2014).

The increased whole-body adiposity of gestationally heat stressed pigs is likely due to greater circulating insulin. Pigs exposed to gestational heat stress show 33% greater insulin concentrations compared to thermoneutral controls (Boddicker et al., 2014). Similarly, Holstein calves born to heat stressed dams had higher insulin concentrations after feeding than did calves born to thermoneutral cows (Tao and Dahl, 2013). This may be the result of either enhanced pancreatic insulin secretion or reduced insulin clearance (Tao and Dahl, 2013).

Citing evidence that poor adolescent growth coupled with increased pre-pubertal fat accumulation negatively correlates with mammary parenchymal DNA and milk production in

Holstein heifers, Tao and Dahl (2013) suggested that maternal heat stress may negatively affect offspring lactational performance. This idea, while certainly logical, is at this time speculative and has not yet been shown experimentally.

The teratogenic effects of heat stress align with principles presented as the Barker Hypothesis. The Barker Hypothesis states that intrauterine growth retardation, lesser than normal birth weights, and poor growth during adolescence is correlated with a greater risk of weight gain, insulin resistance, and cardiovascular disease in adulthood (De Boo and Harding, 2006). For example, in the original findings Barker et al. (1989) showed that the death rate from ischemic heart disease was 3-fold greater for humans who weighed 8.18 kg or less at 1 year of age compared to humans who 12.27 kg or greater. Results of previous research in gestational heat stress show similar lifetime effects of intrauterine growth restricted development, and support the Barker Hypothesis.

Conclusion

The effects of heat stress on swine growth and performance are profound, but only recently have the effects of heat stress on the pregnant pig been sufficiently shown to impact the growth and development of the exposed fetuses into adulthood. Notably, pigs exposed to gestational heat stress gain adipose tissue at a greater rate than lean protein, and tend to experience a reduction in feed efficiency and average daily growth, as well. This in turn leads to greater whole-body adiposity in the growing pig and a carcass with more fat and less lean content at slaughter. No studies at this time exist to explain the possible effects of hyperthermic exposure *in utero* on aspects of reproduction and lactation in swine.

Part II: Swine Lactation

Introduction

In order to improve productivity, sows have been selected for greater prolificacy, resulting in substantial increases in litter size (Knauer and Hostetler, 2013). Similar selection pressure, however, is generally not placed on sow colostrum production or milking ability (Serenius et al., 2004). As a result, the hyperprolific sow lines used in modern swine production are unable to nurse large litters to their maximum growth potential (Miller et al., 2012). To this end, sow lactation has become a limiting production factor of the breeding swine farm (Quesnel et al., 2015).

Mammogenesis

Unlike the bovine udder, which is divided into quarters, the sow's mammary system is composed of a variable number of mammary glands extending in two parallel rows longitudinally from the thoracic to inguinal regions. Glands are named according to their position on the ventral body: thoracic glands (first and second pairs), abdominal glands (third, fourth, and fifth pairs), and inguinal glands (sixth, seventh, and greater, if existing). Each gland contains a complete lobulo-alveolar framework that is anatomically independent from the neighboring glands. Each gland terminates in a teat with two separate teat canals (Farmer and Hurley, 2015).

The sow's mammary system is supported by a complicated vasculature. The thoracic glands are supplied by the lateral cranial, middle cranial, and medial cranial divisions of the external pudendal (pudic) artery. The inguinal glands are supplied oxygen-rich blood by the cranial epigastric artery, which is an extension of the internal thoracic artery. Abdominal glands

receive blood from both networks. Venous return utilizes the subcutaneous abdominal vein. Anterior glands are drained in a cranial direction by two parallel subcutaneous abdominal veins, which ultimately drain into the internal thoracic vein. Posterior glands are drained caudally by the same subcutaneous abdominal vein, which then drains into the external pudic vein. Unique to pigs among other litter-bearing species with similar udder structure, such as rodents and canines, the paired glands located on either side of the udder are connected by a venous anastomosis (Farmer et al., 2015).

Differentiated mammary tissue is apparent in early embryonic life, although little development actually occurs prenatally. At birth, the mammary system consists almost entirely of non-functional connective stroma (Hughes and Varley, 1980). Mammary development is isometric from birth (0.3 mg mammary DNA) to three months of age (7 mg mammary DNA), but after this time undergoes a four-fold increase in mammary DNA and six-fold increase in mammary tissue mass (Sørensen et al., 2002). Attainment of puberty induces a 51% increase in parenchymal mammary tissue and a 16% decrease in extra-parenchymal tissue compared to non-pubertal gilts of the same age (Farmer et al., 2004).

During pregnancy, mammary development continues to progress at a slow pace during the first two-thirds of gestation, but increases substantially during the final trimester. Between d 75 and 112 of pregnancy, Sørensen et al. (2012) reported massive accumulation of mammary tissue (80 g at d 75 and 373 g at d 112) and mammary DNA (40 mg at d 75 and 838 mg at d 112). Similarly, Ji et al. (2006) reported a three-fold increase in mammary growth during the third trimester. The authors also noted differentiation in growth rates, with the abdominal glands (defined in the study as the third, fourth and fifth pairs of glands) growing fastest, inguinal

glands (the sixth, seventh, and eighth pairs) the slowest, and thoracic gland (the first and second pairs) growing at an intermediate rate (Ji et al., 2006).

Also occurring during the last trimester is a shift in adipose and protein content within the mammary parenchyma. Between 75 and 112 d of gestation, the percent crude protein of the mammary increases from 11.4% to 38.3%, while the percent adipose decreases from 87.6% to 58.8% (Ji et al., 2006). In general, abdominal glands exhibit the greatest protein and least fat content during this time, although relative differences in gland composition are not statistically significant at d 112 (Ji et al., 2006). Percent crude ash increases notably during the final trimester as well (d 45: 0.50%; d 75: 0.73%; and d 112: 1.88%; Ji et al., 2006).

Protein accumulation in the late-gestation mammary gland is indicative of functional tissue development. Histological data reported by Ji et al. (2006) indicates some ductal branching visible at d 45 of gestation with terminal ductal lobular unit formation apparent by d 60. The terminal ductal lobular unit is the functional unit of the mammary gland and incorporates an alveolus lobule and both extralobular and intralobular terminal ducts (Hovey et al., 1999). Lobular formation is observed at d 75 of gestation, and lobules continue to grow in size until term (Ji et al., 2006). Alveoli may be viewed histologically at any point in gestation, although alveolar distension is not visible until d 102 of gestation. Distension of alveoli then increases progressively towards term (Ji et al., 2006).

Endocrine Control of Mammogenesis

Mammary growth and development before and after puberty, and during gestation and lactation is largely under endocrine control. It is postulated that pre-pubertal mammary development occurs secondary to the appearance of steroidogenic antral follicles and the

corresponding appearance of gonadal estrogen (Camous et al., 1985; Sørensen et al., 2002; Farmer and Hurley, 2015). Experiments utilizing estrogen receptor knockout mice confirm that in the absence of estrogen signaling mammary development does not occur (Bocchinfuso et al., 2000). Cessation of pre-pubertal mammary development following ovariectomy has also been observed in Holstein heifers (Purup et al., 1993).

Ovarian-produced relaxin also contributes to peri-pubertal mammary development although exogenous administration of relaxin alone in gilts has little effect on mammary development. However, Winn and others (1994) showed that when relaxin is provided in combination with estrogen, as would be physiologically appropriate given the gonadal origins of the hormones, the effects on mammary development are greater than when either relaxin or estrogen is given independently. This indicates that gonadal relaxin and estrogen act in a synergistic manner on mammary development.

Perhaps more so than estrogen and relaxin, the adenohipophyseal hormone prolactin may provide the greatest stimulation to peri-pubertal mammogenesis (Farmer and Hurley, 2015). Using prolactin receptor gene knockout mice, Brisken et al. (1999) showed that mammogenesis is normal until puberty, at which time these animals exhibit decreased ductal branching and failed alveolar development. In pre-pubertal growing pigs, Farmer and Palin (2005) induced a 116% increase in parenchymal tissue mass and a 161% increase in parenchymal DNA by providing daily injections of 4 mg recombinant porcine prolactin for 29 d.

A major increase in circulating estrogen occurs between d 60 and 75 of pregnancy in the gilt, and continues to rise until parturition (DeHoff et al., 1986). This rise in estrogen occurs at the time of lobulo-alveolar development in the mammary gland (Ji et al., 2006). Knight et al. (1973) identified the increased estrogen in late-gestation as being of fetal origin. One study

comparing mammary development in pregnant and pseudo-pregnant gilts noted that pseudo-pregnant gilts exhibit only 22% of the mammary DNA and 23 to 24% of the mammary RNA found in pregnant gilts at d 100 of gestation (Kensinger et al., 1986). The remainder of the mammary DNA is presumed to be derived as a result of fetal influence. Interestingly, no correlation has been found between conceptus number (4 to 7 piglets vs 8 to 11 piglets) and amount of mammary DNA or RNA (Kensinger et al., 1986).

Although the conceptus does provide the major mammogenic stimulus during pregnancy, the role of the gonads cannot be discounted. In ovariectomized gilts, lobulo-alveolar development is absent at d 60, and is markedly reduced at d 100 (Buttle, 1987). Supplementation of exogenous relaxin to ovariectomized gilts does restore mammogenesis, albeit with no observable benefits to lactational performance (Zaleski et al., 1996).

Similar to its role in the peri-pubertal female, pituitary prolactin also contributes to mammogenesis in late-gestation. Daily feeding of the prolactin inhibitor bromocriptine from d 70 to d 110 of gestation did not affect total weight of the udder, but did decrease mass of parenchymal tissue (581 g vs. 1,011 g), mammary dry matter (39.9% vs. 45.6%), percent protein (25.4% vs. 33.5%), total DNA (1.7 g vs. 3.2 g) and total RNA (1.5 g vs. 3.1 g; Farmer et al., 2000). Interestingly, the fetuses of bromocriptine-fed gilts were smaller when harvested on d 110 of gestation (Farmer et al., 2000).

In contrast to most primates and ruminants, the porcine placenta does not produce placental lactogen (Forsyth, 1984). Placental lactogen is a somatotropic hormone with homology similar to growth hormone and prolactin (Forsyth, 1984). In ruminants, the concentration of placental lactogen in late-gestation is highly correlated with mammary development and post-

partum milk production (Forsyth, 1984). No homologous placenta-derived hormone has been discovered in swine.

