INTERACTIVE EFFECTS OF NUTRITION, ENVIRONMENT, AND PROCESSING ON FRESH PORK QUALITY, INTESTINAL BIOMARKERS OF HEAT STRESS IN SWINE, AND CAREER SUCCESS FACTORS FOR AGRICULTURAL STUDENTS

by

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For my family.

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"I would maintain that thanks are the highest form of thought,
and that gratitude is happiness doubled by wonder."
- G. K. Chesterton

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ABSTRACT

Author: Feldpausch, Julie, A. PhD
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Title: Interactive Effects of Nutrition, Environment, and Processing on Fresh Pork Quality, Intestinal Biomarkers of Heat Stress in Swine, and Career Success Factors for Agricultural Students.
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Heat stress (HS) induced changes in energy metabolism, proteolysis, lipogenesis, and oxidative balance have meat quality ramifications for livestock. However, several knowledge gaps exist in understanding heat stressed finishing pig physiology and pork quality characteristics and how dietary zinc may ameliorate undesirable outcomes. Research was completed to determine zinc supplementation effects on carcass composition, meat quality, and oxidative stability of fresh and processed pork from pigs subjected to a chronic, cyclic heat stress using a 2×2×2 factorial arrangement of treatments with main effects of environment (HS vs. thermoneutral; TN), added zinc level (50 vs. 130 mg kg^{-1} available zinc), and zinc source (inorganic vs. organic). Commercial crossbred mixed-sex pigs (initially 72.0 kg) were group-housed under either TN (18.9-16.7°C) or cycling HS conditions with chronic diurnal heat (30-29°C/26-27°C for 12h:12h) on days 24-71 with acute heat waves (32-33°C/29-30°C for 12h:12h) on days 21-24, 42-45, and 63-65. One representative pig (n=80) per pen was slaughtered on day 64. The HS pigs were lighter bodyweight (P=0.039), yielded lighter carcasses (P=0.011), less last rib backfat (P=0.032), tended to have smaller loin eye area (P=0.062) but similar percent lean in belly center slices (P>0.10). Compared to TN, HS carcasses had higher 24-h pH (P=0.001) and decreased drip loss (P=0.034). Shifts in individual fatty acid profile of sausage product derived from HS carcasses were observed but were of insufficient magnitude to affect iodine value. Initially, sausage from HS carcasses tended (day

0, *P*=0.071) to have less thiobarbituric acid reactive substances than TN but over a 10-day simulated retail display, no treatment induced lipid oxidation differences (*P*>0.05) were observed in either sausage or displayed loin chops. Consistent treatment differences in CIE L*a*b* of products throughout the 10-day display were not observed. The relationships between physiological changes in pigs receiving supplemental zinc and their body and ambient temperatures were also investigated. A representative gilt (n=96) was selected for thermal monitoring from each pen of the 2×2×2 treatments plus 4 additional treatments representing 2 intermediate levels of Zn in both environments. Core body temperatures (T_{core}) during the day 42-45 acute heat wave were continuously recorded via indwelling vaginal thermometers and infrared thermal imaging was used to measure skin temperatures at 12-hour intervals. From a 64-gilt subset of the 2×2×2 treatments, jejunum and ileum samples were collected on day 64 for analysis of villus height, crypt depth, and jejunal gene expression of heat shock proteins (27, 70, 90), occludin, and mucin (MUC2). The HS model induced thermoregulatory changes and increases in T_{core} (*P*<0.05). Day 42-45 ambient temperature was negatively correlated with expression of HSP-27 (r=-0.42, *P*=0.047), HSP-90 (r=-0.49, *P*=0.014), and occludin (r=-0.69, *P*<0.001) in HS pigs. For the organic Zn supplemented pigs, ambient temperature was positively correlated with expression of HSP-27 (r=0.42, *P*=0.034) and MUC2 (r=0.45, *P*=0.017) and negatively correlated with villus height in jejunum (r=-0.42, *P*=0.027) and ileum (r=-0.38, *P*=0.048). Thermal Circulation Index (measure of heat dissipation) of HS pigs was negatively correlated with their ileum villus height (r=-0.51, *P*=0.015) and positively correlated with HSP-70 expression (r=0.46, *P*=0.041). The T_{core} lacked correlation with most variables. This research demonstrates cyclic HS affects carcass composition and quality but does not appear to reduce display shelf-life of pork as indicated by lack of differences in lipid oxidation and color stability. In this HS model, zinc level or source imparted

negligible benefits and thermal correlations with gut integrity characteristics existed for organic zinc supplemented and HS pigs. The degree of heat dissipation by heat stressed pigs appeared to be associated with classic HS damage and intestinal responses which may be useful indicators of HS in the grow-finish pig. Another agricultural challenge is maintaining higher education programming which establishes a successful career trajectory for agricultural students amid generational shifts in attitudes and background experiences. Undergraduates studying Animal Science and/or Agricultural Economics were surveyed to understand their perception of how collegiate curricular, co-curricular, and extracurricular experiences (coursework, club participation, relevant work experience, international experience, advising/mentoring, college life, and professional networking) contribute to their anticipated career success. A best-worst scaling experiment was used to force respondents (n=487) to make unbiased tradeoffs between the collegiate experience attributes. Responses were then related back to additional demographical and experience/perception characteristics of respondents. Students indicated relevant work experience was overwhelmingly the most critical of the 7 factors (57% preference share), followed by professional networking (19%), and coursework (14%). Students solely in a pre-veterinary Animal Science curriculum represented a distinct category of students regarding their beliefs and experiences. Further research is needed to investigate possible disconnects between student perceptions and reality in higher education and agricultural careers.

CHAPTER 1. REVIEW: PORK QUALITY CHANGES CAUSED BY PHYSIOLOGICAL RESPONSES TO HEAT STRESS.

1.1 Introduction

Heat stress (HS) occurs when environmental temperature rises above the thermal neutral zone of the animal and the animal begins to expend energy and/or rely on evaporative heat loss i.e. increased respiration rate, to maintain body temperature (NRC, 1981; Kingma et al., 2012; Brownstein et al., 2017). Short term exposure to heat induces rapid homeostatic responses which can be measured within minutes or hours while long term heat exposure causes acclimatory responses over days or weeks (Bernabucci et al., 2010; Collier et al., 2019). Understanding the responses to both acute and chronic heat are critical, with both being directly relevant to addressing practical industry concerns.

The widespread impact of HS encumbers pork production efficiency at every stage from reproduction (reviewed by Hansen, 2009) to market transport survival (Haley et al., 2008; Correa et al., 2013; Peterson et al., 2017). Heat stressing environmental conditions cause visible disruptions in the production cycle by reducing pig growth rate, decreasing market weight and/or increasing days to market (Renaudeau et al., 2011; Cruzen et al., 2015a; Wu et al., 2019). Meat quality could potentially be affected by chronic heat exposure occurring throughout the grow-finish phase as well as by short term heat exposure during transport handling and shipping (Schrama et al., 1996). Numerous reviews have summarized the effects of HS on various aspects of agricultural production yet there is an absence of any comprehensive review of the impact of HS on pork quality.

Various economic analyses have conjectured the impact of HS on the U.S. pork industry is over $315 million dollars annually, negatively impacting every aspect of production from animal

health to reproductive losses (Key et al., 2014; St-Pierre et al., 2003). Minimal research has sought to quantify the impact of HS on pork quality, and an industry failure to do so may lead to gross underestimation of the costs associated with HS. If the negative effects of HS are not appropriately allocated, production systems risk forgoing opportunities to maximize product value and could undervalue heat mitigation strategies. Hence, understanding the physiological and meat quality characteristics of heat stressed finishing pigs is critical.

Therefore, the objective of this literature review is to summarize current understanding of the physiologic responses of the pig to antemortem heat exposure and the implications for pork quality. Production-level considerations of the HS response are recounted in Part I, and then the corresponding physiological responses are summarized in Part II. The physiological context is the biochemical underpinning for the pork quality considerations which are then reviewed in Part III. Several knowledge gaps and outstanding areas for future research are identified throughout the review.

1.2 Part I. Productive Responses to Heat Stress

Wholistic survival mechanisms during hyperthermia include both reducing endogenous heat production as well as increasing heat dissipation to the surrounding environment. The pig modulates heat production through behavioral changes such as decreased physical activity (Kerr et al., 2003) and decreased feed consumption. These changes are important to note because they affect the physiological changes which are observed under HS.

1.2.1 Feed Intake

As part of their behavioral acclimation to HS, hyperthermic pigs have reduced nutrient intake due to lower feed consumption (White et al., 2008; Le Bellego et al., 2002). The magnitude

of reduction depends not only upon the magnitude and duration of temperature, but also on genotype, body weight, diet, housing, and health status (see reviews by Nyachoti et al., 2004; Qingyun and Patience, 2017). Older data suggested a 60 - 100 g reduction in daily voluntary feed intake for every 1 °C increase in ambient temperature above the pig's thermoneutral zone (NRC, 1981) while more recent studies have placed the reduction between 40 - 106 g (Nyachoti et al., 2004; Huynh et al., 2005b). A recent meta-analysis by da Fonseca de Oliveira and colleagues (2019) reported a 266 g (12%) average total reduction in feed intake among grow-finish pigs experiencing high temperatures at or above 29 °C for durations ranging from 5 days to 42 days.

Feed intake modeling predicts less of a reduction in feed intake during diurnal (cyclic) heat compared to constant exposure; yet as ambient temperatures increase above 30 °C, the advantage of the diurnal cooling is less anticipated due to greater time spent above the thermoneutral zone (White et al., 2015). Feed intake suppression also appears to wane as duration of thermal exposure increases although more detailed time series data is needed to better quantify this phenomenon (Patience et al., 2005; White et al., 2015). In heat stressed animals, the postprandial rise in body temperature appears to be exacerbated in the later hours of the day, possibly influenced by additive thermal stress (Cervantes et al., 2018). Indeed, behavioral responses to diurnal heat such as shifting meal consumption to cooler nighttime hours (Quiniou et al., 2000) may lessen the effects of long-term heat exposure on feed intake and growth performance.

1.2.2 Water Intake

Relative to pigs housed under thermoneutral conditions, finishing pigs housed under experimentally induced HS have been reported to increase their daily water intake by 34% (Song et al., 2011). In another experiment, water disappearance rate almost doubled (Mills et al., 2018) as finishing pigs sought to cool themselves and maintain satiety. Water consumption is a means of

cooling internal body temperature through transfer of body heat from internal tissues to gastrointestinal water content and also facilitates evaporative cooling from the surface of the animal when pigs spray themselves with water (see Figure 1.1). The effectiveness of these cooling mechanisms are dependent upon relative temperature of the water and upon relative humidity and air movement.

1.2.3 Body Composition

Commercial data clearly documents the dramatic reduction in body weight and hot carcass weight of finishing pigs experiencing naturally occurring environmental heat (Fragomeni et al., 2016). An attempt to replicate these effects in research settings by long-term exposure to constant high temperature (30 °C) for 55 days yielded an 18 kg growth reduction due to decreases in both protein and lipid accretion (dos Santos et al., 2018). As reviewed by Ross et al. (2015), HS induced compositional changes have been documented in pigs including the accumulation of triglycerides in body lipid rather than partitioning the reduced nutrient intake to lean growth according to classical growth curves. Heat stress has been shown to reduce protein accretion in high-lean-growth pigs but may not reduce protein deposition among moderate growth lines (Nienaber et al., 1997). Similarly in poultry, chronic HS has been shown to decrease protein content and proportional breast muscle weight (Zhang et al., 2012; Zeferino et al., 2016). Simultaneously, this long-term heat exposure can increase lipid content in poultry carcasses, breast muscle, and abdomen (Lu et al., 2007; Zhang et al., 2012; Zeferino et al., 2016; Lu et al., 2017).

Body composition can also be altered with reduced liver and heart organ weights which have been observed in chronically heat stressed broilers (Zeferino et al., 2016). Reduction in porcine organ weights and maintenance requirements (Nienaber and Hahn, 2002) impart adaptive advantage to pigs under HS. In addition, chronic heat stressing conditions stimulate water

consumption by animals and have been shown to cause water retention in broilers (Zhang et al., 2012; Sayed and Downing, 2015) although acute heat exposure (12 hours) can actually decrease breast muscle moisture (Hu et al., 2016). Altogether, these compositional changes reflect metabolic adaptations which are occurring within the heat stressed animal.

1.3 Part II. Physiological Changes in Response to Chronic Heat Stress

1.3.1 Energy and Cellular Metabolism

At any given instant, the disrupting impact of HS is being more or less countered by the animal's adaptive response to the stress. Thus, determination of the stressor's impact on a dynamic system such as energetic signaling can be obscured by the degree of adaptation occurring within the animal's system. Nevertheless, the study of the energetic processes induced by HS establishes the foundation for understanding the proteolytic and lipogenic processes occurring under HS.

It is generally thought that HS induces a non-diabetic insulin resistant phenotype where circulating insulin levels rise without concomitant increase in blood glucose clearance and uptake by cells (reviewed by Baumgard and Rhoads, 2013; summarized by Zhao et al., 2018). In pigs, inhibited insulin signaling and reduced glycogen synthesis have been observed under short-term ($\leq$ 12 hours) exposure to heat (Ganesan et al., 2018c) while after extended (7 days) heat exposure, differences in insulin and glucose to insulin ratio were not detectable (Zhao et al., 2018). The impact of this hormonal status under chronic HS on metabolic signaling within the cell is not yet clearly delineated.

Several studies indicate that constant, chronic HS disturbs mitochondrial function and anaerobic metabolism. Studies of avian and porcine skeletal muscle under chronic heat exposure have demonstrated increased pyruvate dehydrogenase lipoamide kinase isozyme 4 (PDK4), a mitochondrial enzyme which increases flux through oxidative rather than glycolytic energy

pathways (Hao et al., 2016; Lu et al., 2017). Accordingly, chronic HS has been found to transcriptionally downregulate several glycolytic enzymes including glucose-6-phosphate isomerase, phosphofructokinase, triosephosphate isomerase, pyruvate kinase isozymes, and lactate dehydrogenase-A in porcine skeletal muscle (Hao et al., 2016).

However, there is not strong evidence that oxidative metabolism is concomitantly upregulated in lieu of glycolysis. Poultry research indicates oxidative metabolism might be suppressed under chronic heat stress due to decreased gene expression of peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) and its activator sirtuin1 (SIRT1) (Liang and Ward, 2006; Rahman and Islam, 2011; Wan et al., 2018). Indeed, despite the reported reduction in porcine glycolytic gene expression (Hao et al., 2016), other data indicate chronic HS does not change the actual activity of porcine phosphofructokinase (rate limiting enzyme for glycolytic pathway; Zhao et al., 2018) and actually increases activities of both pyruvate kinase (catalyzes final step of glycolysis; Zhang et al., 2012; Lu et al., 2017) and lactate dehydrogenase (converts pyruvate to lactate at the completion of glycolysis; Lu et al., 2017) in broilers. The apparent increase in activity of lactate dehydrogenase is corroborated by observations of increased lactic acid accumulation in skeletal muscle of both species under chronic HS (Zhang et al., 2012; Hao et al., 2016). A reduction in pyruvate substrate for entrance into the tricarboxylic acid (TCA) cycle and oxidative phosphorylation might explain the apparent decrease in citrate synthase activity which catalyzes the first step of the TCA cycle across species (Lu et al., 2017; Zhao et al., 2018). Detailed investigation by Zhao et al. (2018) showed that HS did not affect either the phosphorylation or the activity of the porcine pyruvate dehydrogenase complex (PDH) which determines pyruvate flux into the TCA cycle. The decreased citrate synthase activity may indicate less oxidative metabolism through the TCA cycle and might also indicate less intact mitochondria (Zhao et al., 2018).