Lactogenesis and Lactation

Colostrum is secreted immediately before and up to 24 h following the commencement of parturition. Compared to milk, sow colostrum is lower in lactose and fat, but higher in protein and immunoglobulins (Quesnel et al., 2015). Immunoglobulins are antibodies secreted by cells of the adaptive immune system to halt the spread of foreign pathogens. Maternal immunoglobulins are passed from dam to offspring through colostrum to provide the immunodeficient neonate with basic immune function and disease protection (Butler, 1983). The most common subtypes of immunoglobulins present in colostrum are immunoglobulin A (IgA), IgG, and IgM.

Despite the biological necessity of colostrum for good piglet growth and development, few studies to date have focused on the factors affecting colostrogenesis. In general, younger sows (≤ 3 parities) produce more colostrum than older sows (≥ 4 parities; Quesnel et al., 2015), and it has been suggested that parity two and three sows produce more colostrum than primiparous gilts (Devillers et al., 2007). No association has been found linking colostrum production and litter size or suckling intensity (Devillers et al., 2007). However, a positive correlation with piglet birth weight has been demonstrated, and may indicate that colostrogenesis is in some way positively influenced by pre-natal litter weight (Devillers et al., 2007).

Removal of the progesterone block prior to parturition induces prolactin synthesis in the sow (Taverne et al., 1982). The resulting inverse concentrations of prolactin and progesterone are correlated with colostrum production: sows with lesser progesterone and greater prolactin

concentrations produce a greater volume of colostrum than do sows with relatively greater progesterone and lesser prolactin (Quesnel et al., 2013). Additionally, the piglets of sows with greater pre-farrowing prolactin profiles are more likely to survive and exhibit better pre-weaning growth compared to the piglets of sows with lesser pre-farrowing prolactin (Quesnel et al., 2013).

Colostrum quality is transient, and the colostrum secreted at parturition and 24 h after farrowing differ greatly in composition. For example, Theil et al. (2014) reported significant differences in the 0 h and 24 h concentrations of lipids (5.1% vs. 6.9%), protein (17.7% vs. 8.6%), lactose (3.5% vs. 4.4%), dry matter (27.3% vs. 20.6%), and energy (260 kJ/100 g vs. 346 kJ/100 g).

Similarly, the concentrations of colostral immunoglobulins begin to change nearly immediately after parturition. The humoral antibodies IgG, IgM, and IgA, are greatest at the onset of parturition (95.6 mg/mL, 9.1 mg/mL, and 21.2 mg/mL, respectively). After six h, however, concentrations of each immunoglobulin have decreased by roughly one-third, and at 12 h post-farrowing have decreased by over one-half (32.1 mg/mL, 4.2 mg/mL, and 10.1 mg/mL, respectively). In mature milk, defined as the milk produced after d 10 of lactation, the concentrations of IgG, IgM, and IgA are substantially less than their colostral values (1.5 mg/mL, 1.8 mg/mL, and 4.8 mg/mL, respectively; Klobasa et al., 1987).

The nutrient composition of transient milk (d 2-10 of lactation) changes slightly in early lactation. The nutrient composition of mature milk (d 10+), though, is stable (Quesnel et al., 2015). The nutrient profile of lipids, protein, and lactose in mature milk is: 8.2%, 4.7%, and 5.1%, respectively (Theil et al., 2014).

The factors dictating mature milk production are quite variable. A genetic basis for milking ability does exist, as demonstrated by breed hierarchies in milk production, with Chinese breeds (Meishan) producing the greatest amount of milk, followed by maternal breeds (Landrace, Yorkshire, Large White), and finally terminal breeds (Duroc, Hampshire, Berkshire) (Quesnel et al., 2015). The heritability of milking ability in terminal breed swine is moderate ($h^2 = 0.27$) (York and Robison, 1985).

Toner et al. (1996) illustrated that milk production increases linearly with increasing litter size. The time and intensity of teat massage also increases milk production in early lactation (Algers and Jensen, 1991). As lactation progresses, the piglets become heavier and elicit a greater suckling pressure on the dam. Using cross-fostering strategies, King et al. (1997) observed a 26% increase in milk production when two-week old piglets were fostered onto dams that had farrowed only 2 d prior, and a 22% decrease in milk production when 2 d old piglets were placed on sows in their third week of lactation. In the absence of piglet suckling, milk synthesis is stopped and alveolar cells begin to degenerate (Quesnel and Prunier, 1995).

These results suggest that sow lactation is reciprocal to piglet demand. It has been observed that piglet milk consumption and growth decreases linearly with increasing litter size due to greater teat competition (Auldism et al., 1998). Late lactation milk yield of sows nursing 6, 10, and 14 piglets was 9.80 kg/d, 13.05 kg/d, and 15.52 kg/d, respectively; however, the growth rate of piglets in these same litters over the same time period was 309 g/d, 245 g/d, and 199 g/d, respectively, due to less milk available per piglet (Auldism et al., 1998).

Additional factors contribute to total milk production, albeit to a lesser degree than those previously mentioned. In general, multiparous sows produce a greater milk volume than primiparous gilts (Speer and Cox, 1984). Stress during gestation (Janczak et al., 2003) and

lactation (Algers and Jensen, 1991) decreases maternal responsiveness after farrowing and piglet milk consumption. Further, teat suckling throughout one lactation increases the milk producing abilities of that specific teat in subsequent lactations, as well as the growth of the piglet suckling that gland in future parities (Fraser and Thompson, 1986; Dyck et al., 1987).

According to information reviewed by Quesnel et al. (2015), many studies find no association between milk production and lactation diet composition or lactation feed intake. In their words, this may be because: “(1) improper feeding is compensated by body mobilization; (2) milk yield is difficult to quantify; (3) the genetic potential for producing milk varies between individuals; and (4) many different factors affect milk yield” (Quesnel et al., 2015).

The ability of the lactating sow to preserve milk production by mobilizing her own body reserves was well illustrated by King and Dunkin (1986), who limited the amount of daily feed offered to lactating sows (1.5 kg, 2.2 kg, 2.9 kg, 3.6 kg, 4.3 kg, or 5.0 kg). Piglet growth was numerically but not significantly different between the greatest and least feeding levels over the course of the 21 d lactation (180.9 g/d vs 192.9 g/d, respectively). However, sows allotted the lowest amount of feed per day experienced severe weight loss over the course of lactation compared to sows provided the greatest amount of feed (44.5 kg vs 9.0 kg body weight loss, respectively). These sows also experienced much greater losses in backfat thickness (8.9 mm vs 4.0 mm backfat loss, respectively). Although future ovulation rate did not differ between treatments, feed restricted sows had an extended wean-to-estrus interval, indicating a greater time requirement to recover from the consequences of poor feed intake during lactation. Additional studies have confirmed that the wean-to-service interval and total litter size in subsequent farrowings are negatively impacted by maternal weight loss greater than 10% during lactation (Thaker and Bilkei, 2005).

Effects of Heat Stress on Milk Production and Composition

Heat stress reduces voluntary feed intake (20 to 40%) in sows, and this has been associated with concomitant reductions in milk production and piglet growth (10 to 20%; Black et al., 1993; Spencer et al., 2003; Williams et al., 2013). However, reduced feed intake during heat stress is insufficient to explain the observed production losses. Messias de Bragança et al. (1998) showed that when feed intake was controlled, the average litter daily gain of heat stressed sows was less than the litters of sows housed under thermoneutral conditions ($1,618 \pm 88$ g/d heat stressed; $1,965 \pm 85$ g/d thermoneutral). Similarly, Rhoads et al. (2009) reported a 40% decrease in milk production of heat-stressed Holstein cattle, but found that only 35% of this loss could be attributed to reduced dry matter intake. These results suggest a direct effect of heat stress on mammalian lactational physiology. Indeed, daily growth of piglets nursing heat stressed sows is more closely associated with sow weight loss and backfat mobilization than with sow feed intake (Prunier et al., 1997).

Sows heat stressed during gestation and lactation limit energy reserve mobilization (Messias de Bragança et al., 1998; Williams et al., 2013), likely in an attempt to lessen the heat produced as a result of the necessary biochemical reactions (Prunier et al., 1997). Cortisol and the thyroid hormones, T_3 and T_4 , promote catabolism of body energy reserves and secretion is suppressed during chronic heat stress (Christison and Johnson, 1972; Messias de Bragança et al., 1998). The reduction of these hormones, then, maintains sow energy reserves (i.e., backfat) and impedes milk production. Additionally, T_3 and T_4 likely have direct effects on the mammary gland in terms of nutrient partitioning and milk synthesis, and suppression of secretion may further contribute to poor lactational performance (Neville et al., 2002).

In dairy cattle, heat stress induces a decrease in circulating somatotropin (ST; Rhoads et al., 2009). Pro-lactogenic ST stimulates milk production by diverting nutrients from extra-mammary tissues toward the mammary gland, as well as stimulating the release of hepatic insulin-like growth factor-I (IGF-I; Baumgard and Rhoads, 2013). Similar to what occurs during negative energy balance, the ST-IGF-I axis is uncoupled during heat stress and concentrations of the pro-lactogenic and galactopoietic IGF-I hormone are reduced. Rhoads et al. (2010) discovered that ST-IGF-I uncoupling occurs independently of feed intake via reduced hepatic ST receptor abundance. The physiological purpose for reduced IGF-I during heat stress is unclear, although one hypothesis is to divert glucose usage from milk synthesis towards more critical glucose-dependent body systems, such as the central nervous system (Baumgard and Rhoads, 2013).

It is noteworthy that prolactin increases during chronic heat stress in multiple species (Huerley et al., 1980; Sanz Fernandez et al., 2012). It may be that the pro-lactogenic effects of prolactin exist as a mechanism to mediate the anti-lactogenic effects of heat stress (Neville et al., 2002), however, the increase in circulating prolactin accompanies a decrease in prolactin receptor abundance in the mammary gland (Chilton and Heweston, 2005), which would negate physiological significance for milk production. As such, alternative roles of prolactin must then be considered, including promotion of feed intake (Gerardo-Gettens et al., 1989), fluid and electrolyte balance (Horrobin, 1980), mediation of mammary cell apoptosis (Flint et al., 2001), and control of milk lipid:lactose ratios (Flint and Gardner, 1994). Conversely, increased prolactin concentrations may be relatively inconsequential, and occur simply as a consequence of failed negative feedback resulting from the reduced mammary receptor numbers. More research into the role of prolactin during chronic heat stress is required.

In addition to endocrine responses, heat stress inflicts direct effects on mammary development. Tao et al. (2011) found that dry cows provided shade and misters for heat abatement during the late-gestation dry period (i.e. the period of greatest mammary growth) had greater mammary epithelial cell proliferation than did heat stressed cows. Epithelial cell number is associated with milk production, and the increased milk production of cooled cows over heat stressed cows was reflected in their study (Tao et al., 2011). Although no differences in mammary cell apoptosis were observed in this experiment, results of *in vitro* studies have determined that exposure to acute heat stress (40°C for 1 h) does instigate epithelial cell death (Du et al., 2008). It is questionable, however, if the experimental conditions imposed in the *in vitro* culture (1 h at 40°C) are representative of on-farm conditions.

During heat stress, blood flow is shunted to the periphery for radiative heat dissipation, and it has been suggested that reduced blood flow to the mammary may account for some of the observed reductions in milk production (Black et al., 1993). However, Renaudeau et al. (2003) used blood flow transducers implanted into the sow pudic artery and demonstrated that blood flow at d 8 to 10 of lactation is actually increased when sows were housed at 28°C compared to sows housed at 20°C (945 mL/min vs 872 mL/min, respectively). This may occur as an adaptation to heat stress in confinement farrowing crates constructed of concrete or metal flooring that facilitate conductive heat transfer. Sows spend approximately 85% of the day in ventral or lateral recumbency in farrowing crates (Lou and Hurnik, 1998). Similarly, heat stress has been shown to induce a slight (3 to 6%) increase in mammary blood flow of rabbits during peak lactation (Lublin and Wolfenson, 1996).

Milk composition is altered during heat stress and largely reflects the biochemical environment of the bloodstream. Hypoglycemia reduces the availability of one of the simple

sugars necessary for lactose production, and as such lactose is reduced in lactating sows (Renaudeau et al., 2003) and cows (Rhoads et al., 2009) that are heat stressed. Glucose is converted to lactose in mammary epithelial cells, and the rate of lactose production is dependent on glucose availability (McManaman and Neville, 2003). Interestingly, Renaudeau et al. (2003) noted that even though heat stressed sows were slightly hypoglycemic, pudic artery extraction rate of glucose was actually greater under heat stressed (28°C) than thermoneutral conditions (20°C; 28 mg/L vs. 25 mg/L, respectively), and glucose uptake as a percent of total plasma nutrients was identical between environments. Even so, heat stress decreases milk lactose (5.7% vs. 5.4%; Renaudeau et al., 2003). This seemingly contradictory information may indicate a direct effect of heat stress on GLUT1 transporter function in the mammary.

Additionally, Bernabucci et al. (2002) found that milk crude protein is decreased in heat stressed dairy cows, and this reduction is driven mostly by a reduction in the casein components rather than whey lactalbumin or lactoglobulin. This is significant, as milk casein content has been linked with offspring growth in mice (Kumar et al., 1994). In sows, heat stress induces a slight decrease in milk crude protein as a function of reduced arterial amino acid concentrations. Mammary extraction rate of essential amino acids is either maintained or increased under heat stress, and preference is given to essential over non-essential amino acids, likely as an attempt to maintain milk protein synthesis (Renaudeau et al., 2003).

Percent milk fat has been shown to either decrease (Renaudeau et al., 2003) or be unaffected by heat stress (Rhoads et al., 2009). Mammary extraction rate of triglycerides, which are the major lipid component of milk fat, is held relatively constant at varying degrees of heat stress (Renaudeau et al., 2003). This would suggest that epithelial lipoprotein lipase activity is

adjusted according to the milk synthesis needs of the mammary (Neville and Picciano, 1997). At this time, it is assumed that heat stress exerts greater effects on milk lactose and protein than fat.

Critical to piglets is immune transfer in colostrum during the first 24 h of life.

Information reviewed by Farmer and Quesnel (2009) indicate that colostral concentrations of both IgA and IgG are decreased during summer. Similarly, IgG is also reduced in heat stressed sows during the last two weeks of pregnancy, and this is associated with greater pre-weaning piglet mortality (Machado-Neto et al., 1987). In dairy cattle, Tao et al. (2012) failed to find a relationship between heat stress and colostral IgG content, however the offspring of these heat stressed dams did have less serum IgG during the first 28 d of life. Because there is no intrauterine immune transfer across the epitheliochorial placental of large animals (Churci et al., 2010) the rate of immunoglobulin intestinal absorption during environmental stress is called into question (Tao et al., 2012). Indeed, damage to the intestinal microvilli (shown experimentally as decreased villus height and more shallow crypt depth) of mature pigs occurs within 3 d after exposure to heat stress (Liu et al., 2009).

Methods for Measuring Milk Production

Multiple methods may be used to measure sow milk production. The simplest method is to record piglet weight gain during lactation, provided the piglets have no access to creep feed. Estimates compiled and reported by Quesnel et al. (2015) indicate that the amount of milk required for one gram of piglet growth increases throughout lactation, from 3.78 g milk required for 1 g growth at d 3, to 4.89 g milk for 1 g growth at d 17. Using these published values, it would be possible to estimate milk production using only piglet growth data.

The weigh-suckle-weigh technique is commonly used to estimate milk production by weighing either the sow or the entire litter immediately before and after each suckling event (Lewis et al., 1978). Because the sow suckling interval is relatively short (35 to 50 minutes; Spinka et al., 1997), this measure may be repeated multiple times to achieve statistical robustness. However, difficulties in controlling piglet urination and defecation between weighings as well as the disturbance stress associated with repeated handling of piglets reduces the accuracy of the weigh-suckle-weigh technique by 20% compared to deuterium oxide dilution (Quesnel et al., 2015).

The deuterium oxide dilution technique is generally accepted as the most accurate method of measuring sow colostrum and milk production (Quesnel et al., 2015). In this method, milk samples are analyzed for water content. The piglet is then fasted for a defined period before receiving an injection of deuterated water. After a period of allowed suckling, blood samples are taken to compare the dilution of deuterium oxide and the ingestion of water from milk consumption. Total milk intake, and hence, total milk production, may then be calculated based on the previously analyzed milk water content (Quesnel et al., 2015).

Conclusion

Piglet health and growth is highly dependent on the ability of the gilt or sow to produce a large amount of high-quality colostrum and milk. However, genetic selection for large litter size in commercial breeds has produced a strain on sow milk production, such that milk has become the limiting factor in piglet development. Accordingly, any factors that inhibit milk production or decrease the nutrient quality of the milk are of large consequence to piglet well-being.

It is well known that heat stress decreases sow milk production through direct effects that occur independent of reduced feed intake. Additionally, lactating sows exposed to heat stress produce milk with lower lactose concentrations. Effects of heat stress on percent milk fat and protein are not as substantial, although some studies do show a slight reduction of these nutrients. Decreased nutrient content of sow milk during periods of heat stress depresses piglet health and growth up to and after weaning. No studies to date have investigated the effects of gestational heat stress on milk production and nutrient composition in swine.

Part III: Conclusion

The negative effects of heat stress on the reproductive and lactational performance of gilts and sows is significant, and are most commonly observed on the farm through decreased farrowing rates, smaller litter sizes, poor milk yield, and reduced piglet growth to weaning. Additionally, heat stress decreases pig growth rate, feed efficiency, and promotes an anabolic metabolism as evident by increases in circulating insulin concentration and an increase in the ratio of lipid to lean protein deposition. Collectively, the direct effects of heat stress cost US swine producers over \$300 million each year.

Recent research has suggested that piglets subjected to heat stress during intrauterine development experience an altered physiology that persists into adulthood. The consequences of gestational heat stress reflect those observed in animals directly exposed to heat stress, most notably a reduction in feed efficiency and an increase in whole-body adiposity at slaughter.

Results of experiments in lower-order organisms have shown that thermal stress during pregnancy persist to negatively impact second-generation offspring (i.e., the grandchildren of

adult animals that are exposed to heat stress during pregnancy), and could possibly indicate modification to the epigenome. Phenotypic evidence of multi-generational responses to developmental heat stress have not yet been studied in higher-order mammals.

The hypothesis of the forthcoming experiments are that the altered physiology and metabolism resulting from gestational heat stress negatively impacts pig production beyond what has been previously described. The first experiment was conducted to determine if differences in nutrient availability and body composition would lead to differences in the milk production and nutrient composition of gestational heat stressed gilts. In the second experiment, the offspring of gestational heat stressed gilts were raised to market weights, and their growth performance and carcass composition was observed to determine if the effects of heat stress are trans-generational. The results of these experiments will provide greater insight to the physiology of pigs affected by gestational heat stress, and phenotypic evidence of the trans-generational properties of developmental hyperthermia in domestic mammals.

Chapter II: Milk yield and nutrient composition of gestational heat stressed gilts exposed to a secondary heat stress during pregnancy

Abstract:

Exposure to chronic gestational heat stress (gHS) has been recently shown to alter the growth performance and carcass composition of affected swine. Notably, pigs having developed under gHS show a heightened anabolic metabolism causing increased whole-body adiposity and reduced lean tissue accretion. Given these circumstances, the objective of the present study was to determine the effects of gHS on milk production and colostrum and milk nutrient composition of gilts. Twenty-four post-pubertal gilts were bred and exposed to thermoneutral (gTN) or heat stressed conditions for the entirety of gestation. Female offspring were then retained, grown to breeding age, and bred to farrow in spring (SPR) or summer (SUM; $n = 16$ gHS/SPR; 18 gHS/SUM; 19 gTN/SPR; 15 gTN/SUM). Colostrum samples were collected 15 h after farrowing and milk samples were collected on d 7, 14, and 21 of lactation. All samples were analyzed for percent milk fat, lactose (LAC), protein (PRO), and solids-non-fat (SNF). Additionally, milk production was determined during peak lactation using the weigh-suckle-weigh method on a subset of gilts ($n = 6$ gHS/SPR; 13 gHS/SUM; 6 gTN/SPR; 9 gHS/SUM). Milk production did not differ based upon gestational treatment nor season of farrowing. Gilts that had been in gTN *in utero* produced more LAC than gHS gilts ($P < 0.05$). There was also a season by day interaction effect on milk LAC ($P < 0.01$). There was a tendency for gHS gilts to produce more PRO than gTN gilts ($P = 0.07$), but no effect of season of farrowing on colostrum or milk PRO content. Gilts that farrowed in the SUM produced more milk fat than those that farrowed in the SPR, but milk fat production was not affected by gestational treatment. Milk SNF did not differ as a result of gestational treatment, but also exhibited a season by day interaction effect ($P <$

0.01). These results indicate that heat stress experienced *in utero* has long-term effects that alter milk composition. The mechanisms responsible for the observed differences in milk composition have not been elucidated.