In accordance with classical metabolic prioritization under energy deficient conditions, an extended period of HS with its concomitant reduction in feed intake has been shown to increase fatty acid β-oxidation in skeletal muscle of pigs. Heat stress increased the activity of β-hydroxyacyl coenzyme A dehydrogenase (required for fatty acid β-oxidation in the mitochondria; HAD); this increase coincided with the aforementioned downregulation of major glycolytic pathway genes and upregulation of PDK4 (Hao et al., 2016).

However, there is other evidence suggesting β-oxidation of free fatty acids is actually suppressed by chronic exposure to HS. Gene expression of mitochondrial carnitine palmitoyltransferase I (CPT1), which is critical for the transport of long-chain free fatty acids into the mitochondria for β-oxidation, has been shown to be downregulated under HS in both broilers and pigs (Lu et al., 2017) although the transcript level does not necessarily correspond to any change in protein abundance (Zhao et al., 2018). Under pair-feeding situations, HS has been shown to decrease activity of the β-oxidation enzyme HAD in skeletal muscle (Wu et al., 2016). Thus, it has been hypothesized that a HS induced reduction in β-oxidation contributes to elevated levels of non-esterified fatty acids (NEFA) in circulation (Wu et al., 2016).

This lack of conclusivity has spawned intensive research to identify possible disconnects between oxidative metabolism signaling and apparent lack of mitochondrial function.

Mitochondrial degradation has been observed within the first 6 hours after the onset of heat exposure; this mitophagy is not sustained but subsides with time (Ganesan et al., 2018b). Following 1 to 3 days of HS, the semitendinosus muscle of pigs showed decreased (in oxidative portion; STR) or unchanged (in glycolytic portion; STW) markers of mitophagy, as well as increased mitochondrial protein abundance in the STR (Brownstein et al., 2017). After 7 days of HS, the STR muscle exhibited no change in mitophagy or mitochondrial content (Ganesan et al.,

2018a). These studies further substantiate the temporal and spatial complexity of heat stress's metabolic impact and elicitation of the adaptive response in the pig.

1.3.2 Lipid Deposition

Heat stress has important implications for lipid metabolism across species, appearing to depress lipolytic potential and result in greater lipid accumulation than energetically expected (reviewed by Baumgard and Rhoads, 2013; reviewed by Rhoads et al., 2013a; Qu et al., 2015; Kellner et al., 2016; Qu and Ajuwon, 2018). Through controlled pair-feeding experiments, it has been shown that exposure to constant, chronic HS increases abdominal and intermuscular lipid deposition relative to thermoneutral reared animals on an isocaloric basis (Lu et al., 2007). However, anticipating practical differences in body fat deposition under HS is complicated by the fact that one of the most ubiquitous responses to HS is a reduction in feed intake. This nutrient reduction causes growth attrition and slows anabolic tissue accretion consequently masking thermally induced changes to lipid metabolism and composition. Accordingly, numerous reports exist of extended periods of HS reducing pork carcass adiposity relative to that of thermoneutral counterparts (White et al., 2008; Hausman et al., 2014; Cruzen et al. 2015a; Kellner et al., 2016; Cui et al., 2018; Simonetti et al., 2018). However, comprehensive analyses of some data sets reveal subtleties which imply certain fat depots i.e. abdominal and intramuscular, might be more adept at maintaining adiposity amid HS conditions while other depots such as subcutaneous concurrently show reduced lipid accretion (Rinaldo and Le Dividich, 1991; Lu et al., 2007; Cruzen et al. 2015a). Indeed, different fat depots may respond differently to nutrient restriction with the middle layer subcutaneous backfat being the most metabolically active backfat layer in the pig (Hausman et al., 2014).

It has been suggested that reduced β-oxidation and increased circulating NEFA stimulates increased hepatic VLDL release which adipocytes work to clear by increasing lipoprotein lipase (LPL) in heat stressed pigs (Wu et al., 2016; Kouba et al., 2001). Increased circulating NEFA levels, indicative of a shift in the relative rates of systemic lipolysis and cellular uptake, have been observed in grower pigs after 12 hours of HS (Pearce et al, 2015) and in weaned pigs after 3 weeks of HS (Kouba et al., 2001). However, HS models are not always successful in eliciting the increased NEFA response (Wheelock et al., 2010). The interplay between hepatic, muscle, and adipose lipid metabolism in the heat stressed grow-finish pig is poorly characterized and in need of further investigation.

Fatty acid synthesis appears to be downregulated in heat stressed pigs. Chronic heat exposure has been reported by Hao and colleagues (2016) to decrease protein abundance of the rate-limiting reaction catalyst for fatty acid synthesis, acetyl coenzyme A carboxylase (ACC), as well as that of fatty acid synthase (FAS). Furthermore, the gene expression of stearoyl-CoA desaturase (SCD) (also known as delta-9 desaturase, or acyl CoA desaturase) was also decreased (Hao et al., 2016). In porcine skeletal muscle, 7 days of HS depressed ACC transcript and protein abundance while ACC tended to be less phosphorylated (Zhao et al., 2018) perhaps in response to glucagon, an endocrine signal upregulated under stress (Berg et al., 2002; Jones et al., 2012; Rhoads et al., 2013b). Consistent with these observations, de novo synthesis appears to be suppressed in the pig even after accounting for the disparate feed intake; thermal stress reduced the activity of malic enzyme (ME) in adipose tissue, decreased ACC level in skeletal muscle, and decreased FAS level in both skeletal muscle and in adipose tissue (Wu et al., 2016). Reduced abundance of FAS has also been reported in skeletal muscle of pigs under acute HS (Ganesan et al., 2018c). Contradictorily, in skeletal muscle of pair-fed chickens, HS purportedly increases

intramuscular lipid content and upregulates gene expression of ACC and FAS (Lu et al., 2017). Further research is needed to determine phosphorylation status of ACC and validate the downstream effects of this apparent upregulation of de novo synthesis across tissue types in the bird.

In addition to changes in accretion and depletion, HS can affect other aspects of adipose tissue in the pig. Unless validated with analytical chemistry, apparent changes in adipose accretion as measured by weight or depth may be wholly or partially reflective of a higher moisture content in adipose tissue which has been observed in some fat depots of thermally challenged pigs (Seibert et al., 2018). It has been proposed that this phenomenon could be due to more immature adipocytes with smaller lipid droplets. However, increased water content may not be limited to adipose tissue as increased moisture relative to protein content in muscle tissue has been reported under chronic HS (Cruzen et al., 2015a). Further research on porcine adipocyte development and metabolism under HS at various stages of production would be edifying.

1.3.3 Muscle Proteolysis

Degradation of myofibrillar proteins occurs through the action of the calpain proteases and their inhibitor, calpastatin (Huff-Lonergan et al., 2010). Faster growth rates in swine antemortem correspond to greater activity levels of μ- and m-calpain, with μ-calpain and calpastatin activity at time of slaughter affecting postmortem tenderization (Kristensen et al., 2002). Thus, it might be anticipated that the reduced growth rate of heat stressed animals results in less tender meat yield.

The impact of HS on meat tenderness is not fully characterized, however. Insulin resistance, a phenomenon seemingly induced by HS, appears to activate the ubiquitin-proteasome proteolytic pathway thus increasing muscular protein degradation; furthermore, this proteolysis is empirically augmented by glucocorticoid production and reduced level of adiponectin in circulation (Wang et

al., 2006). Metabolomic analysis of blood metabolites indicates degradative proteolysis of skeletal muscle antemortem may increase in porcine muscle under HS (Wang et al., 2016).

Autophagy, another degrative pathway, is typically associated with a low energetic state because of its catabolic release of macromolecules which can be repurposed in the body amid nutrient-depletion; autophagy also functions to clear damaged proteins and other structures from cells (Rubinsztein et al., 2012). The degrative impact of HS appears transient, especially during the first 12 hours of heat exposure, and dependent upon muscle fiber type (Cruzen et al., 2017). Within 6 hours of HS, increased muscular autophagy is apparent but not sustained over time (Ganesan et al., 2018b). After 1 - 3 days of HS, an accumulation of autophagosomes and decreased autophagosome degradation by lysosomes has been observed in oxidative semitendinosus (STR) muscle; neither of these changes was evident in the glycolytic portion of the semitendinosus (STW) (Brownstein et al., 2017). After 7 days of HS, the STR muscle shows increased autophagic initiation signaling and phagophore formation but no corresponding change in autophagosome degradation (Ganesan et al., 2018a). Moreover, the abundance of lysosomal protease cathepsin-L present in the STR is increased after 1 - 3 days of HS (Brownstein et al., 2017) but is reportedly decreased after 7 days of HS (Ganesan et al., 2018a) further reinforcing the transient nature of this intracellular metabolism. Further research is needed to quantify the impact of thermal stress and its associated reduction in animal growth rate on meat tenderness factors across muscles and heat stress duration (see Figure 1.2).

1.3.4 Acid-Base Homeostasis

The physiologic balance between acidity and alkalinity is affected by changes in intake, endogenous production, and elimination of acidic and alkaline compounds. The balance is globally assessed by the blood pH, carbon dioxide (CO_2), and bicarbonate (HCO_3^-) levels in circulation.

The acid/base homeostasis is maintained via the rapid action of the buffering system and lungs, and over a period of days by the kidneys (Hopkins and Sharma, 2019). As experienced by the heat stressed pig, an increase in respiration rate accelerates elimination of CO_2, a mildly acidic compound, thereby increasing blood pH and causing respiratory alkalosis (Bartko et al., 1984; Hamm et al., 2015). An acute heat exposure for 4 hours has been shown to reduce blood pCO_2 and increase pH in stress susceptible pigs (Aberle et al., 1974).

However, metabolic acidosis has also been reported in pigs under HS. This may be due to prevailing anaerobic metabolism elevating cytosolic lactic acid levels in skeletal muscle (Zhang et al., 2012; Hao et al., 2016), blood lactate, and lowering pH (Judge et al., 1973), as well as the buffering activity of the kidneys. Under chronic (7 - 11 days) diurnal heat exposure which induced a 7 to 8-fold increase in respiration rate, a reduction in blood HCO_3^- has been observed (Patience et al., 2005; Cottrell et al., 2017). While high levels of HCO_3^- reflect an alkalotic state, a reduction of HCO_3^- may be reflective of the endogenous homeostatic buffering response to alkalosis; indeed, reduced blood pH and pCO_2 were concomitantly reported (Cottrell et al., 2017). However, impaired renal filtration and resorption have also been implicated in heat stressed pigs due to observations of elevated levels of urinary metabolites and of circulatory creatine and blood urea nitrogen (BUN) (He et al., 2019). Granted, the creatine and BUN concentrations could also reflect increased proteolysis under HS. Metabolic acidosis has been shown to induce insulin resistance (DeFronzo and Beckles, 1979) which in turn is responsible for muscle protein degradation and wasting (Wang et al., 2006).

Chronic heat stressing conditions greatly increase water consumption, excretion through the respiratory, exocrine and renal systems, and have been shown to cause water retention in broilers (Sayed and Downing, 2015) when emission is outpaced by water intake and metabolic

production. It is conceivable, therefore, that heat stressing conditions strain renal functioning and jeopardize the maintenance of osmotic balance in the body. In broilers, 4 days of heat exposure depleted K^+ and Na^+ cation concentrations in the blood although pH, *p*CO2, HCO_3^-, and Cl^- concentrations were successfully maintained compared to thermoneutral levels (Sayed and Downing, 2015).

Further research would be useful to understand the impacts of thermal stress duration and severity on acid-base balance of swine under commercial conditions. Prevention of short-term respiratory alkalosis and long-term metabolic acidosis is fundamental in minimizing mortality and maximizing lean tissue growth. Under normal physiological conditions, short term bicarbonate feeding to pigs preharvest can improve early postmortem muscle pH and ATP levels, and improvements in muscle water holding capacity correlate with blood pH and HCO_3^- (Ahn et al., 1992). Prophylactic measures may exist to ameliorate the harmful effects of HS on acid-base balance in pigs.

1.3.5 Global Oxidative Stress

Increased oxidative phosphorylation under oxidative metabolism can negatively impact the redox status of an animal through the associated generation of reactive oxygen species which initiate further oxidation. However, endogenous enzymes such as superoxide dismutase (SOD) reduce harmful radicals into semi-stable hydrogen peroxide (H_2O_2) while glutathione peroxidase (GPx) and catalase convert the H_2O_2 into water (Lamprecht, 2015). Oxidative stress occurs when the endogenous reducing systems fail to counteract oxidation caused by endogenous and exogenous factors.

Pigs acutely exposed for $\leq$ 24 hours to temperatures of 35 - 37°C exhibit markers of musculoskeletal oxidative stress such as increased malondialdehyde (MDA) level, a measure of

lipid peroxidation (Volodina et al., 2017; Montilla et al., 2014). Although changes in protein carbonyl content (indicator of protein oxidation) did not accompany the transient increase in lipid peroxidation (Volodina et al., 2017), the antioxidant enzymatic response appears to be rapid and also transient with transcriptional upregulation of the antioxidative enzymes catalase and SOD-1, increased SOD-1 protein concentration, and increased activities of catalase and SOD-1 (Volodina et al., 2017; Montilla et al., 2014). Corroborative proteomics research also reveals redox changes with increased Mn-SOD and decreased peroxidases (Cruzen et al., 2015b).

After a longer heat duration over several days, indicators of global oxidative stress have been reported in pigs. Seven days of HS elevated MDA concentration in the oxidative portion of the semitendinosus (STR) despite higher amounts of SOD-2 protein; levels of SOD-1 and catalase were unaffected, and no differences noted in the activity of catalase or total SOD (Ganesan et al., 2018a). After 8 days of thermal exposure, plasma advanced oxidized protein products (uremic toxins formed by oxidative stress) were increased and a concurrent reduction found in red blood cell lysate GPx activity and plasma levels of metabolites of reactive oxygen species (Liu et al., 2017). In broiler breast meat, an extended 3-4 weeks of diurnal heat appears to suppress SOD and glutathione peroxidase activity, leading to increased MDA and free radicals; radical scavenging activity has also been reported to increase, and conflicting reports indicate catalase activity may or may not be impacted (Cramer et al., 2018a; Wan et al., 2018). It appears that the antemortem assaults on redox balance due to HS may be responsible for altered oxidative stability of meat postmortem but localized effects may depend on certain muscle specific factors. Ongoing research is being conducted to assess other antemortem factors inducing oxidative stress such as dietary components, disease, and mycotoxins (Boler et al., 2012; Kerr et al., 2018; Frame et al., 2019) which could exacerbate the redox balance under HS.

1.3.6 Cellular Stress Response - Heat Shock Proteins

Small heat shock proteins (HSP), characteristically known for their molecular chaperone activity, are involved in the cellular stress response. The HSP serve to correct misfolding of proteins and counteract apoptotic activity under normal physiological conditions and are further induced under various types of stress (Lanneau et al., 2007; Batulan et al., 2016) including heat. In poultry, chronic diurnal heat exposure has been shown to upregulate gene expression of HSP70 and HSP90 (Wan et al., 2018) and there is some evidence of chronic HS increasing HSP27 and HSP70 prevalence in the muscle (Cramer et al., 2018a). Similarly, long term (3 weeks) exposure to a hot environment upregulates gene expression of HSP27 (Hao et al., 2016) and HSP90 (Cervantes et al., 2016; Hao et al., 2016) in *longissimus dorsi* of pigs; however, this upregulation is not observed across all muscles (Cervantes et al., 2016) nor does it indicate how protein abundance and/or activity are affected by heat.