Introduction:

Sow milk production and nutrient composition are influenced by heat stress (Black et al., 1993; Renaudeau et al., 2003; Farmer and Quesnel, 2009). Indeed, studies reviewed by Black et al. (1993) indicate a 10 to 35% decrease in milk production and associated piglet growth when sows were housed in heat stress conditions. Additionally, Renaudeau et al. (2003) reported a decrease in the lactose and fat content following heat stress in mid-lactation. Similar results have been observed in heat-stressed dairy cattle (Rhoads et al., 2009).

Heat stress causes a precipitous decline in feed intake, and this reduction in intake is often blamed for poor lactational performance (Black et al., 1993; Spencer et al., 2003). Results of pair feeding trials indicate, however, that heat stress directly affects sow milk production and nutrient composition (Messias de Bragança et al., 1998; Renaudeau et al., 2003). These assertions are corroborated by Prunier et al. (1997), who noted that daily piglet growth during heat stress is more closely associated with sow weight loss and backfat mobilization than lactation feed intake.

In swine, heat stress promotes an anabolic metabolism that heightens adipose conservation through hyperinsulinemia and reduced secretion of the catabolic hormones cortisol, T₃ and T₄ (Messias de Bragança et al., 1998; Baumgard and Rhoads, 2013). It has recently been shown that pigs exposed to chronic heat stress during gestation (gestational heat stress [gHS])

exhibit a similar anabolic state throughout life, with increased blood insulin and a 95% increase in the ratio of lipid:protein accretion compared to gestationally thermoneutral (gTN) controls (Boddicker et al., 2014; Johnson et al., 2015). To the best of our knowledge, no previous studies have investigated the consequences of this long-term anabolic state for gilts that enter the breeding herd. The presumptive metabolic programming that occurs as a result of gHS could be particularly detrimental for replacement females during their first lactation, thereby affecting the efficiency of production. As such, the objective of the present experiment was to determine whether *in utero* exposure to heat stress affects the milk production and milk nutrient composition of primiparous gilts.

Materials and Methods

All experimental and animal husbandry procedures were approved by the Institutional Animal Care and Use Committees at the University of Missouri and Virginia Tech and followed the guidelines set forth for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010).

Production of F1 Generation

Twenty-four post-pubertal gilts (214 ± 11 d of age; Landrace x Large White; Newsham Choice Genetics [NCG], West Des Moines, IA) were synchronized by top-dress feeding of an oral progestogen (Altrenogest [Matrix, Merck Animal Health, Millsboro, DE]) for 14 d. Gilts were transported from the University of Missouri Swine Research Complex to the Brody Environmental Center (Columbia, MO) on the last d of progestogen feeding. Upon introduction to the Brody Environmental Center, gilts were equally divided between two chambers ($n = 12$

gilts per chamber) and housed in individual gestation stalls (2.4 x 0.6 m). Daily boar exposure commenced the day following chamber introduction. Gilts were artificially inseminated 0 and 24 h after exhibiting standing heat using semen pooled from Landrace x Large White boars [NCG] 4.8 ± 0.9 d after introduction to the chambers.

Heat treatment commenced in one chamber on d 13 after placement in chambers (8.2 ± 1.0 d after breeding). The second chamber was maintained at thermoneutral conditions. The chambers were programmed with 24-h temperature cycles of 18 to 20°C for the TN room and 28 to 32°C for the HS room. Relative humidity was not controlled. Temperature and humidity of both the heated and thermoneutral chambers were recorded every 15 minutes by in-room data loggers. Photoperiod in both chambers was maintained at 15 h light, 9 h dark using fluorescent lighting. Both chambers were ventilated with outside air with a minimum of ten air changes per h (10 to 14 air changes per h range; air was not recycled). Gilts in both treatment groups were offered 1.8 kg of the same diet throughout gestation. The diet was a standard corn/soybean meal mixture with appropriate vitamin and minerals to meet maintenance requirements (NRC, 2012).

At 107.2 ± 0.9 d of gestation, gilts were moved from the environmental chambers into a single farrowing room maintained at thermoneutral conditions (18 to 20°C). Gilts farrowed in standard farrowing crates and were provided *ad libitum* access to feed throughout lactation (NRC, 2012). Piglets were processed on d 2 post-farrowing (ear notching, needle teeth clipping, tail docking, and a 2 mL intramuscular injection of dehydrogenated iron dextran [100 mg/mL]). Piglets were weaned at 23.6 ± 1.2 d of age.

F1 Generation Post-Weaning through Breeding

A total of 12 HS and 12 TN gilts farrowed. Female F1 piglets (n=124) were retained at weaning and grown in split treatment pens at the University of Missouri South Farm according to standard farm production practices. Gilts were transported to the Virginia Tech Tidewater Agricultural Research and Extension Center (**TAREC**) in Suffolk, VA, at 189 d post-weaning. At TAREC, gilts from both treatments were co-mingled in group pens (approximately 30 gilts per pen). Gilts were divided into four replicate farrowing groups (n = 20 to 25 gilts per breeding group; equal number of gHS and gTN gilts in each breeding) and, prior to breeding, moved into gestation stalls in fan-ventilated barns with no additional heating or cooling provisions. Estrus was synchronized by a top-dress feeding of progestogen for 14 d. After withdrawal of progestogen, gilts were checked for estrus in the presence of a mature boar and mated using AI at 0 and 24 h after first displaying lordosis to purebred Yorkshire boars (semen from Swine Genetics International, Cambridge, IA was employed). Breeding groups were planned so that farrowing occurred during the spring or summer months. Two spring-farrowing groups (SPR) were bred in early November and early December and farrowed in late February and early April, respectively. Two summer-farrowing groups (SUM) were bred in late March and May, and farrowed in July and August, respectively. Thus, gilts that had been gTN and gHS were exposed to summer or spring conditions as a consequence of season during late pregnancy and farrowing. High and low ambient temperatures were recorded at the TAREC station daily. At approximately d 110 of gestation, gilts were moved into one large environmentally controlled farrowing room maintained at TN conditions.

Milk Production and Quality

Farrowings were attended, and a colostrum sample was obtained approximately 14-16 hours (d 1) following the initiation of farrowing. Milk samples were taken during the transient (d 7) and mature stages of lactation (d 14 and 21). In each case, letdown was induced using a 1 mL (10 IU) i.m. injection of oxytocin (Vedco Inc., St. Joseph, MO). Gilts were hand-milked into collection cups, with milk from all teats represented in the sample. Approximately 10 mL of milk was collected per gilt per collection, although less colostrum was available for collection compared to mature milk. Milk was thoroughly mixed in the collection cup and transferred into vials with a Broad Spectrum Microtabs II preservative (D & F Control Systems, Inc., Norwood, MA). Samples were refrigerated for one wk or less until infrared spectrophotometry analysis was conducted for percent fat, protein (PRO), lactose (LAC), and solids-non-fat (SNF). Spectrophotometry analysis was conducted by the United Dairy Herd Information Association (DHIA) lab (Radford, VA).

Peak milk production was measured at $d 19 \pm 1.4$ of lactation using the weigh-suckle-weigh method as described by Lewis et al., (1978). Briefly, piglets were separated from their dams and allowed no access to creep feed or water. After 50 min of separation from the dam, piglets were weighed, returned to the gilt, and suckling was observed. After suckling ceased (evident as two or more piglets defecting from the udder, or the dam changing positions to prevent piglets from accessing the udder) the piglets were again weighed. This procedure was repeated consecutively nine times, with the first three replicates used to acclimate the pigs and dams to the stress of handling. Data from these three initial weighings was not included in the analysis. Weights from the final six replicates were used to calculate total milk production.

Statistics

Milk production data was analyzed using the mixed procedure (PROC MIXED) of SAS (SAS Institute, Inc., Cary, NC). The model included the main effects of gestational treatment (gHS or gTN) and season (SPR or SUM) as well as interactions of these effects. Days in milk and litter size, and average daily feed intake during lactation were included as covariates.

Milk nutrient composition was also analyzed using the mixed procedure in SAS. The model included the main effects of gestational treatment, season of farrowing, and days in milk (d 1, 7, 14, or 21) as well as interactions of these effects. In instances where there was no effect of season of farrowing, this variable was removed from the model.

For all analyses, seven covariance structures were tested and the most appropriate was chosen based upon Akaike's information criterion, Akaike's information criterion with correction, and Bayesian information criterion values. Gilt nested within treatment was included as the random effect. Separation of means was conducted using the Tukey-Kramer procedure of SAS. Results are reported as least squares means \pm SEM. Significance was set at $P \leq 0.05$, and tendencies were defined as $P \leq 0.10$.

Results

Environmental Data

For the gHS component of the study, average temperature and relative humidity of the heated and thermoneutral chambers at the Brody Environmental Chambers was $31.0 \pm 4.0^\circ\text{C}$ and $78.5 \pm 8.6\%$, and $18.1 \pm 2.1^\circ\text{C}$ and $59.7 \pm 5.3\%$, respectively. Average temperature and relative

humidity of the farrowing room was similar to the temperature and humidity of the thermoneutral chambers. Average 24 hour temperatures for both treatments are presented in

Figure 2.1.