The unique functional niches of the different mammalian HSP may have important ramifications under the metabolic conditions induced by heat stressing environments. Upregulated under oxidative stress, HSP27 (also known as HSPβ1) induces an antioxidative response by lowering iron and elevating glutathione levels intracellularly, and by augmenting the degradative clearance of oxidatively damaged proteins (Arrigo, 2001; Arrigo et al., 2005; Lanneau et al., 2007; Batulan et al., 2016). Recent data also suggests HSP27 has a role in muscular glucose uptake thereby improving insulin sensitivity (Yuan et al., 2016). The HSP60 and HSP70 chaperones facilitate protein folding and translocation (reviewed by Voos, 2013). The HSP70 (also known as HSP72) can associate with lipid bilayers of membranes (Batulan et al., 2016) and transports other proteins across cellular membranes (Lanneau et al., 2007) having a distinct role in bringing partially-folded proteins into the mitochondria (see reviews by Voos, 2013, and by Craig, 2018). Its action is ATP dependent and blocks cathepsin release (Lanneau et al., 2007). The ATP

dependent HSP90 exists as either HSP90α or HSP90β proteins (Lanneau et al., 2007). Recent data suggests HSP90 sustains muscle mass by preventing muscle atrophy and promoting synthesis by phosphorylitic activation of protein kinase B (Akt) and the mTOR pathway (Hafen et al., 2019).

Because the small HSP may slow postmortem apoptosis and protein degradation, HSP potentially affects meat quality attributes such as tenderness and water holding capacity. Degradation of HSP27 has been strongly correlated to myofibrillar protein degradation, proteolytic and apoptotic enzymes, and meat tenderness in lamb (Cramer et al., 2018b). However, neither the mRNA expression nor abundance of HSP27 and HSP70 have proven to be useful biomarkers for beef quality (Moncau et al., 2018; Temizkan et al., 2019). Meanwhile, lower expression of HSP60 and HSP90 in the broiler breast muscle has been shown to correspond to poorer water holding capacity while HSP70 lacked this association (Zhang et al., 2017).

In pork, the relationship between HSP and quality is not yet established under either normal or stress-induced physiologic conditions. There is some evidence that greater levels of HSP90 correspond to more desirable pork color and water holding capacity under normal physiologic conditions (Zhang et al., 2014). Pigs with reduced HSP27, HSP70, and HSP90 gene expression in the *longissimus dorsi* due to acute transport stress also had poorer pH, drip loss, and meat color (Yu et al., 2009). However, upregulated HSP27 gene expression due to antemortem chronic heat exposure has been noted as undesirably correlating with pork lactic acid content and shear force (Hao et al., 2016). Whether these are strictly associations or reflect causal relationships needs to be determined. A genome-wide association study indicates differential expression of HSP genes might confer HS resistant phenotypes in pigs (Cross et al., 2018). Selection for greater HS tolerance among pigs could inadvertently reduce pork quality; however, a more thorough

understanding of how pork quality is impacted by HS induced changes in HSP regulation, abundance, and activity will help inform sound breeding selection priorities.

1.4 Part III. Impact of Chronic Heat Stress on Pork Quality

Little research has investigated the impact of HS on pork quality. In comparison, more extensive documentation of HS negatively impacting poultry meat quality exists. In poultry, the impact of HS has been predominantly studied in the pectoralis muscle. The pectoralis muscle is comprised of mostly type IIB muscle fibers which are highly glycolytic in their metabolism (Verdiglione and Cassandro, 2013). The HS responses of this muscle type might not be ubiquitous across other muscles types in a carcass. However, the longissimus muscle of pork is similarly characterized by predominance of type IIB muscle fibers and a highly glycolytic metabolism such that it might be similarly impacted by HS (Listrat et al., 2016).

1.4.1 Carcass Temperature

The combination of postmortem muscle pH and temperature, a function of chill rate, is an important determinant of pork quality due to a combined impact on postmortem glycogenolysis and metabolism (Scheffler and Gerrard, 2007). Carcass temperature decline is a function of several factors, including carcass size and composition, cooler chilling method, and postmortem metabolic heat generation. Research has demonstrated that numerical differences in longissimus temperature 45 minutes after exsanguination might not converge until 10 hours postmortem (Allison et al., 2003). Thus, differences in initial body temperature pre-exsanguination could have a prolonged impact on the postmortem temperature decline. Presently, there is little published data indicating how antemortem elevation of body temperature by HS impacts subsequent chilling rate and pork quality.

The extent to which carcass temperature throughout the early postmortem period is influenced by initial body temperature and/or by postmortem metabolic heat generation can be difficult to establish. At 45 minutes postmortem, there exists an inverse relationship between muscle temperature and muscle pH which is indicative of postmortem metabolism (Allison et al., 2003). Moreover, antemortem management which increases muscle temperature 45 minutes postmortem has been shown to correspond to lower pH while management which increases muscle pH 45 minutes postmortem has been shown to correspond to lower temperature (van der Wal et al., 1999; Tikk et al., 2006). Thus, any impact of initial muscle temperature on pork quality may be confounded by metabolic rate. However, Rosenvold et al. (2001) observed that antemortem exercise could induce higher muscle temperatures without concurrent changes in glycogen level and pH. Further research is needed to elucidate the impact of HS on muscle temperature and carcass cooling rate independent of postmortem metabolism and consequent pH.

1.4.2 pH

Both the rate of pH decline as well as the extent of the decline as indicated by the ultimate pH circa 24-hours postmortem are important indicators of meat quality. Carcass chilling has an impact on rate of pH decline although there is some evidence that the rate may be affected by carcass temperature only once carcass temperatures fall below 37 °C (Maribo et al., 1998). The extent of pH decline is affected by the accumulation of hydrogen ions produced through metabolic hydrolysis of ATP; the ultimate pH is therefore dependent on glycolytic substrate levels, enzymatic activity, and other factors presently undetermined (Allison et al., 2003; Scheffler et al., 2013; England et al., 2017).

In swine, prolonged heat exposure appears to reduce carcass pH (Hao et al., 2014; Cui et al., 2018; Simonetti et al., 2018) or cause no pH difference at all (Kellner et al., 2016). Similarly,

poultry breast muscle pH appears to be lowered by acute (Hu et al., 2016) and chronic HS (Akşit et al., 2006; Lu et al., 2017; Wan et al., 2018). However, 2 weeks of chronic heat exposure has been shown to increase poultry breast meat pH (Zeferino et al., 2016) and seasonal HS has been associated with greater ultimate pH values in ruminants (Kadim et al., 2004).

1.4.3 Water Holding Capacity

Drip loss is associated with the movement of water from within muscle fibers to the extracellular space (Pearce et al., 2011), a phenomenon which may be heightened under poor lipid membrane integrity and damaging conditions. Increased water loss has been reported in breast muscle from poultry exposed to both acute (Hu et al., 2016) and chronic heat (Dai et al., 2009; Lu et al., 2007; 2017; Wan et al., 2018) and in pork from pigs exposed to chronic heat (Simonetti et al., 2018). Most often, the observed reductions in water holding capacity correspond with reports of lower muscle pH but not all reports of HS reduced pH have found a difference in water holding capacity (Lu et al., 2007; Cui et al., 2018). Particularly for pork, more research is needed to delineate possible mechanisms responsible for reduced water holding capacity under HS.

1.4.4 Color

Meat color is largely determined by the concentration of color pigments, predominantly the heme iron containing molecule myoglobin, as well as by the form of the myoglobin molecule, which is determined by the oxidative and binding status of the heme iron within the molecule (Lindahl et al., 2001). Meat pH is considered one the greatest factors affecting meat color (AMSA, 2012). Low muscle pH increases denaturation risk of the myoglobin molecule and because of reduced water holding capacity, pH can indirectly affect light absorption and reflectance, sarcoplasmic protein precipitation, and myofilament space which are all associated with color changes (Joo et al., 1999; Lindahl et al., 2001; Swatland, 2004). Under low pH conditions,

formation of metmyoglobin is greater (Yin and Faustman, 1993) and reduction in oxygen consumption rate by mitochondria can augment the formation of oxymyoglobin, thus further lightening color and increasing redness (Faustman and Cassens, 1990; Li et al., 2011).

In poultry, increased lightness (L*) (Akşit et al., 2006; Dai et al., 2009; Zhang et al., 2012; Hu et al., 2016; Lu et al., 2017; Wan et al., 2018), and decreased redness (a*) (Akşit et al., 2006; Dai et al., 2009; Zhang et al., 2012; Hu et al., 2016; Wan et al., 2018) of breast muscle with lowered pH have been reported under both acute and chronic HS rearing conditions. However, there are also reports of increased L* (Lu et al., 2007; Zeferino et al., 2016) and lower a* (Zeferino et al., 2016) without concomitant reduction in pH of chicken breast from chronically heat stressed birds. Acute heat exposure has been shown to decrease b* in the breast (Hu et al., 2016). Yet there is a lack of evidence that chronic heat exposure affects yellow hue (b*) of poultry breast. However, Zhang et al. (2012) found decreased b* in the thigh but not the breast, suggesting chronic HS might differentially affect color of different muscles in the carcass.

In pigs, reported increases in L* and decreases in both red hue (a*) and yellow hue (b*) values in pork from HS carcasses corresponded to lower pH attributable to heat (Cui et al., 2018; Simonetti et al., 2018). In a longitudinal study by dalla Costa and colleagues (2007), pigs harvested during the summer season yielded paler meat compared to carcasses harvested during the winter season. Lower heme iron content in pork from heat exposed pigs has been reported by Simonetti et al. (2018). In addition, increased percentages of oxymyoglobin and metmyoglobin formation due to heat exposure (Simonetti et al., 2018) could indicate a suppression of endogenous reducing systems amid a HS induced oxidizing state. To date, there is a lack of data indicating whether or not chronic HS impacts color stability in chicken or pork in addition to initial color differences.

1.4.5 Tenderness

Meat tenderness is dependent upon several factors including background toughness (collagen content and type), sarcomere length or degree of shortening, and the rate and extent of postmortem proteolysis via the calpain system (Weston et al., 2002; Huff-Lonergan et al., 2010). Minor roles in postmortem tenderization are attributed to the lysosomal cathepsin proteases and to the proteases of the multicatalytic proteinase complex (Koohmaraie and Geesink, 2006). Activity of the calpain system is believed to be influenced by a myriad of variables including rate of muscle pH decline early postmortem (Huff-Lonergan et al., 2010). Under the protein denaturing conditions of low pH, autolytic activation of μ-calpain is reduced thereby stunting postmortem proteolysis (Kim et al., 2010).

Accordingly, 3 weeks of constant HS has been shown to increase porcine longissimus muscle shear force early postmortem. This change was accompanied by higher lactic acid levels, lower muscle pH, and upregulated HSP90 and HSP27 mRNA expression; the HSP27 expression positively correlated with shear force (Hao et al., 2016). Increased shear force with concomitant reduction in pH has also been reported for extensively raised swine during seasonal heat exposure (Simonetti et al., 2018). Thus, the effect of HS on pork tenderness is seemingly confounded with reductions in muscle pH.

The impact of HS on shear force of poultry breast meat has also been extensively reported. Results are inconsistent as there are reports of diurnal and constant chronic HS lowering pH and increasing shear force (Dai et al., 2009; Zhang et al., 2012), chronic constant HS (3 weeks of 34 °C) failing to impact either pH or shear force (Lu et al., 2007), and chronic 14 days of constant 32 °C HS decreasing both pH and shear force (Lu et al., 2017). Future research would be helpful to understand the inconsistency of the HS impact on meat tenderness, whether relative rates of proteolysis antemortem are maintained postmortem in the heat stressed animal, and how initial

tenderness of heat stressed pork is related to tenderness development (aging potential) under different storage conditions.

1.4.6 Oxidative Stability

Phospholipids, the chief constituent of cellular membrane bilayers, undergo greater oxidation under conditions of high temperature and low pH (Yin and Faustman, 1993). Lipid oxidation produces reactive aldehydes and ketones which in turn catalyze further oxidation in cellular molecules and has long been known to contribute to rancid meat odor (Pearson et al., 1977). Relative to ruminant species, pork is characterized by a high polyunsaturated fat content which is more susceptible to peroxidation than monounsaturated or saturated fatty acids. It should be noted that the myoglobin in pork is believed to be less susceptible to direct oxidation by the secondary products of lipid oxidation (aldehydes) compared other species' myoglobin stability due to fewer histidines of the pork myoglobin structure (Suman and Joseph, 2013).

Thermal challenge to the live animal has the potential to negatively impact the oxidative stability of postmortem muscle and fat deposits of meat cuts and products. Under acute heat exposure (2 hours at 40° C), increases in lipid peroxidation (MDA content) and antioxidative enzyme super oxide dismutase (SOD) activity have been observed in both pork and broiler muscle (Hao and Gu, 2014; Volodina et al., 2017; Montilla et al., 2014). Longer exposure of 3-4 weeks of diurnal heat yielded broiler breast meat containing considerably higher malondialdehyde (MDA) content compared to thermoneutral counterparts (Habibian et al., 2016; Wan et al., 2018). Chronic exposure might also decrease SOD and catalase antioxidant enzyme activity in the muscle of poultry (Wan et al., 2018). In swine, stress associated with chronic heat (35 days of 32° C) and spatial allocation negatively impacted pork fat quality by decreasing saturated:unsaturated fatty

acid ratios (White et al., 2008) thus increasing a risk factor for peroxidation. Additional data on oxidative stability of pork produced under antemortem HS conditions is needed.

Muscles with greater oxidative metabolism (mitochondrial oxidative phosphorylation) may be more susceptible to alterations in muscle redox balance caused by HS as compared to those of highly glycolytic metabolism where energy is predominantly obtained through anaerobic glycolysis. Although HS elevated MDA level in oxidative muscle along with upregulation of catalase and SOD-1, MDA was not elevated nor was the antioxidative response as strong in glycolytic muscle thus indicating muscle specificity (Montilla et al., 2014). It is commonly understood that muscle fiber type and corresponding metabolism affect postmortem meat quality and recent data suggests that indicators of pork quality measured on the loin (*longissimus dorsi*) are not strongly correlated to quality traits of other cuts (ex. belly, ham; Arkfeld et al., 2016). Hence, investigating the postmortem oxidative stability across different muscle types is useful.

1.5 Conclusion

Behavioral and nutrient intake changes under HS have well-recognized effects on pig growth and carcass yield while differences in composition and quality attributes are observed less consistently. The numerous physiologic adaptations to HS might have counterbalancing effects on pork quality attributes thus making net changes in quality parameters difficult to detect. As depicted in Figure 1.3, changes in postmortem tenderness could be masked by the counteractive effects of increased proteolysis and increased HSP. The inconsistency in altered pH could reflect pH being influenced positively by lessened glycolytic substrate and negatively by higher temperature and metabolic acidosis under HS. Muscle pH is a large determinant of water holding capacity such that any proteolytic-driven improvement in water holding capacity might not be realized under low pH conditions. Because shifts in energy metabolism, proteolysis, lipogenesis,

and oxidative balance appear to occur under chronic HS, further research is warranted to better understand both the isolated effects of HS which have the potential to impact quality, as well as any measurable interactive impact which they may have on pork quality.

Considering the altered energetic status of the heat stressed animal and observations in poultry, comparative specie data is needed in swine to clarify how chill rate and pork pH are affected by magnitude and duration of HS. Muscle pH has an overwhelming impact on WHC, color, and tenderness, thus determining these other pork quality attributes to a large extent. Lipogenesis is altered by HS and a more precise quantification is needed of the relative rates of lipolysis and cellular utilization of NEFA. Understanding the maturation of adipocytes under chronic HS holds tremendous value as the process has ramifications for pork fat quality. Because HS induced proteolysis in skeletal muscle of pigs appears to be temporal and muscle specific, postmortem proteolysis in HS pork is largely unexplored. Although tenderness is not a heavily emphasized pork quality attribute, the degree of proteolysis also has implications for WHC and therefore is worthy of study so genetic selection and different processing and storage protocols can be developed to optimize postmortem tenderization and WHC of different cuts. Future research is also needed to identify thresholds at which cumulative oxidative challenges overwhelm endogenous reducing systems which become stressed under heat exposure. This research should focus on the postmortem oxidative stability of pork cuts and products having high oxidation potential.