Daily high and low temperature for SPR and SUM farrowings at TAREC are presented in **Figure 2.2**. Average high and low temperature for spring farrowings was $12.8 \pm 7.2^{\circ}\text{C}$ and $-1.6 \pm 5.9^{\circ}\text{C}$, respectively. Average high and low temperature for summer farrowings was $28.0 \pm 5.1^{\circ}\text{C}$ and $14.0 \pm 5.4^{\circ}\text{C}$, respectively. Relative humidity was not recorded.

Milk Production

There was no effect of pre-natal or post-natal environment on milk production observed using the weigh-suckle-weigh technique at peak lactation (6.49 ± 0.37 kg gHS vs 6.00 ± 0.40 kg gTN; $P = 0.40$). Additionally, there was no interaction between seasons (6.19 ± 0.42 kg SPR vs 6.30 ± 0.32 kg SUM; $P = 0.84$).

Colostrum and Milk Nutrient Composition

Among gilts, fat was least at d 21 compared to other observations (**Table 2.1**; $P < 0.01$). Additionally, fat was higher in gilts that farrowed in SUM compared SPR (**Table 2.1**; $P < 0.05$). There was no main effect of gestational environment ($P = 0.33$). Gilts farrowing in SUM produced milk with greater fat on d 7 compared to gilts farrowing in SPR (9.34 ± 0.19 % vs 8.01 ± 0.29 %; $P < 0.01$).

There was an effect of gestational treatment on milk LAC, wherein gHS gilts produced less LAC than gTN gilts ($5.10 \pm 0.04\%$ vs $5.21 \pm 0.04\%$, respectively; $P < 0.05$). There was also an interaction between season of farrowing and days in milk (**Table 2.2**). Milk LAC was lower

in the colostrum of SUM farrowing gilts compared to SPR farrowing gilts ($P < 0.01$), and tended to be lower at d 14 of lactation ($P = 0.08$).

Milk SNF was not affected by gestational treatment ($P = 0.20$), nor by the interactions of treatment by season of farrowing ($P = 0.38$) or treatment by days in milk ($P = 0.43$). There was an interaction between season of farrowing and days in milk as a result of differences in SNF content in the colostrum (d 1, **Table 2.3**; $P < 0.01$). The nature of this difference was opposite of what was observed for LAC, with SUM farrowing gilts having greater SNF in colostrum compared to SPR farrowing gilts. There was a tendency for gHS gilts to produce more PRO than gTN gilts (**Figure 2.3**; $P = 0.07$). Expectedly, PRO was greatest in colostrum in both treatments ($P < 0.01$).

Discussion

There were no differences observed in the milk production of gHS and gTN gilts measured at peak lactation ($d 19 \pm 1.4$). Previously, heat stress inflicted during late gestation and lactation decreased milk production and piglet growth by as much as 35% (Black et al., 1993; Spencer et al., 2003). Although often considered to be a function of reduced feed intake (indirect actions), much of the reduction in milk production can be attributed to direct actions on the sow endocrine and mammary systems. For example, heat stress impairs energy reserve mobilization by reducing concentrations of the catabolic hormones cortisol, T_3 and T_4 (Messias de Bragança et al., 1998). Indeed, daily growth of piglets nursing heat stressed sows is more closely associated with sow weight loss and backfat mobilization than with sow feed intake during lactation (Prunier et al., 1997). Reports in dairy cattle have indicated decreased proliferation and

increased apoptosis of mammary epithelial cells under heat stress (Du et al., 2008; Tao et al., 2011). These results have not yet been repeated in swine.

Even though gHS did not affect milk production in the present study, Tao and Dahl (2013) cite evidence that poor adolescent growth coupled with increased pre-pubertal fat accumulation negatively correlates with mammary parenchyma DNA and milk production in dairy heifers, and theorize that gHS may in fact decrease milk production. Greater insulin concentrations and adolescent adiposity have been confirmed in both growing swine and dairy heifers (Tao et al., 2013; Johnson et al., 2015b).

It may be that gHS did indeed have a biological effect on milk production, but treatment differences were not observable using the weigh-suckle-weight method. Inherent difficulties in controlling piglet urination and defecation between weighings, as well as the disturbance stress associated with repeated piglet handling, undoubtedly reduces the accuracy of this technique. Indeed, studies reviewed by Quesnel et al. (2015) indicate a 20% decrease in the accuracy of milk production estimates generated through weigh-suckle-weigh compared to other methods, such as deuterium oxide dilution.

As expected, milk nutrient composition changed during the course of the 21 d lactation. Values for percent milk fat, LAC, SNF and PRO in colostrum and milk were within the range of values reported previously (for review of swine milk nutrient composition, see: Hurley, 2015). In the present experiment, milk fat was slightly greater than the average fat content presented by Hurley (2015), but still within the range of previously reported values. One explanation for this elevated fat content may be breed type used in the present experiment. It is known that maternal breed sows produce colostrum and milk with greater percent milk fat (Fahmy, 1972). Use of a

hyperprolific sow line, as well as emphasis on maternal-breed boars (Yorkshire and Landrace x Large White), in the present experiment may explain the slightly elevated fat content observed in the present experiment.

Expected differences were observed in the LAC, PRO, and SNF content of colostrum as compared to milk. Indeed, differences based on days in milk (PRO) and the interaction between season of farrowing and days in milk (LAC, SNF) were primarily due to differences in the colostrum (as indicated by the Tukey-Kramer comparisons of means). It is well known that sow colostrum protein content is greatest in the immediate post-partum period due to the necessity for transfer of passive immunity to the neonate (Rooke and Bland, 2002). In particular, colostrum has a large concentration of IgG, IgM, and IgA. These immunoglobulins are included in the spectrophotometry analysis of PRO and SNF. Conversely, in sows, percent milk lactose increases around 3 d post-farrowing (Hurley, 2015). The increased lactose content at this time coincides with upregulation of intestinal lactase in piglets (Le Huërou-Luron and Ferret-Bernard, 2015).

Milk fat was lesser at peak lactation (d 21) than at any other time during lactation. Schoenherr et al. (1989) reported a linear decrease in milk fat percentage of multiparous sows fed a starch-based diet from d 0 to 22 of lactation. Interestingly, a linear decrease in milk fat content was not observed in the present experiment, but rather, the gilts showed a numeric, but non-significant, increase in milk fat between d 7 and 14, followed by a precipitous drop between d 14 and 21. Schoenherr et al. (1989) hypothesize that the decrease in milk fat occurs due to the metabolic requirements of milk production exceeding the capacity of the sow to ingest adequate nutrients. Gilts in the present study were allowed *ad libitum* diet access to feed and so it is likely, then, that the animals were in a state of positive energy balance through d 14 of lactation, which

would account for the slight increase in fat. However, energy balance likely switched to a negative state at peak milk production and gilts may have become unable to meet their nutrient intake demands. Although unverifiable, this hypothesis would explain the large decrease in milk fat observed at d 21 in the present study.

Heat stress as a consequence of season increased milk fat content while decreasing milk LAC. Similar reductions in milk LAC during heat stress have been reported previously in both sows (Renaudeau et al., 2003) and cows (Rhoads et al., 2009). The decrease in milk LAC during heat stress likely occurs as a function of the hypoglycemic state spurred by a whole-body shift towards anabolism and a preferential increase in tissue glucose utilization (Wheelock et al., 2010).

The increased fat percentage observed during summer is at odds with previous studies. The majority of studies indicate either no effect (Shurson et al., 1986; Renaudeau and Noblet, 2001; Prunier et al., 1997) or a slight decrease in percent milk fat during heat stress (Schoenherr et al., 1989; Renaudeau et al., 2003). However, confounding factors in experimental design make direct comparisons to these studies difficult. Notable differences occur in use of multiparous sows (Shurson et al., 1986; Schoenherr et al., 1989; Renaudeau and Noblet, 2001; Renaudeau et al., 2003) and having inflicted heat stress either in late-gestation (Schoenherr et al., 1989), at farrowing (Prunier et al., 1997; Renaudeau and Noblet, 2001) or in mid-lactation (Renaudeau et al., 2003). It is unknown what effect, if any, the duration of heat stress may have on milk fat secretion. More studies will be required to understand the effects of acute vs chronic heat stress on fat content of milk.

There was no effect of season on milk PRO content, which is consistent with previous reports (Renaudeau et al., 2003; Hurley, 2015). Indeed, Renaudeau et al. (2003) determined that the mammary preferentially selects essential amino acids at the expense of non-essential amino acids (which may be synthesized from essential amino acids) to maintain PRO synthesis during heat stress. Concentrations of amino acids were not measured in the present study.

The decrease in milk LAC content, and tendency for greater milk PRO content, of gHS gilts provides evidence that the metabolic state imparted by hyperthermic intrauterine development persists into adulthood to alter the milk nutrient content of lactating gilts. In particular, it is known that LAC, which is the primary carbohydrate present in sow milk, is positively correlated with both milk yield and piglet growth through weaning (Noble et al., 2002). The reduced LAC present in the milk of gHS gilts did not decrease milk yield in the present study. Piglet body weight at weaning was not assessed in the present study.

This experiment was designed to simulate normal gilt production systems, and as such is highly relevant to breeding swine farms. Gilts are generally expected to farrow their first litter between 10 and 11 mo of age, meaning that female piglets developing in heat stressed dams during the summer months will be exposed to similar conditions during pregnancy when they enter the breeding herd the following year. It would be expected, then, that the differences observed in milk nutrient composition between gHS/SUM and gTN/SPR treatments would be reflected on production sow farms.

Figure 2.1. Average 24 hour temperature in Brody Environmental Chambers at the University of Missouri (Columbia, MO)

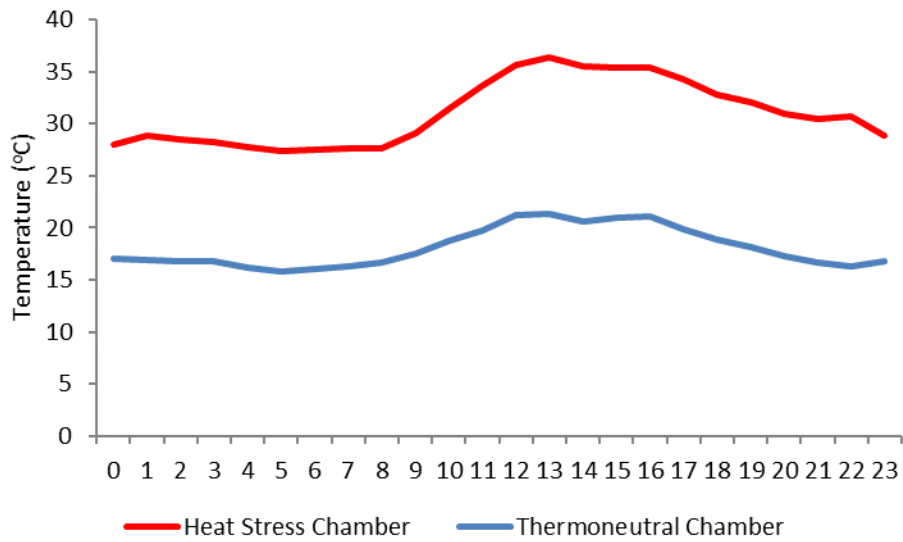


Figure 2.2. Daily high and low temperatures from breeding through weaning recorded at TAREC for the spring (SPR) and summer (SUM) farrowings

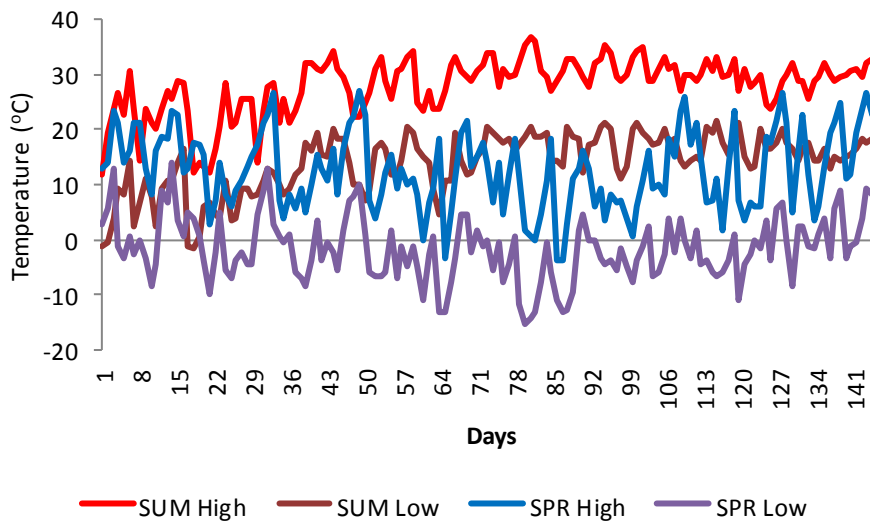


Table 2.1: Percent milk fat of gilts farrowing in spring (SPR) and summer (SUM) at 1, 7, 14, and 21 days in milk

	d 1	d 7	d 14	d 21	Mean Fat
SPR	8.87 ± 0.34 ^x	8.01 ± 0.29 ^{a, r}	8.60 ± 0.41 ^x	6.75 ± 0.30 ^{y, s}	8.06 ± 0.21 ^a
SUM	8.93 ± 0.33	9.34 ± 0.19 ^{b, x}	9.13 ± 0.33 ^x	7.80 ± 0.31 ^y	8.80 ± 0.21 ^a
Mean Obs.	8.90 ± 0.24 ^x	8.68 ± 0.18 ^x	8.86 ± 0.26 ^x	7.28 ± 0.22 ^y	

* **x, y:** means within row are different ($P < 0.01$)

* **r, s:** means within row tend to differ ($P = 0.07$)

* **a, b:** means within column are different ($P < 0.01$)

Table 2.2: Percent milk lactose of gilts farrowing in spring (SPR) and summer (SUM) at 1, 7, 14, and 21 days in milk

	d 1	d 7	d 14	d 21	Mean Lactose
SPR	4.46 ± 0.07 ^{a, x}	5.52 ± 0.06 ^y	5.64 ± 0.08 ^{r, y}	5.44 ± 0.07 ^y	5.27 ± 0.04 ^a
SUM	3.99 ± 0.07 ^{b, x}	5.37 ± 0.04 ^y	5.34 ± 0.06 ^{s, y}	5.44 ± 0.07 ^y	5.04 ± 0.04 ^b
Mean Obs.	4.23 ± 0.05 ^x	5.44 ± 0.04 ^y	5.49 ± 0.05 ^y	5.44 ± 0.05 ^y	

* **x, y:** means within row are different ($P < 0.01$)

* **a, b:** means within column are different ($P < 0.01$)

* **r, s:** means within column tend to differ ($P = 0.08$)

Table 2.3: Percent milk solid-non-fat of gilts farrowing in spring (SPR) and summer (SUM) at 1, 7, 14, and 21 days in milk

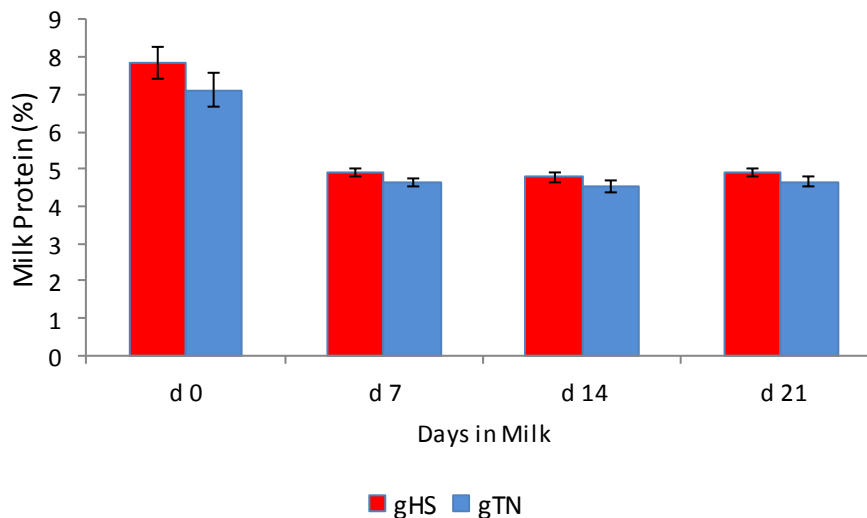
	d 1	d 7	d 14	d 21	Mean SNF
SPR	11.48 ± 0.33 ^a	11.07 ± 0.09	10.86 ± 0.12	11.10 ± 0.08	11.13 ± 0.11 ^a
SUM	13.60 ± 0.32 ^{b, x}	11.07 ± 0.06 ^y	10.97 ± 0.08 ^y	11.04 ± 0.09 ^y	11.67 ± 0.10 ^b
Mean Obs.	12.54 ± 0.23 ^x	11.07 ± 0.06 ^{r, y}	10.91 ± 0.07 ^{s, y}	11.07 ± 0.06 ^y	

* *x, y*: means within row are different ($P < 0.01$)

* *r, s*: means within row are different ($P = 0.01$)

* *a, b*: means within column are different ($P < 0.01$)

Figure 2.3. Percent milk protein of gestational heat stressed (gHS) and gestational thermoneutral (gTN) gilts at 1, 7, 14, and 21 days in milk (TRT $P = 0.07$; TRT x DIM $P = 0.88$)



Chapter III: Growth performance and carcass composition of offspring having developed in gestational heat stressed dams

Abstract

Exposure to chronic heat stress during fetal development has long-term effects on offspring growth and development. The present experiment was conducted to determine if differences in growth and development are transgenerational, and evident in the offspring of gestationally heat stressed dams (OgHS). Breeding females that had been exposed to heat stress or thermoneutral conditions while *in utero* were obtained for use prior to this experiment. Those females were inseminated so that they farrowed in two replicate groups. At weaning, two male and two female offspring were retained from 16 gestational heat stress (gHS) dams and 17 gestational thermoneutral (gTN) dams and grown to market weight in pens of mixed treatment and gender. At 113.6 ± 0.3 kg adjusted BW, pigs were marketed to a commercial packing plant. Liveweight (LW) was recorded before leaving the farm, and hot carcass weight (HCW) was determined at slaughter. At 24 hr post-mortem, carcass length (CRC L) was measured on one intact side. The side was then split and backfat thickness (BF) and loin eye area (LEA) was measured between the 10th and 11th rib. Dressing percentage (DRESS %) and lean percentage (LEAN %) were calculated. There was no difference in the growth of pigs to market, nor in the days to market weight ($P = 0.93$). At slaughter, there were no treatment-related differences in LW ($P = 0.30$) or HCW ($P = 0.52$). Barrows born to gHS dams had the shortest CRC L ($P < 0.05$). Pigs born to gHS dams tended to have greater BF ($P = 0.11$) than OgTN. There was also a tendency for greater DRESS % in OgHS ($P = 0.14$). There were no differences in LEA ($P = 0.22$) or LEAN % ($P = 0.21$). These results demonstrate exposure to heat stress during pregnancy

creates lasting impressions in carcass measurements in first-generation offspring are evident, albeit muted, in the second-generation offspring of gHS dams.

Introduction

Heat stress negatively impacts swine feed intake, average daily gain, and the days on feed necessary to reach target market weights (Baumgard and Rhoads, 2013). Heat stressed pigs exhibit decreased overall activity and spend less time at the feeder (Hicks et al., 1998), presumably as an evolutionary adaptation to decrease the heat increment associated with digestion. Collectively, heat stress in the growing-finishing herd costs the US swine industry over \$200 million annually (St-Pierre et al., 2003).

Recent research has demonstrated the effects of chronic gestational heat stress (gHS) on swine growth performance and physiology (Johnson et al., 2015a; 2015b). Pigs exposed to heat stress while *in utero* subsequently exhibit decreased feed conversion efficiency and require additional days on feed to reach target market weight compared to pigs exposed to gestationally thermoneutral (gTN) conditions (Wilmoth et al., 2014). Further, gHS promotes hyperinsulinemia and heightened anabolic metabolism in offspring throughout life, contributing to greater carcass adiposity and reduced lean percentage (Johnson et al., 2015b).

Recent research has shown that thermal stress early in life induces epigenetic modifications to the genome that persist through multiple generations in fruit flies (Seong et al., 2011), rodents (Tetievsky and Horowitz, 2010), fish (Meier et al., 2014), and plants (Molinier et al., 2006). It is unknown, however, if the altered physiology and production traits of pigs exposed to gHS could be passed on to the next generation of offspring (F2). Therefore, the

objective of this study was to determine if the altered growth and carcass characteristics of pigs affected by gHS persists across multiple generations.