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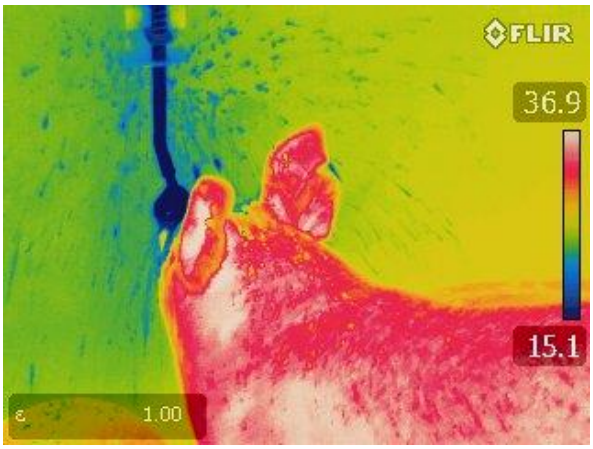

Figure 1.1. The infrared thermal image shows a heat stressed grow-finish pig drinking cool water from a nipple water drinker line affixed to the pen wall. The water droplets spraying from the waterer are lower in temperature than the surrounding walls and animal.

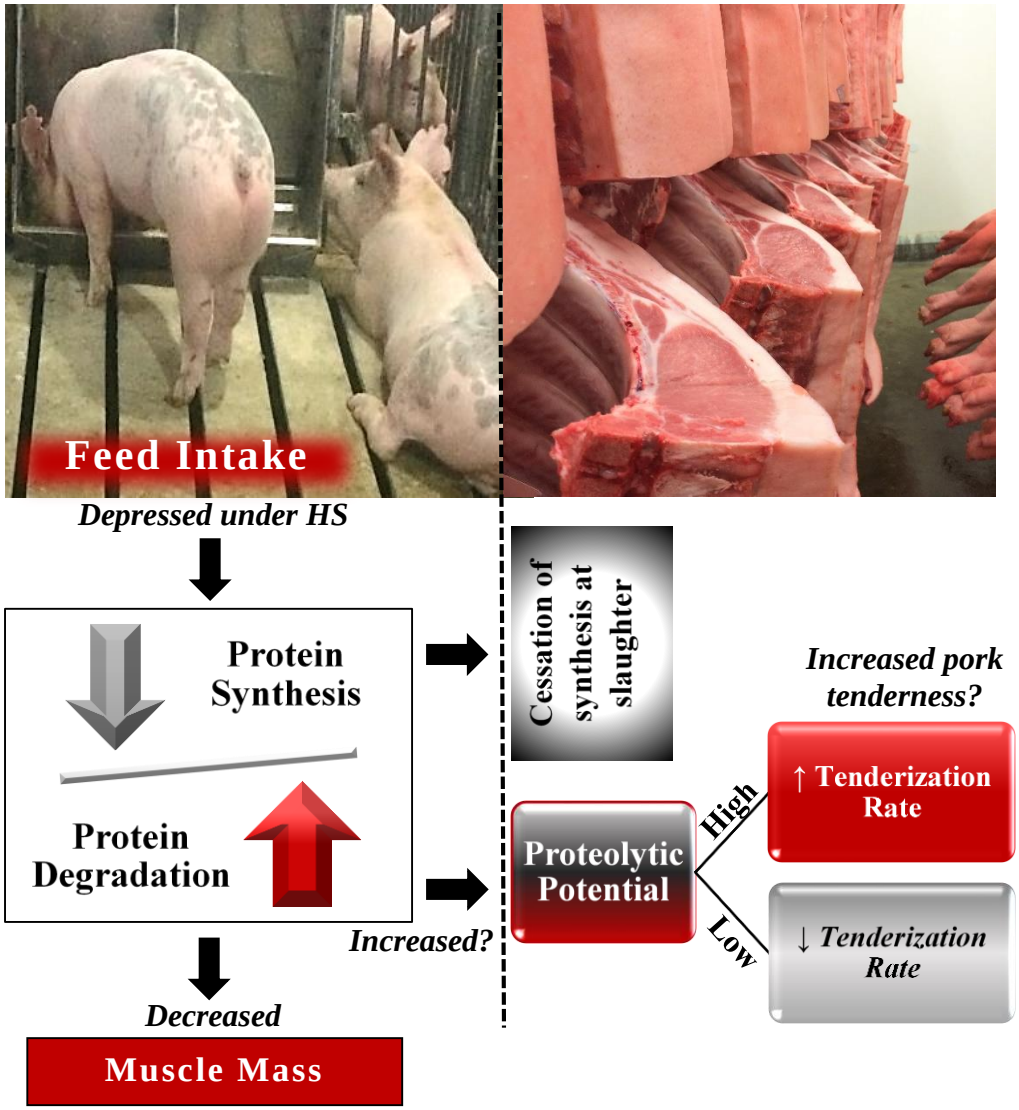

Figure 1.2. Hypothesized effect of heat stress (HS) on the relation between feeding intensity, in vivo protein turnover, and postmortem tenderization rate proposed by Kristensen et al. (2002).

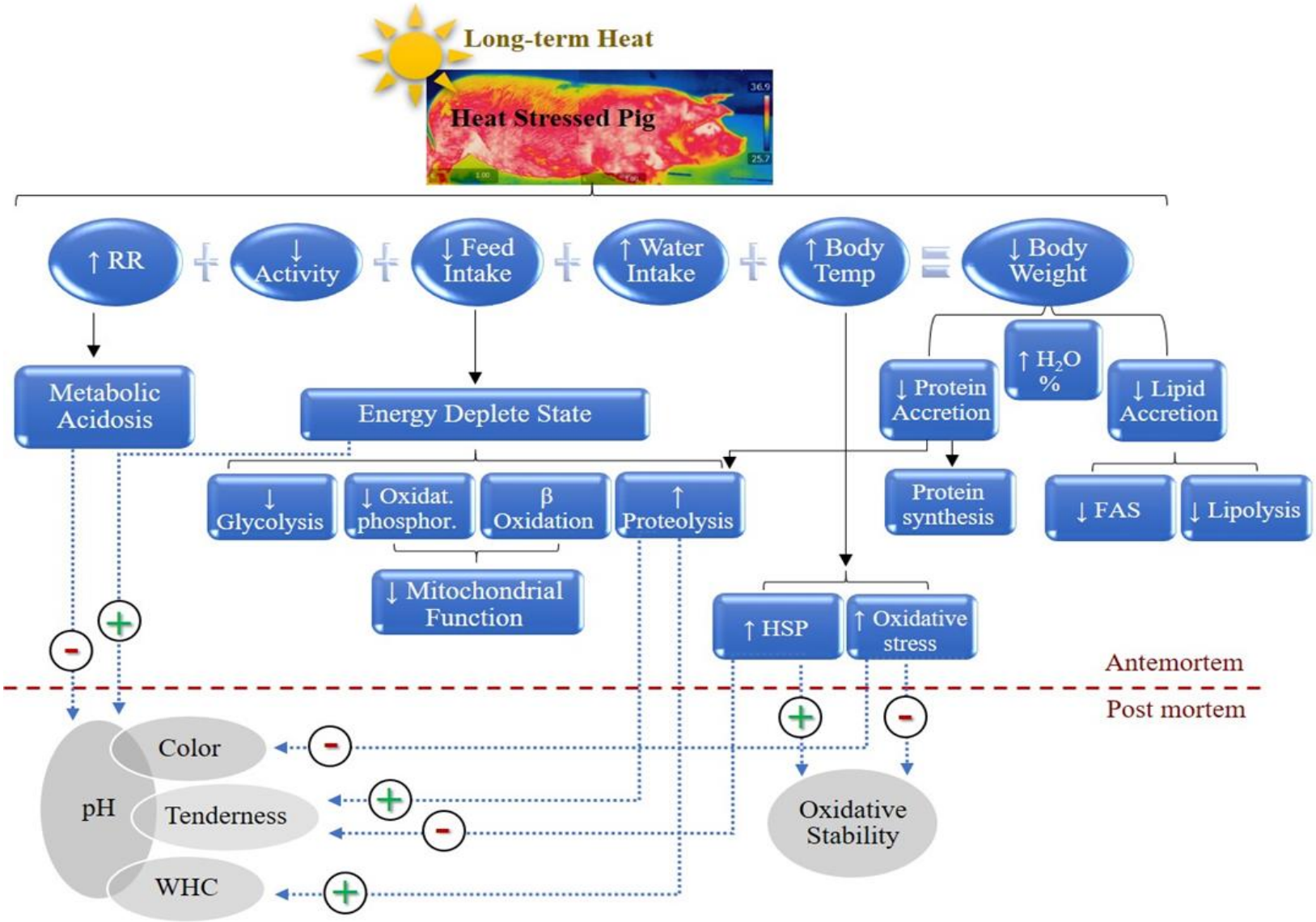

Figure 1.3. Top-down model of the proposed effects of chronic heat stress (HS) on porcine antemortem metabolism and postmortem meat quality attributes (FAS: Fatty Acid Synthesis; HSP: Heat Shock Proteins; WHC: Water Holding Capacity; RR: Respiration Rate).

CHAPTER 2. DISPLAYED PORK SHELF-LIFE AND CARCASS QUALITY OF CHRONICALLY HEAT STRESSED PIGS RECEIVING DIETARY ZINC SUPPLEMENTATION

2.1 Abstract

Heat stress (HS) suppresses animal growth and physiologic adaptation to stress induces metabolic changes, outcomes which may be ameliorated by dietary zinc. The study objective was to determine zinc supplementation effects on carcass composition, quality, and oxidative stability of fresh and processed pork from pigs subjected to a chronic, cyclic heat using a 2×2×2 treatment factorial with main effects of environment (HS vs. thermoneutral; TN), added zinc level (50 vs. 130 mg kg^{-1} available zinc), and zinc source (inorganic vs. organic). Commercial crossbred mixed-sex pigs (initially 72.0 kg) were group-housed under either TN (18.9–16.7°C) or cycling HS conditions with diurnal chronic heat (30 °C/26.7 °C for 12 hours:12 hours) on days 24-71 with diurnal acute heat waves (32-33 °C/29-30 °C for 12 hours:12 hours) on days 21-24, 42-45, and 63-65. One representative pig (n=80) per pen was slaughtered on day 64. The HS pigs had lighter bodyweight (P=0.039), yielded lighter carcasses (P=0.011), less last rib backfat (P=0.032), tended to have smaller (P=0.062) loin eye area but similar percent lean in belly center slices (P>0.10). Compared to TN, HS carcasses had higher (P=0.001) 24-h loin pH and decreased (P=0.034) drip loss. Shifts in individual fatty acid profile of sausage product derived from HS carcasses were observed but were not of sufficient magnitude to affect iodine value. Initially, sausage from HS carcasses tended (day 0, P=0.071) to have less thiobarbituric acid reactive substances than TN but over a 10-day simulated retail display, no treatment induced lipid oxidation differences (P>0.05) were observed in either sausage or displayed loin chops. Consistent treatment differences in CIE L*a*b* of products throughout the display were not observed. Thus, cyclic HS affected carcass

composition and quality but zinc level and source imparted negligible benefits. The HS model did not appear to reduce display shelf-life of pork as indicated by lack of differences in lipid oxidation and color stability.

Keywords: pork, heat stress, zinc, carcass quality, shelf-life, lipid oxidation.

2.2 Introduction

Pigs experiencing heat stress (**HS**) have decreased productive performance resulting from both the direct and indirect effects of heat. These effects include direct thermal impacts as well as indirect impacts on behavior and *ad libitum* nutrient consumption (Collin et al., 2001; Baumgard and Rhoads, 2013). The combined effects of heat reduce pig growth rate and encumber productive efficiency at the farm level by decreasing market weight and/or increasing days to market (Renaudeau et al., 2011; Cruzen et al., 2015a; Wu et al., 2019). Genetic advances in productivity have concomitantly increased the susceptibility of pigs to HS (Brown-Brandl et al., 2013) thereby making thermoregulatory adaptations even more critical to HS survival.

Among the physiologic adaptations to heat stress, there is evidence that metabolic changes in the pig alter energy metabolism (Kellner et al., 2016) which may differentially affect adiposity at different carcass fat depots (Cruzen et al., 2015a). Metabolic changes also alter the musculoskeletal redox balance in pigs exposed to acute heat, which may then translate to poorer redox function postmortem and decreased product oxidative stability. Pigs exposed to thermal challenge exhibit indicators of global oxidative stress (Liu et al., 2017) and markers of musculoskeletal oxidative stress under acute heat (Montilla et al., 2014; Volodina et al., 2017). However, the impact of chronic seasonal heat during the grow-finish period of swine on resultant fresh pork quality remains largely uninvestigated.

Maintaining animal welfare and productivity of animals under duress of heat requires identification of genetic, management, and nutritional strategies to reduce the risk of thermal stress on animal welfare and productivity. Although standard NRC (2012) mineral supplementation levels appear sufficient in maintaining pork quality under conventional environmental conditions (Shelton et al., 2004; Gowanlock et al., 2013), there is an absence of data to indicate whether requirements may be altered during seasonal heat conditions. Zinc (**Zn**) may be particularly beneficial to HS animals due to its involvement in nutrient and insulin mediated metabolism. Furthermore, Zn fed in an amino acid complex (organic) form has been shown to improve the growth response of pigs when fed in combination with a metabolic modifier (Patience et al., 2011; Rambo et al., 2012). Although Zn itself is redox neutral, roles in redox signaling have been implicated and there is some indication that Zn supplementation could improve pork metmyoglobin reducing ability (MRA) (Maret, 2013; Paulk et al., 2014).

Thus, the hypothesis to be tested was that a chronic seasonal heat would decrease carcass weight and yield, reduce pork quality and oxidative stability but be ameliorated through dietary supplementation of Zn to the animal. Therefore, the study objective was to determine the effects of supplemental Zn level and source on pork carcass composition, fresh pork quality, and oxidative stability of pork products derived from pigs exposed to cycling thermal stress in the last nine weeks preslaughter.

2.3 Materials and Methods

2.3.1 Animal Care

The activities of this experiment were approved by the Purdue Animal Care and Use Committee (Protocol No. 1112000447). Animals were housed in 10 rooms of the Purdue University Swine Environmental Research Building in West Lafayette, IN. Each room was

independently environmentally controlled and equipped with a single propane heater, automated ventilation system, pit manure storage, artificial lighting, and contained 12 group pens over fully slatted concrete flooring. Each pen contained a single-hole stainless steel dry self-feeder and an adjustable wall mounted nipple drinker to provide pigs with *ad libitum* access to feed and water. The study utilized 120 mixed-sex crossbred commercial pigs (71.9 ± 4.3 kg BW), a subset from a larger (600 pigs) study on growth performance (Mills et al., 2018). Animals were group housed 5 pigs per pen (0.84 m^2 per head) across 10 different rooms and were part of 3 different grow-finish pig groups over a 28-day range.

2.3.2 Experimental and Treatment Designs

Treatments were arranged in a 2 × 2 × 2 factorial with main effects of environment (ENV; heat stress vs. thermoneutral), added Zn level (LEV; 50 vs. 130 mg kg^{-1} available Zn), and added Zn source (SOR). The Zn SOR effect was the comparison of 100% inorganic Zn from ZnO relative to predominantly organic Zn from Availa®Zn (Zinpro Corporation, Eden Prairie, MN) consisting of 100% Zn from Availa®Zn at 50 mg kg^{-1} level or 62% Zn from Availa®Zn and 38% from ZnO at the 130 mg kg^{-1} level. Dietary treatments were fed from study day 0 until slaughter on either day 64 or day 71. The thermoneutral (TN) treatment consisted of thermoneutral conditions (target of 18.9–16.7 °C; Myer and Bucklin, 2001) maintained at constant temperatures for the duration of the trial. Pigs experiencing HS received a dietary acclimation period prior to the first heat exposure. Cycling diurnal heat stressing conditions simulating seasonal chronic heat (target of 30 °C/26.7 °C for 12 hours:12 hours) occurred on days 24-71 with acute heat waves (target of 32-33 °C/29-30 °C for 12 hours:12 hours) on days 21-24, 42-45, and 63-65. A brief heat acclimation period (target of 27.8/24.4 for 12 hours:12 hours) on days 18-21 occurred prior to the first acute event. The upper temperatures selected for the acute heat stress events mimicked average historical summer daytime

temperatures for swine-dense geographic locations in the United States (Ames, IA; Le Mars, IA; Raleigh, NC) and were slightly greater than average historical summer daytime high temperatures in Worthington, MN. The diurnal temperature changes were designed to simulate normal daily temperature fluxes with warming and cooling periods occurring gradually over a several hour time period. Humidity was monitored, but not controlled, throughout the study.