Materials and Methods:

All animal procedures were approved by the Institutional Animal Care and Use Committees at the University of Missouri and Virginia Tech and followed the guidelines set forth for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010).

Production and Management of the F2 Generation

The methods used to generate the F1 and F2 generations were previously described (Chapter II). Gilts were bred to produce F2 piglets in two separate farrowing replicates. Farrowings were attended and piglet BW were recorded before the first suckle. Piglets were weighed again at processing (procedures similar to those described previously), which occurred approximately 15 hours after farrowing. Castration and vaccination (*Mycoplasma hyopneumonia*) occurred at d 7 post-farrowing. Creep feed was not provided at any point. Weaning occurred when the average age of the farrowing group reached 21 d. At weaning, piglets were vaccinated for Bordatella, Erysipelas, and Circovirus. Two male and two female offspring from a subset of litters (n = 16 gHS and n = 17 gTN litters) were retained at weaning and grown to market weight. Pigs were retained based that most closely represented the mean piglet weaning weight within the litter. Weights of the pigs were recorded during the growth phase for calculation and analysis of average daily gain. For both replicates, BW at 113.7 ± 0.4 d of age was evaluated. One pig from a gHS litter and two pigs from gTN litters exhibited poor growth and were not marketed with their contemporaries. Their growth data was excluded from

the dataset. Pigs from the first replicate were marketed in October, and pigs from the second replicate were marketed in January.

The retained pigs were housed in mixed treatment and gender nursery pens (n = 8 pigs per pen) for six weeks post-weaning and provided *ad libitum* access to a fortified corn/soybean meal diet that met or exceeded requirements (NRC, 2012). Following the nursery phase of production, pigs were removed from the climate controlled barns and moved to large concrete-floored outdoor finishing lots where all pigs were mixed according to treatment and gender (n = 20 pigs per pen). Weights were determined on all pigs approximately half-way through the growing period and again prior to market. All pigs (n = 32 male OgHS, 31 female OgHS, 33 male OgTN, and 33 female OgTN) were marketed to a commercial packer for carcass analysis (The Pork Company, Warsaw, NC).

Liveweight (LW) was recorded on all pigs before loading, and after slaughter hot carcass weight (HCW) was determined. After 24 hr in the cooler, carcass length (CRC L) was recorded on one side of the carcass as the linear distance between the first rib and the aitch bone. The side was then separated with a hand saw between the 10th and 11th rib and backfat thickness (BF) and loin eye area (LEA) were measured. From these values, dressing percentage (DRESS %) and lean percentage (LEAN %) were determined using calculations described by Cromwell et al. (2002).

Statistics

For all analyses, values greater than two standard deviations from the mean were considered outliers and excluded. Post-weaning growth of pigs was evaluated as average daily gain, BW at 113.7 ± 0.4 d of age and adjusted d of age at a common market weight. Pig d of age

at a common market weight (113.4 kg) was calculated using National Swine Improvement Federation adjustment calculations (NSIF, 2003):

$$\text{adjusted days} = \text{actual age} + [(\text{desired weight} - \text{actual weight}) * (\text{actual age} - \mathbf{a}) / \text{actual weight}],$$

where \mathbf{a} = 50 for barrows, and 40 for gilts.

Post-weaning growth variables were then analyzed for a main effect of treatment using the mixed procedure (PROC MIXED) of SAS (SAS Institute, Inc., Cary, NC). For analyses of average daily gain and BW, d of age was included in the model as a covariate. Carcass characteristics were analyzed for main effects of treatment, gender and the treatment x gender interaction using the PROC-MIXED procedure of SAS with HCW as a covariate. When significant, replicate farrowing group was also included in the model. For all analyses (growth and carcass characteristics), seven covariance structures were tested and the most appropriate was chosen based upon Akaike's information criterion, Akaike's information criterion with correction, and Bayesian information criterion values. Pig nested within litter was used as a random effect in all analyses. Separation of means was conducted using the Tukey-Kramer with adjustments procedure of SAS. Results are reported as least squares means \pm SEM. Statistical significance was set at $P \leq 0.05$.

Results

There were no treatment-related differences in post-weaning growth owing to the dams gestational environment (OgHS vs OgTN) as assessed by average daily gain, BW at 113.7 ± 0.4 d of age and adjusted d of age at a common market weight ($P = 0.93$). There also was no

difference in weight or age at marketing between OgHS and OgTN pigs. Barrows were heavier (LW and HCW) at slaughter compared to gilts (127.3 ± 0.4 vs 125.5 ± 0.4 kg LW; 96.6 ± 0.8 vs 89.9 ± 0.9 kg HCW for barrows and gilts, respectively; $P < 0.01$).

Loin depth and LEA did not differ between pigs born to gHS or gTN dams ($P = 0.21$). Barrows yielded carcasses with greater BF compared to gilts (**Figure 3.1**; $P < 0.01$), and there was a tendency for pigs born to gHS dams to have greater BF than pigs born to gTN dams ($P = 0.11$). There were no gender x treatment interactions for BF thickness ($P = 0.97$). Barrows born to gHS dams had the shortest CRC L compared to other gender x treatment groups (**Figure 3.2**; $P = 0.02$)

Gilts had greater DRESS % than did barrows (**Figure 3.3**; $P = 0.04$), and there was a tendency for pigs born to gHS dams to have greater DRESS % than pigs born to gTN dams ($P = 0.14$). Similarly, gilts had greater LEAN % than did barrows ($63.4 \pm 0.4\%$ vs $60.3\% \pm 0.4\%$ for gilts and barrows, respectively; $P < 0.01$), but there were no differences related to the dam's gestational environment.

Discussion

The differences in LW at slaughter and carcass composition between barrows and gilts were expected. Newell and Bowland (1972) had previously reported increased ADG, decreased days on feed necessary to reach target market weights, and greater carcass adiposity of barrows compared to gilts, and these results were in agreement with those from the current study.

Regardless of the dam's gestational environment, barrows had greater LW at slaughter, HCW, and BF thickness, and lesser LEAN %, DRESS %, and CRC L than did gilts.

Formulas published by the National Swine Improvement Federation (NSIF, 2003) were used to calculate the days of age at a common market weight between treatments. There were no differences in the adjusted days of age at market weight between OgHS and OgTN pigs. Previously, Wilmoth et al. (2014) reported reduced weight at finishing of barrows exposed to gHS. Other studies, however, have failed to show differences between pigs directly exposed to either gHS or gTN (Johnson et al., 2015a; 2015b). Low numbers of observations in these studies (n = 6 pigs per treatment) may have contributed to the lack of treatment effect. The studies conducted by Johnson et al. (2015a; 2015b), however, did show reduced feed conversion efficiency of F1 gHS pigs. Results of the present experiment indicate that impaired growth rate is not detectable in the F2 generation.

Differences in the season of growth, finishing and slaughter would also make detecting differences in growth rate difficult on the farm. For example, pigs that are gestated under heat stress conditions in the summer grow to market in the mild temperatures of the fall and winter. Pigs gestated during the winter and farrowed in the spring, though, grow to market during the heat of the summer. Accurately comparing the on-farm growth performance, then, of pigs gestated during different seasons would be complicated, if not impossible. Similar season-related differences were observed in the current study, and were accounted for by including farrowing replicate in the statistical model.

Previous carcass analysis of market pigs has revealed an increase in the rate of lipid accretion (292 g/d vs 220 g/d) and a 95% increase in the ratio of lipid to lean protein accretion of pigs in utero during gHS (Johnson et al., 2015b). Likely, this increased body adiposity is due to increased circulating insulin concentrations. Pigs exposed to gHS exhibit 33% greater insulin levels compared to pigs gestated under TN conditions (Boddicker et al., 2014). Similar

hyperinsulinemia and body adiposity has been found in calves exposed to gHS in late gestation (Tao and Dahl, 2013).

The carcass characteristics of OgHS and OgTN pigs in this study indicate that the adipose-promoting effects of gestational hyperthermia may be inheritable. Pigs born to gHS dams tended to have greater carcass adiposity, as indicated by tendencies for greater BF thickness at slaughter. Although not statistically significant in this study ($P = 0.21$), the numerically greater calculated carcass LEAN % of pigs born to gTN dams ($62.2 \pm 0.4\%$ vs $61.5 \pm 0.4\%$, respectively) may further imply persistent effects of gHS across generations.

The reduced CRC L of only barrow OgHS pigs is interesting. Stahly and Cromwell (1979) reported a positive linear effect of increasing ambient temperature on CRC L. The greater body length of swine grown under heat stress conditions, as well as the reduced length of swine grown during cold stress, likely exists as a mechanism through which the animal alters their surface area:body mass ratio in an effort to conserve or dissipate heat. It is also known that gHS can influence body development. Alexander and Williams (1971) showed that developmentally heat stress lambs exhibited comparatively longer bodies with larger skulls, brains, kidneys, and pituitary and adrenal glands, but smaller livers, thyroids, and bicep muscles. A longer CRC L would therefore be anticipated in OgHS pigs. It is unknown why the CRC L of only OgHS barrows was reduced, and not increased.

The increased DRESS % of OgHS pigs could provide further phenotypic evidence of increased whole-body adiposity in the F2 generations. Zu et al. (2010) previously showed that increased body adiposity of finishing pigs resulted in greater DRESS % at slaughter. However, the increased DRESS % of OgHS pigs in the present study may also have been due to the previously described differences in organ weights commonly observed during gHS. The weights

of the head and visceral organs were not collected and recorded in the present study, and therefore the precise reason for differences in DRESS % between treatments cannot be determined.