On study day 0, pens of 5 pigs each were blocked on initial bodyweight (**BW**) then randomly allotted to 1 of the 8 interactive environment and diet treatments across a total of 10 complete replicate blocks balanced across rooms (n = 10 pens per treatment). In 8 of the 10 blocks, a representative gilt from each pen (n = 64 gilts) closest to pen mean body weight (**BW**) on day 18 was selected for thermal monitoring and future meat quality analysis. In the other 2 blocks, a representative animal from each pen was selected for meat quality analysis but not thermal monitoring (n = 8 barrows and 8 gilts).

2.3.3 Diets

The grow-finish diets were corn–soybean meal based and fed in meal form. The Zn treatments were achieved by additions of respective Zn levels and sources to a fine-ground corn premix to facilitate a constant trace mineral (**TM**) premix inclusion rate in the final diet. For the diets which contained 50 mg kg^{-1} of available Zn from ZnO, a mineral premix containing ZnO was added at 0.052% of the 0.20% dietary inclusion of the fine-ground corn premix. To this basal premix, an additional 80 mg kg^{-1} of available Zn from ZnO for the 130 mg kg^{-1} inorganic source treatment and from Availa®Zn 120 (12% Zn; Zinpro Corporation, Eden Prairie, MN) for the 130 mg kg^{-1} organic source treatment was added in place of fine-ground corn. For the diet containing 50 mg kg^{-1} of available Zn from an organic source, a separate TM premix was made solely with Zn from AvailaZn®. For all treatments, the TM premix also supplied per kg of diet: 50 mg Fe, 6.2

mg Mn, 4.66 mg Cu, and 0.19 mg I. Diets were formulated to be fed in 3 grow-finish phases for BW of 68.0 to 88.5 kg (Study Phase 1; days 0 – 21), 88.5 to 108.9 kg (Study Phase 2; days 21 – 42), and 108.9 to 129.3 kg (Study Phase 3; days 42 – 72). Feed samples from each batch of feed were obtained and pooled within each diet treatment and phase for Zn and proximate analysis (Mills, 2018).

2.3.4 Thermal Monitoring

Throughout the study, the daily temperature extremes (high and low over 24-hour period) in each room was recorded via an AcuRite thermometer (Model 00305SBDI; Chaney Instrument Co., Lake Geneva, WI) and ambient temperature and relative humidity were also recorded in each room every 5 minutes by an EasyLog Data Logger (Lascar Electronics, Erie, PA). Throughout the study humidity was monitored but not controlled; daily average humidity recorded in the rooms during the acute heat periods ranged from 46 – 61% for the HS rooms and 41 – 60% for the TN rooms while during the chronic heat periods daily average humidity ranged from 45 – 59% for the HS rooms and 36 – 63% for the TN rooms. Indwelling vaginal thermometers (Maxim Integrated™ ibutton®, San Jose, CA; secured to a progesterone extracted sheep CIDR® insert, Pfizer Inc, New York, NY) were inserted into the representative gilt of each pen in 8 of the blocks (n = 64 gilts) for determination of core body temperature ($\mathbf{T_{core}}$) responses to heat stress. Real time body temperature responses were logged at 10 minute intervals before, during, and after each of the major heat events during the trial. Thermometers were inserted on the morning of day 17 prior to initiation of the acclimation heat period and remained inserted for the duration of the heat event (days 18 – 24) until removal on day 26. Thermometers were again inserted the afternoon of day 41 prior to the start of the second heat event and remained inserted for the duration of the heat event (days 42 – 45) until removal on day 48.

On the days which T_{core} was measured, infrared thermal images were taken of the gilts with the indwelling thermometers at approximately 07:00 hours and 19:00 hours daily using a FLIR T440 camera with an emission factor setting of 0.90 - 1.00. Images were analyzed using FLIR Tools (version 5.11.16357.2007; 2016) software to determine skin temperatures of the trunk and posterior face of the ear of each animal. Thermal Circulation Index (**TCI**) was calculated at each AM and PM timepoint to obtain a measure of heat dissipation by the animal for the regulation of core body temperature (Kingma et al., 2014):

$$[T_{trunk\ skin} - T_{ambient}] \div [T_{core} - T_{trunk\ skin}]$$

2.3.5 Carcass Data and Sample Collection

At a fixed time of 64.3 ± 1.2 days and after 24 - 48 hours of the third acute heat exposure, the representative gilt from each pen in the 8 blocks which had undergone thermal monitoring (*n* = 64), plus representative pigs from each pen in the other 2 weight blocks (*n* = 8 barrows and 8 gilts), were slaughtered at the Purdue University Meats Laboratory state inspected harvest facility. Forty additional HS pigs (1 pig from each HS pen with BW closest to day 70 pen mean; 4 barrows and 36 gilts) were also slaughtered on day 71 ± 1.7 after attaining a common weight compared to that of TN pigs previously slaughtered on day 64 (126.4 kg TN vs. 125.8 kg HS, *P* = 0.674). Prior to slaughter, access to feed was maintained until pigs were transported by body weight blocks approximately 16 km to the Purdue University Meats Laboratory under ambient conditions. After 2 hours or less of holding at the abattoir, pigs were electrically stunned and exsanguinated. Leaf fat was removed from each carcass prior to measuring hot carcass weight (**HCW**) and calculating dressing percentage.

Following the final rinse and carcass inspection, a 15-cm boning knife was used to make an incision 10 cm caudal to the aitch bone into the deep ham muscle (*semimembranosus*) of the 8

carcasses of the first BW block and the 8 carcasses of the last BW block to be slaughtered on the second harvest day and again on the third harvest day for a total of 32 carcasses (4 per treatment combination). An ibutton® recording temperatures at 2-minute intervals was secured inside a latex glove finger and inserted along the incision into the deep ham muscle to monitor carcass cooling. For each carcass, the average recorded temperature over 10-minute intervals was calculated beginning with the first temperature recorded after insertion (peak temperature for each carcass).

After an 18 - 24 hour chill, the left side of each carcass was ribbed between the 10th and 11th rib. The pH of the *longissimus thoracis* was measured using a calibrated pH meter (HI 99161 with FC202 pH electrode; Hanna Instruments, Woonsocket, RI) inserted in the center of the muscle posterior to the 10th and 11th rib interface. Subjective color, firmness, and marbling were evaluated after 20 minutes of bloom time on the anterior loin face according to National Pork Producers Council standards (NPPC, 2000).

Carcasses were ribbed and measurements were taken of the *longissimus thoracis* area and of backfat depths at the 10th rib (including skin and measured perpendicular to the skin at ¾ the distance of the *longissimus* from the medial edge), last rib, and last lumbar vertebra. Carcass lean was calculated using these measurements according to the following equation: % lean = 100 × {[(11.08 + (0.218×BW live, kg) + (-0.715 × 10^{th} rib BF, cm) + (-3.31 × last rib BF, cm) + (0.346 × LEA, cm^2)] ÷ (BW live, kg × 0.74)} (A. Schinckel, personal communication).

Approximately 24 hours postmortem, day 64 carcasses (*n* = 80) were fabricated for additional sample and data collection. Water holding capacity for each carcass was assessed on the *longissimus thoracis* muscle. Three 20 mm cores were taken from a 2.54 cm thick loin chop immediately anterior to the 10^{th}/11^{th} rib interface where subjective quality had been assessed. Each muscle core sample was individually placed into a capped exudate collection tube (EZ DripLoss

container; Danish Technological Institute, Taastrup, Denmark) and stored at 4 °C for 24 hours. The weight of each tube with its contents was recorded, the muscle core discarded, then the weight of each tube containing exudate was recorded. Exudate from each core was calculated as the difference between the weight of the tube containing exudate and the tare weight of each tube. Percentage drip loss was subsequently expressed as the average of the triplicate observations. The remainder of the chop (muscle and subcutaneous fat layer) from which the triplicate drip loss cores were taken was packaged in a Whirl-pack bag (Nasco, Fort Atkinson, WI) and frozen at -20 °C.

At the time of fabrication and from the opposite side of the carcass upon which subjective loin quality was assessed, bellies were cut according to an adaptation of Item No. 408 specifications (IMPS Fresh Pork Series 400; USDA, 2014). Belly weight, length from cranial to caudal edges, width from ventral to dorsal edge at 25%, 50%, and 75% the length (3 locations averaged to obtain overall width), and thickness at the 25%, 50%, and 75% depths at each of the 25%, 50%, and 75% length locations of the belly (all 9 locations averaged to obtain overall thickness) were recorded. Temperature was assessed in the *obliquus abdominis* at the time of bend assessment. Belly bend was determined using a standard 2.54 cm grid belly board where the belly was centered on the fulcrum with the dorsal edge proximal to the board (ventral edge distal to the board; see Rentfrow et al., 2003). Vertical distance from the top of the fulcrum to the lowest hanging point of the belly was recorded for the cranial and caudal ends (averaged to determine vertical flex) and the horizontal distance from the fulcrum center to the proximal edge of the lowest hanging point determined for the cranial and caudal edges (averaged for lateral flex). A sample of subcutaneous fat was removed from the ventral corner of the caudal edge of the fabricated belly and frozen at -20 °C in Whirl-pack bags (Nasco, Fort Atkinson, WI). A 2.54 cm center slice was also cut from the belly so that the anterior face of the slice represented the belly at 50% of the belly

length. A Nikon DSLR camera (Nikon Corporation; Minato, Tokyo) was used to capture digital images of both the anterior and posterior faces of the slice; photos included a ruler for future reference. Adobe Photoshop CC 2017 (Adobe Systems Inc; San Jose, California) was subsequently used to measure the % lean:fat ratio of each anterior face. Following imaging, center cut slices were packaged in Whirl-pack bags (Nasco, Fort Atkinson, WI) and frozen at -20 °C.

2.3.6 Pork Quality Analysis

Four boneless, closely-trimmed chops 2.54 cm thick were fabricated from the 4th to 10th rib portion of the full loin of each carcass. These 4 remaining chops were subjected to a simulated retail display. Each chop was weighed, individually packaged with a soaker pad on a white Styrofoam tray with PVC film overwrap, arbitrarily assigned a display time of either 0, 3, 7, or 10 days, and displayed under constant 24-hour cool white fluorescent 40-watt lighting (1,227 lux illumination) at 4 °C for its respective time of display. Product was displayed on shelving in a walk-in cooler and lighting consistency maintained by controlling ambient light sources. Instrumental color (CIE L*, a*, b*) was measured on each PVC overwrapped chop initially (d 0) and on the final day of its respective display time (d 3, 7, or 10) utilizing a Hunter MiniScan EZ colorimeter (Hunter; Reston, VA, USA). The average of 3 calibrated readings (25 mm viewed area; D65 illuminant/10°) across the loin face was recorded for each chop. The colorimeter was standardized prior to each use with white and black tiles and a clear PVC film covering the viewed area. Hue angle and chroma (saturation index) were calculated as hue angle = $\tan^{-1}(b^* \div a^*)$ and chroma = $\sqrt{(a^{*2} + b^{*2})}$ according to AMSA Meat Color Measurement Guidelines (AMSA, 2012).

On the final day of display for each chop, chops were removed from display, weighed, and instrumental color measured. Individual purge loss was calculated for day 3, 7, and 10 chops as

the weight difference between the initial weight and day of interest weight divided by the initial weight. Chops were subsequently individually vacuum packaged and frozen at -20 °C.

One picnic shoulder from each fabricated carcass was stored in an open container for either 72 or 96 hours postmortem preceding processing into fresh ground pork patties. Shoulders were dissected to obtain lean and trim for individual 2.27 kg batches of 70/30 lean:fat ratio. Meat and trim were ground twice through a 4-mm plate and uniformly mixed for 30 seconds in an electric Cuisinart stand mixer (Conair Corp; Stamford, Connecticut) with 50 g of a commercial sausage seasoning blend (A.C. Legg, Inc; Calera, Alabama) for a final formulation of 1.9% NaCl. Sausage was stuffed in a synthetic collagen casing, chilled until firm (approximately 2 hours), and sliced into 8, 2.54-cm thick patties. Casing was removed from around each patty then patties were packaged 2 per white Styrofoam tray with PVC film overwrap. Each tray of 2 patties each was arbitrarily assigned a display time of either 0, 3, 7, or 10 days and both initial and final instrumental color was measured on the PVC overwrapped patties using a calibrated Hunter MiniScan EZ colorimeter to take 2 color readings per patty (4 readings per package which were subsequently averaged) according to the methods used for assessing loin chop color. Patty packages were displayed under the same conditions as the loin chops, then patties were vacuum packaged and stored at -20 °C.

Lipid oxidation for patties (n = 80 per display timepoint) and chops (balanced subset, n = 48 per display timepoint) was assessed via 2-Thiobarbituric Acid Reactive Substances (TBARS – rapid, wet method) analysis conducted in duplicate according to published methods (Kim et al., 2017). Briefly, chops and patties were pulverized by submersion of cubed pieces in liquid nitrogen then pulverization of the frozen cubes in a grinder. The resulting powder was kept frozen until a 5 g aliquot was combined with 15 mL distilled deionized water and 50 μL 10% butylated

hydroxyanisole/90% ethanol solution. The mixture was homogenized for 10 – 15 seconds using a T25 Ultra Turrax® (IKA® Works, Inc; Wilmington, North Carolina) at 7,000 – 8,000 rpm, then 1 mL of homogenate was mixed with 2 mL of TBA/TCA solution consisting of 20 mM 2-thiobarbituric acid in a 15% trichloroacetic acid solution (15 g TCA/100 mL distilled deionized water), incubated in a 80 °C water bath for 15 minutes, cooled in an ice bath for 10 minutes, and supernatant filtered through Whatman No. 4 filter paper after centrifugation. Absorbance of the supernatant at 531 nm was measured in duplicate by a microplate spectrophotometer (Epoch, Biotek Instruments Inc.; Winooski, Vermont) using a 96-well plate containing a standard curve of 1,1,3,3-tetra-ethoxypropane (TEP) solution. Milligrams of malondialdehyde per kg of meat was calculated from the standard curve and the average of the duplicate readings (absorbance adjusted for standard $\leq$ 0.11 standard deviation between duplicates) for the duplicate analyses was computed for each carcass of origin.

2.3.7 Chemical Analyses

Proximate composition, amino acid, and mineral (Ca and P) concentrations of feed samples were analyzed by the University of Missouri Agriculture Experiment Station Chemical Laboratories (Columbia, MO). Dry matter was determined in a vacuum oven according to Association of Official Analytical Chemists (**AOAC**) Official Method 934.01 (AOAC, 2006), crude protein reported as N content × 6.25 with N determined by combustion analysis (LECO) AOAC Official Method 990.03 (AOAC, 2006), crude fat determined by ether extraction (AOAC Official Method 920.39 (A), 2006), crude fiber using a filter bag digestion technique according to American Oil Chemists' (**AOCS**) Approved Procedure Ba 6a-05, and minerals according to AOAC Official Method 984.27 (2006). The complete amino acid profile was assessed according to AOAC Official Method 982.30 E(a,b,c), chapter 45.3.05 (2006) including separate tryptophan

analysis by alkaline hydrolysis (AOAC Official Method 988.15, chapter 45.4.04, 2006). Diet analysis was also completed in-house on ground diet samples (1 mm screen; Wiley Mill, Thomas Scientific, Swedesboro, NJ). Dry matter was assessed following drying (Blue M Electric Company, Blue Island, IL) at 60° C for 12 hours. Ash content was determined after 8 hours at 500° C (Thermolyne 6000 muffle furnace, Dubuque, IA). Bomb calorimetry (Parr Isoperibol 6200 Calorimeter, Moline, IL) was used to determine gross energy (**GE**). Dietary Zn was assessed via atomic absorption spectrophotometry (Spectra AA 220FS, Varian) after nitric-perchloric digestion; samples were analyzed in duplicate (CV ≤ 5%) and values were adjusted to an internal standard for each run.

Protein content (nitrogen × 6.25) of thawed, pulverized loin chop and sausage patty samples (display d 0 products) was determined via macro combustion and thermal conductivity analysis using a LECO TruMac® N Determinator (LECO Corporation, St. Joseph, MI). Calibration was performed using an EDTA standard with known N content of 9.56% ± 0.02. Samples of 0.5 g were analyzed in triplicate in accordance with AOAC Method 992.15 (2006). Frozen, pulverized samples were thawed and dried for ≥ 12 hours at 75 °C to determine percent moisture content. Ether extractable lipid content of the loin samples was determined via petroleum ether solvent extraction of neutral lipids for 5-6 hours on the previously dried samples (1.6 g wet weight) in triplicate, according to the methods of AOAC Method 960.39 (2006). Ether extractable lipid content of the sausage samples was determined via tandem petroleum-ethyl ether solvent extraction for 16 - 18 hours in triplicate on the previously dried samples (0.75-1.50 g wet weight).

To determine the fatty acid (**FA**) profile of the diets and 30% fat blend sausage patties (pulverized, thawed sample, *n* = 80 display day 0 patties), a modified Folch Method of chloroform-methanol lipid extraction was performed on 1.0 g samples. Each sample was combined with 7 mL

methanol and 14 mL chloroform with homogenization occurring after each addition. Homogenate was filtered through no. 40 Whatman filter paper and combined with 8 mL 0.88% KCl (2.2 g KCl/250 mL distilled water), then shaken for 10 minutes. Samples were refrigerated (4° C) overnight for the formation of biphasic layers. The upper aqueous phase was removed and from the remaining phase the chloroform was evaporated under nitrogen until approximately 3 mL sample remained. The extracted sample was then rinsed with a 2 mL aliquot of 2:1 (v/v) chloroform/methanol. Extracted lipids were transesterified by sodium methoxide (alkaline) catalyzation to form fatty acid methyl esters by dissolving extracted lipids in 1 mL dry toluene and 2 mL 0.5 N sodium methoxide in methanol (2 g NaOH/100 mL methanol). The mixture was heated for 10 minutes at 50° C then combined with 0.1 mL glacial acetic acid and 5 mL NaCl solution (1.202 g NaCl/1 mL distilled water). The organic hexane portion was combined with 0.01 g anhydrous sodium sulfate after twice-repeated extraction accomplished by addition of 3 mL hexane to each sample, 10 minutes of agitation and 10 minutes centrifugation at 1000 x *g*. Hexane was removed from the methylated fatty acids by nitrogen evaporation, then the fatty acid methyl esters were reconstituted in hexane and analyzed using a Varian 3900 gas-liquid chromatograph with a 8400 autosampler, flame-ionization detector, and a WCOT fused silica 30 m x 0.32 mm capillary-channel polymer wax 52 chemically bonded capillary column (Varian Inc., Palo Alto, CA). Analysis was performed on 5 uL injected sample mixed with helium (1:100 dilution rate) at 240° C using PUFA and FAME standards (Sigma-Aldrich, St. Louis, MO) according to the methods of White et al. (2008). For the major FA, duplicate values for a single sample differing more than 5% from each other were reran.

2.3.8 Statistical Analyses

Carcass characteristics, composition, and water holding capacity data were analyzed as a 2 × 2 × 2 treatment structure in a randomized complete block design replicated over 3 harvest days. The 8 treatment combinations were randomly assigned to each pen of pigs at the start of the study; thus, the representative pig from each pen served as the single observational (experimental) unit. The MIXED procedure of SAS (v9.4, SAS Institute Inc., Cary, NC) was used to model treatment as a fixed effect with random effects of harvest day and initial weight block nested within harvest day. The main effects of environment, Zn level, and Zn source, as well as their interactions, were tested using *a priori* orthogonal contrast statements. In the analysis of day 64 characteristics compared to day 71 characteristics, the random effect of harvest day was not included in the model and individual hot carcass weight was included as a covariate in the analysis of loin eye area and carcass fat measurements.

Color and TBARS measurements on the pork products were similarly analyzed using a model which included body weight block and carcass-of-origin nested within body weight block as random effects such that the residual error was attributed to the individual pork product packages. The Kenward Roger method was used to compute denominator degrees of freedom for tests of fixed effects. Differences due to time existed between display days and pairwise comparisons among days were tested using the Tukey-Kramer method. Color differences over time were evaluated on unadjusted values as a repeated measurement on each carcass-of-origin from day 0 to 10. No interactions were observed between display day and the interactive treatment means nor between display day and the factorial main effects, so the effects of ENV, LEV, SOR, as well as their interactions were assessed within each display day. Color measurements on day 3, 7, and 10 were adjusted for the initial day 0 reading taken on that same individual package by including the day 0 reading as a covariate. In the assessment of the day 3, 7, and 10 TBARS

response, inclusion of initial TBARS value associated with the d 0 product from the respective carcass-of-origin as a covariate in the model was non-significant and therefore not included in the final model for TBARS response. Pearson correlation coefficients were generated for various variables. All results were considered statistically significant at $P \leq 0.05$; results with *P*-values > 0.05 and ≤ 0.10 were considered a trend.

2.4 Results

2.4.1 Elevation of Body Temperature

Based on the relative humidity and ambient temperatures (Fig. 2.1) recorded in the HS rooms (see Chapter 3), the HS pigs experienced thermal indices in the "alert" and "danger" zones but were not exposed to "emergency" conditions (Hahn et al., 2009). During the first acute HS event (days 21 - 24), the T_{core} of animals was elevated ($P < 0.05$) by HS conditions at the PM timepoint and during the second acute HS event on days 42 – 45 at the AM (39.69 versus 39.37 °C) and PM timepoints (39.93 versus 39.45 °C; Fig. 2.2). During the initial acute HS exposure on days 18 - 24, increased ($P < 0.10$) TCI corresponded to maintenance of T_{core} among HS animals while the increases in T_{core} observed during acute HS events occurred when the TCI was not increased ($P > 0.10$). On days 45 - 48 following the second acute HS event, despite increased ($P < 0.05$) TCI among HS animals their T_{core} remained higher ($P < 0.05$) than that of the TN animals.

2.4.2 Carcass Composition

Due to their environment, the HS pigs were lighter BW ($P = 0.039$) and yielded lighter carcasses ($P = 0.011$; 96.1 kg HS vs. 99.3 kg TN) which consequently had less backfat at the last rib ($P = 0.032$), and tended to have less leaf fat ($P = 0.054$), smaller loin eye area (LEA; $P = 0.062$), and lower dressing percentage ($P = 0.088$; Table 2.11 and 2.12). No significant differences in

backfat measured at the 10th rib were observed. When 64-day carcass composition measurements were compared on a common HCW basis, there were tendencies for an interaction between ENV, LEV, and SOR on 10th rib backfat (P = 0.098) and on last lumbar backfat (P = 0.100), between ENV and SOR on last rib backfat (P = 0.078), and between ENV and LEV on leaf fat (P = 0.100); however no significant effects of ENV, LEV, or SOR were observed (Table 2.11 and 2.12). No differences among treatments were observed in marbling scores, belly size (weight, average length, average width, or average thickness) or in percent lean of center slices (P > 0.10; Table 2.13 and 2.14).

To determine whether carcass composition observations were simply a function of the HS animals' retarded growth or if differences remained evident when HS pigs had attained a similar body weight to the thermoneutral treatment, the composition of the 71-day HS carcasses was compared to that of the 64-day TN carcasses on a similar weight basis (126.4 kg TN vs. 125.8 kg HS, P = 0.674; Table 2.15 and 2.16). The 71-day HS pigs still had a lower dressing percentage (P = 0.012) compared to 64-day TN pigs. The 71-day HS carcasses had more backfat at the last rib (P = 0.033) when adjusted for HCW but no measurable differences in backfat at the 10th rib and last lumbar locations; these carcasses also had a tendency for lower subjective marbling score (P = 0.088) relative to 64-day TN pigs (Table 2.17 and 2.18). Regardless of environment, 130 mg kg^{-1} Zn improved both live BW (P = 0.017) and HCW (P = 0.014) compared to 50 mg kg^{-1} Zn. Zinc treatment showed little effect on carcass composition with the exception of an interactive effect between ENV and SOR on LEA such that LEA under 71-day HS conditions was greater when pigs received inorganic Zn but under 64-day TN conditions organic Zn increased LEA (ENV × SOR, P = 0.039; Table 2.15 and 2.16). Tendencies for interactive effects of LEV and SOR (P = 0.084)

on 10th rib backfat depth and of ENV and LEV (P = 0.055) on leaf fat were also observed on a common HCW basis.

2.4.3 Fresh Quality Indicators

No main effects of Zn source, level, or interactions were observed (P > 0.050) on carcass pH and water holding capacity measurements (Table 2.13 and 2.14). Compared to 64-day TN pigs, carcasses of 64-day HS pigs had improved 24 h loin pH (P = 0.001) and decreased (P = 0.034) drip loss; however, this initial improvement in water holding capacity did not correspond (P > 0.050) to any differences in chop purge during the display period over 10 d post-fabrication. Across all treatments, the correlation between pH and drip loss measured in this experiment was r = -0.27 (P = 0.014). Upon further analysis, the correlation for carcasses exposed to *ante mortem* HS was r = -0.33 (P = 0.041) while that for carcasses under the TN antemortem conditions was non-significant at only r = -0.12. The 64-day HS loins were also characterized by greater subjective color (P = 0.024) and firmness scores (P = 0.030) relative to the 64-day TN loins; however, no differences in firmness or color scores were observed between the 64-day TN loins and the 71-day HS loins (Table 2.17 and 2.18).

No significant treatment main effects or interactions (P < 0.05) were observed on the carcass cooling temperatures (Fig. 2.3) at any of the following time points succeeding the postmortem peak recorded temperature of each carcass: 10, 20, 30, 40, 50, 60 minutes, and 2, 3, 4, 5, 8, 12 and 24 hours. However, there was a tendency (P = 0.054) for bellies fabricated from HS carcasses to be colder at 24-hours postmortem than bellies from TN carcasses and a tendency for an interaction between ENV and LEV such that the 130 mg kg^{-1} Zn level bellies were colder than the 50 mg kg^{-1} Zn level bellies among the HS treatment but TN bellies were similar temperature between Zn levels (Table 2.13 and 2.14). No main effects (P > 0.10) of environment or Zn

source/level were observed on belly flex. However, lateral belly flex adjusted for belly thickness was greater (indicative of a firmer belly) among TN pigs receiving inorganic Zn and among HS pigs which had been fed organic Zn (ENV × SOR, $P = 0.018$). Tendencies for interactions between ENV and LEV were also observed for adjusted vertical ($P = 0.054$) and adjusted lateral flex ($P = 0.095$); under the HS treatment, vertical flex lessened (more firm) for the 130 mg kg^{-1} Zn level compared to the 50 mg kg^{-1} Zn level while under the TN treatment, lateral flex increased (more firm) for the 50 mg kg^{-1} Zn level compared to the 130 mg kg^{-1} Zn level.

2.4.4 Chemical Composition

Moisture percentage of loin chop samples did not different between environments ($P > 0.10$) but was 3% greater ($P = 0.030$) for the sausage (30% fat) originating from HS carcass tissue compared to sausage made from TN carcasses (Table 2.19, 2.20, 2.21, 2.21). Organic Zn source tended to decrease percent moisture ($P = 0.073$) of loin chop samples. No differences were observed ($P < 0.05$) in ether extract among product samples or in nitrogen content of sausage. In the subset of loin chops analyzed, a 3-way interaction between ENV, LEV, and SOR ($P = 0.028$) was detected for crude protein (**CP**); under TN conditions, organic Zn source increased CP at the 130 mg kg^{-1} level but decreased CP at the 50 mg kg^{-1} level and under HS conditions, organic Zn source decreased CP at the 130 mg kg^{-1} but not at the 50 mg kg^{-1} level. There was also a tendency for a significant interaction between ENV and LEV ($P = 0.080$) on ether extract of chops, where under the HS environment, the 130 mg kg^{-1} Zn level increased ether extractable lipid compared to the 50 mg kg^{-1} Zn level, while under the TN environment the 130 mg kg^{-1} Zn level did not do so. The correlation coefficient between the NPPC marbling scores and the crude fat measurement of the loin was r = 0.46 ($P = 0.001$). For HS carcasses specifically, the correlation was r = 0.41 ($P = 0.049$) and for the TN carcasses, the correlation was r = 0.54 ($P = 0.006$).

Differences were observed in the fatty acid (**FA**) profile of the sausage manufactured using the shoulder fat trim from the carcasses (Table 2.23 and 2.24). However, these shifts were not of sufficient magnitude to affect the associated Iodine Value (**IV**; $P > 0.10$) among any environmental or dietary treatments. Relative to TN carcasses, fat from the picnic shoulder of the HS carcasses tended to have a greater percentage of polyunsaturated fatty acids (**PUFA**; $P = 0.054$) but had a lesser percentage of monounsaturated fatty acids (**MUFA**; $P < 0.001$) which resulted in a lesser percentage of unsaturated fatty acids (**UFA**; $P = 0.007$) under HS than under TN. Both n-3 PUFA increased ($P = 0.001$) and n-6 PUFA tended to increase ($P = 0.056$) under HS relative to TN such that the ratio of n-6:n-3 PUFA did not differ among temperature nor any Zn treatments. Heat stress increased the percentage of saturated fatty acids (**SFA;** $P = 0.019$) and the ratio of SFA:UFA ($P = 0.010$) compared to the TN treatment. However, the percentage of SFA and the ratio of SFA:UFA were dependent on the combination of both temperature and Zn level (ENV × LEV, $P = 0.027$ and 0.039, respectively); under HS conditions, the degree of saturation was increased at 130 mg kg^{-1} compared to the 50 mg kg^{-1} level but under TN conditions the degree of saturation was slightly numerically less at the 130 mg kg^{-1} level compared to the 50 mg kg^{-1} level. There was also a tendency for an interactive effect of LEV and SOR ($P = 0.063$) on percentage of SFA, where at the 130 mg kg^{-1} level the organic source increased the percent SFA while at the 50 mg kg^{-1} level the percent SFA lessened with organic source relative to inorganic source. The percentages of UFA (ENV × LEV, $P = 0.059$) and MUFA (ENV × LEV, $P = 0.085$) tended to increase at the 130 mg kg^{-1} level compared to 50 mg kg^{-1} level under TN but tended to decrease at the 130 mg kg^{-1} level compared to 50 mg kg^{-1} under HS. Tendency for an interactive effect of ENV and SOR on percentage of MUFA was also observed, as percentage MUFA was decreased with organic source compared to inorganic source under TN conditions but increased with organic source compared to

inorganic source under HS conditions (ENV × SOR, $P = 0.070$). The differences in total UFA, SFA, MUFA, and SFA:UFA due to treatment existed independently of differences in individual HCW (HCW covariate; $P > 0.10$).