At this time, it is unknown what mechanisms may be responsible for multi-generational effects. Characteristics of the offspring of the gestationally-treated dams could differ as a result of altered maternal physiology or as a function of true epigenetic modifications to the animal's genome. For example, studies in ruminants have shown that heat stress during gestation reduces placental size, blood flow, and oxygen and glucose transfer to the fetus (Bell et al., 1987; Thureen et al., 1992). These changes result in a smaller fetus that is hypoxic and hypoglycemic compared to fetuses developing under TN conditions (Reynolds et al., 1985). These smaller fetuses also exhibit discrepancies in organ development, with the liver being most severely affected by gHS (Reynolds et al., Dreiling et al., 1991). When adjusted for day of gestational age, Reynolds et al. (1985) found that the livers from heat stressed bovine fetuses weigh only 61% of livers from thermoneutral fetuses (173 g vs 284 g, respectively). These livers also had reduced total RNA and protein content, possibly indicating a reduced capacity for protein synthesis throughout life (Reynolds et al., 1985). Reduced liver weight in pigs as result of gHS has also been documented (Johnson et al., 2015a). Although not measured in the current study, reduced liver size and hyperinsulinemic state of F1 pigs affected by gHS may have created developmental conditions that led to the increased body adiposity of their F2 offspring.

Epigenetic modifications prompting altered phenotypes as a result of heat stress have not yet been described in domestic species, but have been elucidated in lower organisms. Heat shock during embryogenesis in *Drosophila* results in transcription factor phosphorylation and disrupted heterochromatin formation of stress-related genes (Seong et al., 2011). Generational experiments

with these flies show that when heat stress was applied to the F1 generation, similar gene silencing was observed in the F2, but not F3 to F5 generations. However, when heat stress was applied to both the F1 and F2 generations, partial silencing was observed in the F3 to F5 generations (Seong et al., 2011), indicating that the intensity of epigenetic change of some genes may be related to the intensity or duration of stress exposure. Thermal stress induces similar epigenetic outcomes with transgenerational implications in rodents (Tetievsky and Horowitz, 2010), fish (Takle et al., 2005; Meier et al., 2014), and plants (Molinier et al., 2006). Although yet to be demonstrated, it is assumed that the response of higher mammals to developmental hyperthermia is at least partially the result of epigenetic modifications as well (Johnson, 2014). It would be difficult to state from the present experiment whether the differences in body composition in the F2 generation are due to altered maternal physiology or imprinted epigenetic modifications. More research will be required to better understand the effects of gHS across generations.

Figure 3.1: Backfat thickness of gilts and barrows born to either gHS or gTN dams (TRT $P = 0.11$; Gender $P < 0.01$; TRT x Gender $P = 0.97$)

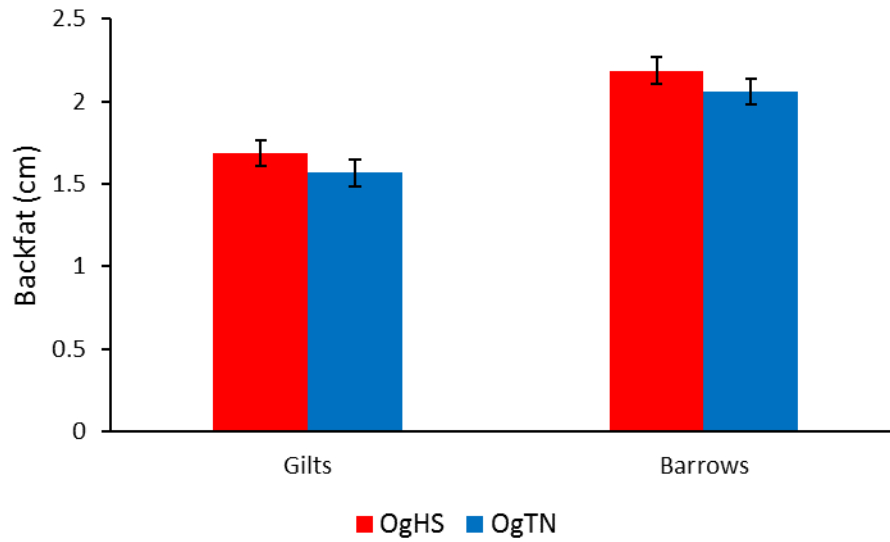


Figure 3.2: Carcass length of gilts and barrows born to either gHS or gTN dams. (TRT $P < 0.01$; Gender $P = 0.07$; TRT x Gender $P = 0.02$)

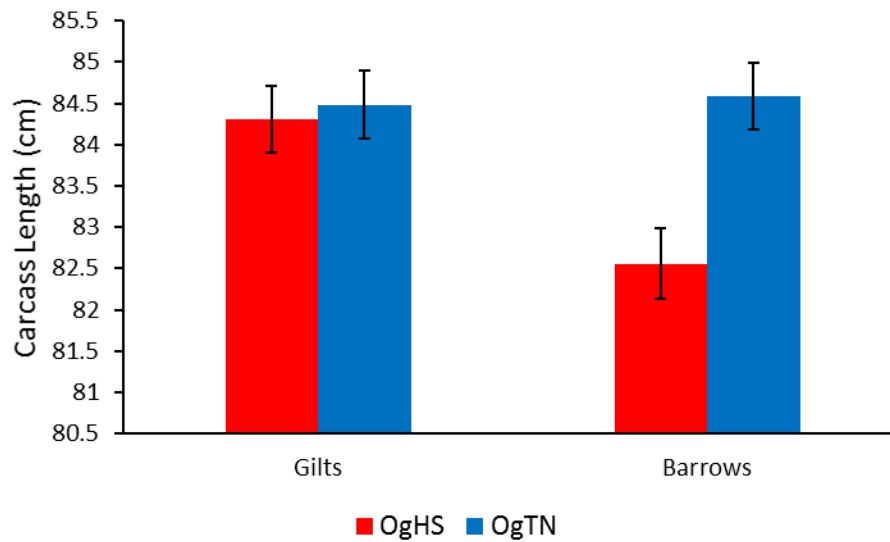
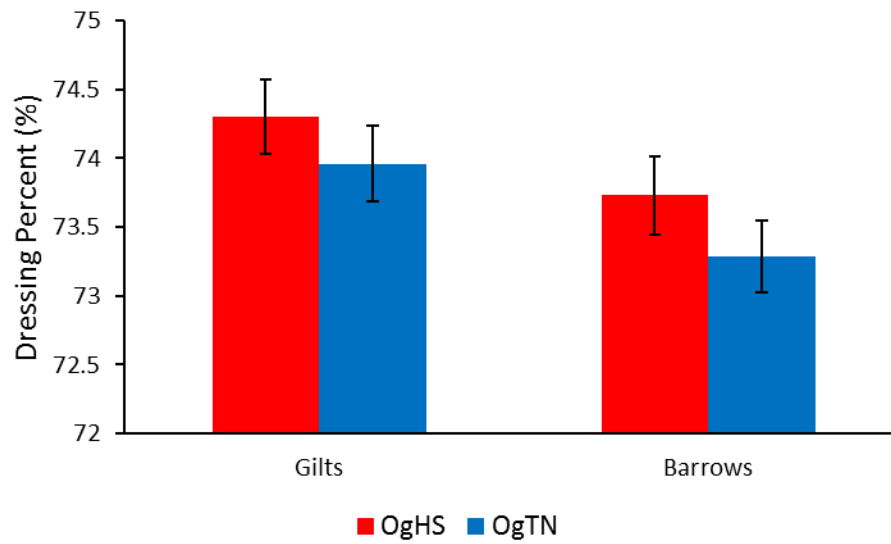


Figure 3.3: Dressing percentage of gilts and barrows born to either gHS or gTN dams (TRT $P = 0.14$; Gender $P = 0.04$; TRT x Gender $P = 0.85$)



Chapter IV: Implications for Science and Industry

The results of the present experiments confirm that gestational heat stress slightly impacts the milk nutrient content of F1 gilts, and suggests that overarching effects may linger through multiple generations to affect the body composition of offspring. This supports previous work completed at collaborating universities, which showed that pigs exposed to hyperthermia during intrauterine development displayed increased their daily adipose deposition at the expense of lean tissue accretion. That the effects of heat stress during pregnancy on the grandparent stock are detectable in the F2 generation is compelling phenotypic evidence, and may indicate epigenetic modifications to the genome of affected fetuses. Future research will be required to understand the responsible physiological and epigenetic mechanisms responsible for these phenotypes. Emphasis should be placed on gilt metabolism, and how the altered hormone profiles previously observed in prior work may interact with reproductive performance. Hyperthermia during fetal development may also impact pig reproductive physiology directly, possibly by negatively affecting primordial germ cells in the developing gonads. Similar pursuits would be worthwhile in the male reproductive tract, as well. The research presented herein provides the grounds to pursue these fundamental endeavors.

Pigs are bred year-round on swine farms, meaning that for at least three months of the year in most locations, breeding females will be under some degree of heat stress while pregnant. At least 25% of the breeding stock and their progeny, then, can be assumed to be impacted by gHS. In the pyramidal organization of the modern swine industry, nucleus farms are likely to be the most affected by gestational heat stress. Nucleus herds are used to make rapid genetic changes within maternal-type sow lines, usually in the areas of litter size, milk yield, and feed-efficient lean gain. Superior females produced on nucleus farms are transported to multiplier or

commercial farms, depending on the scale of the operation. Multiplier herds simply multiply the maternal genetics into a large enough breeding herd to sustain commercial production.

Commercial farms breed the maternal-type females produced on nucleus and multiplier farms to terminal-type boars to produce a good meat-producing market hog.

The results of this experiment indicate that the traits selected for on nucleus farms, especially lean gain, are negatively affected by gestational heat stress and may be passed through generations. Therefore, females suffering gestational heat stress on nucleus farms will produce offspring that are of inferior quality compared to females that are bred during the fall, winter, or spring. These inferior breeding females will then proliferate undesirable phenotypes through multiplier and commercial herds and reduce the total productivity of the entire swine operation. Extension efforts to inform producers of the multi-generational impacts of gestational heat stress should therefore be focused on nucleus production, with emphasis placed on good recordkeeping of swine lineages and analysis of performance data over generations.

The results of this experiment and of previous experiments conducted in the field should also provide incentive for all breeding swine farms to invest in heat abatement strategies to alleviate the negative consequences of heat stress. Many producers are aware of the direct effects of heat stress on their breeding herd (decreased conception rates and increased abortions, in particular) but may fail to realize that the growth and performance of these pigs affected by hyperthermia during intrauterine development will be impaired for many months after summer has ended. Monetizing the true costs of gestational heat stress on large-scale production farms will be difficult, but will be required to convince producers to invest heavily in automated fans, misters, sprinklers, and cooling pads to maintain the production and welfare of their herd.

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