On an individual FA basis, percentage of stearic acid (C18:0) was 4.8% greater ($P = 0.006$) in HS carcasses relative to TN carcasses, and was increased with organic Zn source compared to inorganic Zn source at 130 mg kg^{-1} level but was at a more similar level between Zn sources at the 50 mg kg^{-1} level (LEV × SOR, $P = 0.041$). Compared to TN carcasses, HS carcasses had 2.7% less oleic acid (C18:1n-9; $P = 0.002$), tended to have less percentage of vaccenic acid (C18:1n-7; $P = 0.096$), and tended to have greater percentage of linoleic acid (C18:2n-6; $P = 0.061$). Percentages of palmitic acid (C16:0) and palmitoleic acid (C16:1n-7) did not significantly differ among treatments. The relative percentages of C16:0, C18:0, and C18:1n-9 existed despite HCW differences (HCW covariate; $P > 0.10$). However, there tended to be an interactive effect of ENV and LEV on C14:0 ($P = 0.079$), C16:0 ($P = 0.061$), and C18:0 ($P = 0.099$), as percentages of these saturated fatty acids were lessened with 130 mg kg^{-1} level compared to 50 mg kg^{-1} level under the TN environment but tended to decrease at the 130 mg kg^{-1} level compared to 50 mg kg^{-1} under the HS environment. Concomitantly, there was a tendency for an interaction where the percentage of C18:1n-7 was greater at the 130 mg kg^{-1} level compared to 50 mg kg^{-1} level under the TN environment but was less at the 130 mg kg^{-1} level compared to 50 mg kg^{-1} under the HS environment (ENV × LEV, $P = 0.070$).

Minor fatty acids decreased by HS relative to TN included C18:$2^{10,12}$ ($P = 0.049$) and C22:1 ($P = 0.038$), while HS increased C18:3n-3 ($P = 0.006$), C20:4n-6 ($P = 0.025$), C22:4n-6 ($P = 0.016$) and tended to increase percentages of C18:3n-6 ($P = 0.088$), C22:5n-3 ($P = 0.064$), and C22:6n-3 ($P = 0.091$) relative to TN. Relative to the 50 mg kg^{-1} Zn level, 130 mg kg^{-1} Zn caused small but

significant reductions in the percentages of C16:2n-4 (P = 0.005), C16:3n-4 (P = 0.006), C20:4n-6 (P = 0.047), and tended to increase C20:0 (P = 0.072). Interactions between LEV and SOR were observed for C16:2n-4 (P = 0.011) and C16:3n-4 (tendency, P = 0.068), where percentages of these fatty acids were less with organic source at the 50 mg kg^{-1} Zn level but at the 130 mg kg^{-1} Zn level percentages were greater with organic source. There was also a tendency for a 3-way interaction for C16:2n-4 (ENV × LEV × SOR, P = 0.087) because the increase in C16:2n-4 percentage by organic source at the 130 mg kg^{-1} Zn level was more evident under HS conditions than under TN conditions. An interactive effect of LEV and SOR was also observed on the percentage of C18:$2^{10,12}$ (P = 0.025), which was slightly less with organic Zn source at the 130 mg kg^{-1} Zn level but similar percent with inorganic Zn source at the 50 mg kg^{-1} Zn level; this was driven by a 3-way interaction between ENV, LEV, and SOR **(**P = 0.029) where percent C18:$2^{10,12}$ was less with 130 mg kg^{-1} Zn from organic source under HS conditions, but not less with 130 mg kg^{-1} Zn from organic source under TN conditions. The percentage of C22:5n-3 was increased (P = 0.011) by inorganic Zn source relative to organic Zn source, and organic Zn decreased C24:0 (P = 0.024) and tended to decrease C18:$2^{9,11}$ (P = 0.055) percentages relative to inorganic Zn source. A significant 3-way interaction was also observed for C24:0 (ENV × LEV × SOR, P = 0.021) because under TN conditions, inorganic Zn increased percent C24:0 but failed to do so under HS conditions at the 50 mg kg^{-1} Zn level. A significant interaction was observed on the percentage of C22:5n-6 which was greater with inorganic Zn source under HS conditions but less with inorganic Zn source under TN conditions (ENV × SOR, P = 0.047).

2.4.5 Display Shelf-Life

Color changes occurred over time in loin chops (Table 2.25 and 2.26, Fig. 2.4). Chop L* increased (P < 0.05) at each display day from d 0 to d 7 but did not statistically differ between d 7

and d 10. Initial L* on d 0 chops was found to be significantly ($P < 0.001$) correlated with L* on measured on d 3 (d 3 chops; r = 0.71), d 7 (d 7 chops; r = 0.73), and d 10 (d 10 chops; r = 0.84). Initial a* measurement on d 0 chops was found to be significantly ($P < 0.001$) correlated with a* on d 3 (d 3 chops; r = 0.66), d 7 (d 7 chops; r = 0.40), and d 10 (d 10 chops; r = 0.42). Hunter a* increased ($P < 0.05$) from d 0 to a peak value on d 3, then returned to d 0 level by d 7 and decreased farther by d 10. Color intensity (chroma) followed a similar pattern increasing ($P < 0.05$) from d 0 to a peak value on d 3, then subsequently decreasing from d 3 to d 7 and from d 7 to d 10. Hunter b* increased ($P < 0.05$) from d 0 to d 3 but then remained unchanged from d 3 through to d 10. Initial b* on d 0 chops significantly correlated ($P < 0.001$) to b* measurements on d 3 (d 3 chops; r = 0.50), d 7 (d 7 chops; r = 0.65), and d 10 (d 10 chops; r = 0.76). Discoloration of chops (hue angle) initially decreased ($P < 0.05$) from d 0 to d 3, but by d 7 was greater than either d 0 or d 3 and increased farther from d 7 to d 10. Hue angles observed on d 3, d 7, and d 10 chops significantly correlated to initial hue angle observed on d 0 chops ($P < 0.01$).

No interactions ($P > 0.10$) between display day and treatment were observed for chop color. Heat stress tended to reduce chop d 7 L* ($P = 0.098$), d 10 b* ($P = 0.062$), and d 10 chroma ($P = 0.057$) relative to TN. Day 3 b* was less ($P = 0.048$) at the 50 mg kg^{-1} Zn level compared to the 130 mg kg^{-1} Zn level. Inorganic Zn increased d 10 a* (6.16 vs. 5.83, $P = 0.008$) and decreased discoloration (hue angle, $P = 0.004$) compared to organic Zn. A significant interaction between ENV and SOR existed for d 7 b* ($P = 0.026$), d 10 b* ($P = 0.010$), d 10 chroma ($P = 0.010$), and tended to exist for d 3 a* ($P = 0.053$), because under TN conditions, organic source Zn decreased these color parameters compared to inorganic source Zn while under HS conditions, organic source Zn increased these color parameters relative to inorganic source Zn. Under TN conditions, inorganic source Zn reduced d 3 hue angle compared to organic source Zn yet under HS conditions,

inorganic source Zn increased d 3 hue angle relative to organic source Zn (ENV × SOR, $P = 0.020$). A significant interaction was also observed between LEV and SOR for d 3 L*, which was reduced by organic source Zn at the 50 mg kg^{-1} Zn level but increased by organic source Zn at the 130 mg kg^{-1} Zn level (LEV × SOR, $P = 0.014$). There was also a tendency for an interaction between ENV and LEV ($P = 0.069$) on d 0 L*, which was lower at the 50 mg kg^{-1} Zn level under TN conditions but even lower at 130 mg kg^{-1} Zn level under HS conditions.

For sausage patties, L* increased ($P < 0.05$; Table 2.27 and 2.28, Fig. 2.5) gradually over time, with d 7 values greater than on d 0 and d 10 values greater than on d 3. Initial L* on d 0 patties was significantly ($P < 0.001$) correlated with L* on measured on d 3 (d 3 patties; r = 0.71), d 7 (d 7 patties; r = 0.67), and d 10 (d 10 patties; r = 0.74). Hunter a* decreased ($P < 0.05$) at each display time point from d 0 to d 10. Color intensity (chroma) followed a similar pattern decreasing ($P < 0.05$) from d 0 to d 7 but no farther decrease from d 7 to d 10. Initial a* measurement on d 0 patties significantly ($P < 0.05$) correlated with a* on d 3 (d 3 patties; r = 0.53), d 7 (d 7 patties; r = 0.24), and d 10 (d 10 patties; r = 0.35). Hunter b* decreased ($P < 0.05$) from d 0 to d 3 and to a lesser extent from d 3 to d 7 but by d 10 had returned to d 3 level. Initial b* on d 0 patties failed to correlate ($P > 0.10$) with b* measurements of d 3, d 7, or d 10 patties. Discoloration of sausage patties (hue angle) increased ($P < 0.05$) at each display time point from d 0 to d 10. Hue angles observed on d 3, d 7, and d 10 patties significantly correlated to initial hue angle observed on d 0 patties ($P < 0.001$). No interactions ($P > 0.10$) between display day and treatment were observed. Compared to TN, HS decreased d 0 b* ($P = 0.007$), d 10 b* ($P < 0.001$), d 10 chroma ($P = 0.002$), and tended to decrease d 0 chroma ($P = 0.058$) and d 10 hue angle ($P = 0.096$). Day 3 L* was decreased ($P = 0.016$) at the 50 mg kg^{-1} Zn level relative to 130 mg kg^{-1}. No effects of Zn source

($P > 0.10$) on sausage patty color nor any treatment associated changes in patty a* ($P > 0.10$) were observed.

In loin chops under display, lipid oxidation increased ($P < 0.05$; Table 2.29 and 2.30) from d 0 to d 3 and from d 3 to d 10 but the increase between d 3 and d 7 as well as the increase from d 7 to d 10 were not statistically different. No interactions between display day and treatment nor any main effects of treatment within each display day on lipid oxidation of loin chops were observed ($P > 0.10$). On day 3, there was a tendency for an interaction between LEV and SOR where MDA was lessened by organic Zn source at the 50 mg kg^{-1} level but was increased by organic Zn source at the 130 mg kg^{-1} level (LEV × SOR, $P = 0.094$). A tendency was also observed for a 3-way interaction (ENV × LEV × SOR, $P = 0.099$) because the decrease in d 3 MDA level by the organic Zn source at the 50 mg kg^{-1} level was evident under HS but not TN conditions. The initial MDA content of the 48-chop subset of d 0 chops was negatively correlated with their initial b* (r = -0.37; $P = 0.010$) and saturation index (r = -0.30; $P = 0.037$) values; however, d 10 MDA levels were not significantly correlated ($P > 0.50$) to any of the d 10 color measurements. The level of MDA on d 0 was highly correlated with the MDA content measured in the d 10 chops following 10 days of display (r = 0.77; $P < 0.001$) and the correlation was strong within both the TN (r = 0.82; $P < 0.001$) and HS groups (r = 0.71; $P < 0.001$).

Lipid oxidation increased ($P < 0.05$; Table 2.31 and 2.32, Fig. 2.6) in pork sausage patties at each time point but no significant interactions were observed between display day and treatment. Initially, sausage derived from HS animals tended (d 0, $P = 0.071$) to have less lipid oxidation than that from TN animals but within each subsequent display day, no lipid oxidation differences ($P > 0.10$) due to treatment were observed. Across all treatments, the initial MDA content of d 0 patties was positively correlated with the d 10 MDA content (r = 0.31; $P = 0.005$). However, further

analysis showed that the correlation between d 0 and d 10 lipid oxidation was observed among the portion of sausage patties originating from TN carcasses (r = 0.34; P = 0.033) but did not appear to be significant among those from the HS carcasses (r = 0.29; P = 0.069). Across all sausage samples, the initial MDA content of d 0 patties was positively correlated with initial a* (r = 0.22; P = 0.045), b* (r = 0.44; $P < 0.001$), and saturation index (r = 0.39; $P < 0.001$) values; however, d 10 MDA levels in sausage patties were not significantly correlated ($P > 0.10$) to any of the d 10 color measurements.

The extractable lipid content was not related to the initial TBARS values of the sausage patties (Pearson correlation, $P \geq 0.089$) but was negatively related to the initial TBARS values of the loin chops (r = -0.41, P = 0.004). In the portion of chops from TN animals, the correlation between initial TBARS and extractable lipid content was stronger (r = -0.55, P = 0.006) but among chops from the subset of HS animals, a significant relationship was not observed (Pearson correlation, P = 0.210).

2.5 Discussion

Cortisol level may serve as an indicator of stress associated with acute heat exposure but has not been established as a reliable indicator of chronic thermally induced stress in the pig (Pearce et al., 2013). Consequently, thermal monitoring was used to measure the effects of the HS treatment on core body temperature and an indicator of heat dissipation. The heat challenge model used in this study elevated animals' core body temperature during acute heat exposure. After repeated exposure to the combination of both chronic and acute heat challenge, the thermoregulatory response employed by HS animals was inadequate to preserve core body temperature (days 42 - 45) among the heat challenged animals. The lighter body weight and hot carcass weight of the heat stressed pigs relative to the thermoneutral pigs also indicates the chronic

heat stress model employed in this study successfully induced the classic growth reduction response (Baumgard and Rhoads, 2013) which is of practical concern to the swine industry.

Under research settings, long-term exposure to constant high temperatures (30 °C) has been shown to decrease growth by 18 kg over 55 d resulting in both decreased protein and lipid accretion (Santos et al., 2018). However, behavioral adaption to diurnal heat conditions such as shifting meal consumption to cooler nighttime hours (Quiniou et al., 2000) may lessen the effects of long-term heat exposure on feed intake and growth performance. Feeding behavior was not monitored in this study and the outcome of our model agrees with commercial data indicating naturally occurring heat load reduces body weight and hot carcass weight of pigs (Fragomeni et al., 2016).

In the present study, HS negatively impacted dressing percentage in contrast to Cruzen et al. (2015a) who reported HS tended to increase dressing percentage in addition to decreasing hot carcass weight. All other factors equal, smaller carcasses are associated with lower dressing percentages because less lipid and lean accretion contributing to carcass weight does not dilute out the weight of the non-carcass components. The reduction in dressing percentage which was observed among the HS animals slaughtered on day 71 compared to the day 64 TN animals with similar HCW suggests that the HS tendency to reduce d 64 dressing percentage is not simply the result of smaller carcasses among the HS group.

Considering several compositional changes observed under HS, a decrease in dressing percentage due to HS is surprising. Numerically greater bone weights have been observed under chronic HS (Cruzen et al., 2015a) and could actually contribute to a greater dressing percent in HS carcasses. Gestational HS has been shown to decrease head weight in pigs but this effect appears to be confined to specific developmental periods (Cruzen et al., 2015a). Under different models of

HS, liver and viscera weights have been reported to decrease (Kerr et al., 2003; Johnson et al., 2015; Cui et al., 2016) which could also improve dressing percent.

However, compensatory feed intake post-HS has been reported in research pigs (Kerr et al., 2005) and differences in gastrointestinal tract contents can create differences in dressing percentage. In the present study, compensatory feed intake and water intake in the days succeeding the d 63 - 65 acute heat wave may have increased gut fill among the HS pigs slaughtered on d 71 and thus contributed to their poorer dressing percentage relative to d 64 TN pigs. The tendency for reduced dressing percentage among d 64 HS pigs remains unexplained however and further investigation would be edifying.

Zinc source appeared to affect LEA in a temperature dependent manner; this effect remained significant even when HCW differences were accounted for indicating a possible effect of Zn on skeletal muscle to the exclusion of other tissue types. Zinc is implicated as an important molecule in the mTOR pathway, which is a critical signaling pathway required for protein synthesis (Kim et al., 2000; Szewczyk et al., 2015). Zinc's phosphorylation of insulin receptor substrate (IRS-1) and effect on protein kinase B (Akt) inhibits negative regulation of mTOR activity by tumor suppressors TSC1/TSC2 (Lynch et al., 2001; Brugarolas et al., 2004). Furthermore, Zn may directly stimulate mTOR activity by protein phosphorylation of mTOR (Lynch et al., 2001). Under normal physiological conditions, organic sources of highly bioavailable Zn might stimulate the mTOR pathway resulting in increased muscle synthesis for the pigs of the present study. However under heat stressing conditions, prevailing intracellular hypoxia and low energy levels will inhibit mTOR function (Brugarolas et al., 2004; Laplante and Sabatini, 2009). In addition, heat stress can damage intestinal epithelium and absorptive function, a phenomenon which Zn oxide is known to ameliorate in weanling pigs (Li et al., 2001; Li et al.,

2006; Mills, 2018). Further research would be useful to determine the impact of Zn on the protein synthetic pathway under hypoxic, energy replete conditions.

The combined effects of heat and its associated reduction in feed intake under the present model tended to lessen both adipose and lean tissue accretion on a fixed time basis. Despite the retarded growth of the HS animals, the non-polar lipid content of the *longissimus thoracis* was numerically but not significantly greater under HS relative to TN and no differences were observed visually. Heat stress is known to alter lipid metabolism and depress lipolytic potential resulting in greater lipid accumulation than energetically expected (reviewed by Baumgard and Rhoads, 2013; reviewed by Rhoads et al., 2013; Qu et al., 2015; Kellner et al., 2016; Qu and Ajuwon, 2018). The present experiment, however, was not designed to quantify individual pig energy intake and determine the direct effect of HS on lipid accretion rate.

Despite the clear reduction in hot carcass weight and smaller *longissimus thoracis* area in the present study, lipid accretion was not consistently reduced across all depots i.e. subcutaneous backfat, intramuscular fat, intermuscular deposition in the belly center-slice, etc. Unlike Kellner et al. (2016) who found HS bellies were lighter and thinner, differences in belly size and percent lean under HS were not observed in the present study. Although White et al. (2008) reported no differences in belly weight, they did find that bacon slices had increased percent lean under HS conditions relative to TN.

After 5-8 weeks of diurnal heat load, HS barrows have reportedly yielded carcasses with less backfat despite a lack of difference in loin eye area/depth relative to TN counterparts (Cruzen et al. 2015a; Kellner et al., 2016). In contrast, the HS pigs in the current study (predominantly gilts) exhibited more subcutaneous fat measured at the last rib when compared to the TN pigs of similar weight. Pig sex, genetic composition, rate of feed intake during thermal challenge and re-

alimentation post-heat stress, as well as the stage of growth maturity affecting partitioning of energy to fat versus lean may all contribute to the differences observed between the studies. Further research on the effects of chronic and cyclic heat on individual energy intake, lean growth curve, and carcass composition of low vs. high-lean genetics would be useful.

Zinc supplementation had negligible impact on loin marbling scores similar to the observation of Shelton et al. (2004) who observed trace mineral supplementation failed to impact marbling. The impact of HS on intramuscular fat deposition is not straightforward, as intramuscular fat has been shown to be decreased by 3 weeks of HS (Cui et al., 2018) yet unaffected by 7-10 weeks of constant HS (Cruzen et al., 2015a) and by seasonal differences under extensive rearing conditions (Simonetti et al., 2018). It is unclear why in the present study, despite the deeper subcutaneous fat layer at the last rib due to HS, the subjective assessment of intramuscular fat in the loin tended to be equal (day 64) or less (day 71) in the HS carcasses relative to day 64 TN carcasses. Intramuscular fat deposition is classically the last depot to be filled during development (Gerrard and Grant, 2003) but appeared to be less in the day 71 HS carcasses relative to day 64 HS carcasses. This apparent reduction could be due simply to individual pig variation in intramuscular adipocyte number and size considering the short time period investigated and the relatively low lipolytic and metabolic activities of the porcine intramuscular adipocytes (Gondret et al., 2008). Moreover, intramuscular adipocytes have less than 10% of the fatty acid synthetic activity of subcutaneous adipocytes (Hausman et al., 2009).

Overall moisture, protein, and extractable lipid content of the *longissimus thoracis* samples were similar to composition values reported by others (Joo et al., 2002; Park et al., 2008; Sobotka et al., 2012; Jung et al., 2015) and were not affected by the chronic HS model employed in this study. However, there was a 13% numerical increase in the extractable lipid content of *longissimus*

samples from the HS pigs relative to TN pigs. Furthermore, only the neutral lipid fraction (triacylglycerols, sterols, etc) which is the main lipid storage form was assessed in this study; polar phospholipids and unbound fatty acid levels were assumed to be relatively consistent among treatments but was not assessed.

Comparison of chemical lipid measurements taken on the subset (n = 48) of day 64 carcasses with the corresponding marbling scores of the carcasses revealed the strongest relationship in the TN carcasses and a weaker relationship among the HS carcasses. Visual scores of intramuscular fat have been shown to have high correlation with chemical measurements (Faucitano et al., 2004). Further research would be useful to verify this apparent change in relationship among HS carcasses. Greater adipose tissue moisture has been reported for some fat depots of thermally challenged pigs (Seibert et al., 2018) and might also contribute to the weakened relationship between visual and chemical measurements. Additionally, greater adipose tissue moisture has been reported for some fat depots of thermally challenged pigs (Seibert et al., 2018) and may have contributed to the greater moisture content observed in the high fat (30%) sausage product from HS carcasses in the present study.

The diet IV's calculated using the analyzed fatty acid profile of the diets showed some variability which is likely the result of sampling variability and inability to obtain a representative dietary sample of the small mass required for the fatty acid analysis. Of the primary FA constituents of the pork carcass (16:0, 16:1, 18:0, 18:1, and 18:2; Kloareg et al., 2005), sausage product from HS carcasses in the present study was characterized by lower percentage of oleic acid (C18:1n-9) and greater percentages of stearic acid (C18:0) and linoleic acid (C18:2n-6). Similar shifts have been reported by others, implicating a possible effect of chronic HS on stearoyl-CoA desaturase (**SCD**) enzyme activity which desaturizes palmitic and stearic acids to

palmitoleate and oleate. Based on observations of HS increasing levels of SFA and PUFA with decreases in MUFA level and SCD index, Seibert et al. (2018) proposed the "homeoviscous adaptation concept" where fatty acid saturation is increased under periods of HS to preserve lipid membrane integrity (Péter et al., 2017). In accordance with this theory, Kloareg et al. (2005) reported that HS decreased the desaturation of C18:0 to C18:1n-9, and was partially responsible for the greater concentration of C18:0 under HS compared to TN.

Contrary to this apparent decrease in SCD activity, HS has actually been shown to upregulate SCD transcriptase in skeletal muscle (Hao et al., 2016) as well as in adipose (Kellner et al., 2016), although in the latter tissue no concomitant shift in the ratio of C18:0 to C18:1 was observed. Additional data suggests HS decreases C18:1n-9 in adipose but the mechanism remains obscure because relative to TN conditions, adipocyte SCD mRNA was increased in HS pigs which were also crowded but was reduced in HS pigs not crowded (White et al., 2008).

Upregulated SCD gene expression is believed to precede lipid filling during the maturation of preadipocytes and thus is downregulated under feed restriction (Hausman et al., 2009). However, under conditions of insulin resistance, hepatic SCD activity is increased due to the stimulus of insulin and results in an increase in MUFA (reviewed by Ntambi, 1999; Tan et al., 2015); these MUFA have a greater rate of esterification rate relative to SFA (Aljohani et al., 2017). However, the predominant (Kloareg et al., 2005) product of SCD catalyzed desaturation, oleate, exacerbates insulin resistance by decreasing skeletal muscle GLUT4 and hepatic GLUT2 protein abundance; moreover, a systemic SCD1 knockout increases glucose uptake via increased GLUT4 expression (Aljohani et al., 2017). Consequently, decreasing production of oleate may be an adaptive strategy in pigs to counter the hyperinsulinemia caused by HS.

Because of the central role of SCD in energy metabolism, this enzyme contributes to metabolic cross-talk between hepatic and peripheral lipogenic activity; it has been proposed that hepatic oleate might be a stronger stimulator of lipogenesis in peripheral adipose tissue than local SCD activity (Aljohani et al., 2017). However, under HS physiological conditions White et al. (2008) failed to observe a difference in hepatic SCD1 mRNA. Thus, the inconsistencies in FA saturation and SCD mRNA levels might be better explained by disruption of SCD function possibly via posttranscriptional modification which PUFA's are believed to affect (Ntambi, 1999). Therefore, future investigation into FA desaturase activity and regulation under HS is needed to clarify the inconsistencies in the literature and provide a clear explanation of why and how the changes in FA profile, particularly in oleic acid, are occurring.

In the present study, the differences in individual FA constituents shifted UFA (MUFA, PUFA) and SFA. The decrease in C18:1n-9 caused most of the decrease in MUFA in HS products. The numerical increase in linoleic acid (C18:2n-6) was responsible for the majority of the increase in PUFA and specifically the n-6 PUFA in product from HS compared to TN. However, in the present study the HS increase in linoleic acid (C18:2n-6) was not sufficient to shift the n-6:n-3 ratio nor enough to counter the decrease in MUFA in HS product such that the reduction in total UFA in HS product was driven by the decrease in C18:1n-9. The increase in stearic acid (C18:0) constituted the majority of the increase in SFA in HS product. In addition, the observed fatty acid saturation differences reflect differences in growth rate (and by inference, feed intake) observed under the various environmental and Zn level combinations; the unsaturated percentage was greater among the treatments also yielding heavier body weight animals. This is empirically supported by the fact that deposition of unsaturated fatty acids from the diet outpaces that of endogenously synthesized saturated fatty acids among fast growing animals (Hausman et al., 2009).

However, simply accounting for differences in HCW failed to account for the observed differences in fatty acid saturation.

The interaction between Zn level and environmental temperature in SFA percentage and SFA:UFA drove the main effect of HS increasing SFA and SFA:UFA. Other studies have failed to show an effect of chronic HS on the SFA:UFA ratio in adipose tissue (White et al., 2008; Kellner et al., 2016; Seibert et al., 2018); however, none of these studies considered changes occurring beyond 35 days of HS. Despite the FA profile of the present study being assessed on a 70% skeletal muscle / 30% adipose tissue product, the overall saturation ratio was similar to that observed in the adipose tissue in the other published studies. However, the present observations of shifted fatty acid profile are based singularly on the lipid deposition in the picnic shoulder wholesale cut and may not be reflective of all pork carcass fat depots.

Considering the interaction between Zn level and environmental temperature in SFA percentage and SFA:UFA, there was a tendency for a similarly patterned interaction in the fat percentage of the loin. In the respective tissues, concentration of fat and saturation were similar between TN and HS when Zn was supplemented at the 50 mg kg^{-1} level but when Zn was supplemented at 130 mg kg^{-1}, both lipid content and saturation increased under HS conditions but not in TN conditions. Given the importance of Zn in countering hyperglycemia under insulin resistance and Zn's pivotal roles in anabolic signaling pathways including mTOR and IGF-1 stabilization (Himoto and Masaki, 2018), feeding greater levels may stimulate *de novo* fatty acid synthesis without causing measurable compositional differences on a whole-body level. Furthermore, this action of Zn might also provide partial explanation of the curious interaction between Zn level and source on stearic acid (C18:0) percentage. At a presumably non-limiting Zn level of 130 mg kg^{-1}, the more available organic source of Zn increased C18:0 but at a possibly

limiting Zn level of 50 mg kg^{-1}, organic source impacted C18:0 percentage similarly to inorganic source.

Despite the changes in FA profile, the iodine value (**IV**) of the sausage product was not affected by treatment. Accordingly, several other studies have failed to demonstrate a direct effect of HS on the IV of various fat depots including jowl, abdominal, subcutaneous backfat, and belly despite small changes within the fatty acid profile (White et al., 2008; Kellner et al., 2016; Seibert et al., 2018). The IV of the sausage product of the present study was similar to the IV for back and belly fat reported by White et al. (2008), and a couple points lower than the IV for jowl fat reported by Kellner et al. (2016) when higher IV diets were fed, and the IV for subcutaneous neck fat reported by Seibert et al. (2018) when 15% distiller's dried grains with solubles (DDGS) was fed. Furthermore, there was no impact of HS on belly stiffness in the present study in accordance with the lack of any large magnitude of change in unsaturated lipid content of the sausage product. No reduction in belly firmness due to chronic HS was reported by Kellner et al. (2016), despite the HS bellies being lighter and thinner.

The interaction of organic source Zn improving belly firmness under HS but reducing firmness under TN conditions did not correspond to a shift in FA profile to support this interaction. However, similar interactions between environment and zinc source were observed for LEA which were smaller with organic source under HS compared to organic under TN, and less desirable color (b* and chroma) of displayed loin chops when organic Zn was fed under HS compared to under TN. These interactions indicate the source of Zn fed under different environmental conditions has the potential to impact various aspects of pork quality through either similar or disparate mechanisms.

Increased desaturation of fatty acids in meat product increases the risk of oxidation and reduces the shelf-life stability of pork. Therefore, changes in lipid oxidation (TBARS) were monitored over a 10-day simulated retail display. The lipid oxidation increased over the 10-day display period in both loin chops and sausage patties despite addition of a commercial seasoning blend which contained both salt (a pro-oxidant) and unidentified spices (possible antioxidative effect). However, lipid oxidation in both products was unaffected by preharvest Zn supplementation level or source. Because Zn has dual roles as both an in vivo antioxidant and prooxidant (Lee, 2018), the impact of preharvest Zn supplementation on oxidative stability of pork remains largely unexplored. However, under normal physiological conditions, activity of hepatic antioxidant enzymes was unresponsive to supplemental Zn (Gowanlock et al., 2015) and may indicate activity in peripheral skeletal muscle tissues is also unaffected by Zn supplementation.

Thermal challenge antemortem has been shown to reduce the oxidative stability of postmortem broiler meat (Harsini et al., 2012; Hao and Gu, 2014) and increase pork peroxidation risk when pigs were also exposed to multiple antemortem stressors (White et al., 2008). Therefore, the tendency for lower TBARS in the sausage product from HS animals at the start of the display period was not anticipated. However, acute thermal challenge has been shown to induce antioxidant enzymatic responses in porcine muscle (Montilla et al., 2014; Cruzen et al., 2015b; Ganesan et al., 2017; Volodina et al., 2017). Fabrication of sausage occurred 72-96 h postmortem so early activity of upregulated antioxidant enzymes in HS carcasses may have lessened oxidative byproduct accumulation in the fat tissue which composed 30% of the sausage product. Overholt and colleagues (2018) hypothesized that the seemingly greater susceptibility of poultry meat to oxidative challenge relative to the more robust oxidative stability of pork may be attributable to muscle fiber type differences and more endogenous enzymatic activity in pork relative to poultry.

Further research is needed to understand the effect of chronic, cyclic heat events on redox balance in the pig and antioxidant activity postmortem. Particularly, attention should be given to potential differences across muscle metabolic type as HS has been shown to have a greater oxidative insult and elicit a stronger antioxidative response in oxidative muscle compared to glycolytic muscle (Montilla et al., 2014).

Lipid oxidation in pork generates reactive aldehydes and ketones which in turn catalyze further oxidation in cellular molecules. As reviewed by Suman and Joseph (2013), the myoglobin color pigment in pork is believed to be less susceptible to direct oxidation by these products of lipid oxidation compared to other species' myoglobin stability. Independent of lipid oxidation changes, HS could impact color stability through other mechanisms, such as by increasing antemortem lactate removal through increased peripheral blood flow, overloading cellular radical scavenging antioxidant ability, leaching of sarcoplasmic myoglobin pigment as a consequence of deteriorated lipid membrane integrity, increasing myoglobin concentration as a function of myofiber atrophy, and by shifting metabolism and thereby the regeneration of reducing equivalents (from glycolytic metabolism intermediates) or mitochondrial activity and oxygen consumption rate (Carr et al., 2005; AMSA, 2012).

Heat stress has negatively impacted meat color in poultry (Zeferino et al., 2016) and in pigs (Cui et al., 2018; Simonetti et al., 2018). In pigs, the reported increases in L* and decreases in a* and b* values in the HS carcasses corresponded to lower pH also attributed to HS. In the present study, the subjective color scores 24 h postmortem corresponded to the pH values taken on the carcasses. Overall, color scores were lighter than those Guo et al. (2006a; 2006b) observed on carcasses of greater pH than those observed in this study, but in the present study both color and

firmness scores were improved among the HS carcasses which also had greater pH compared to the TN carcasses.

It appears that the present study is the first to examine the effect of HS on color development over time in displayed pork products from HS carcasses compared to TN. Despite the initial improvement in subjective loin color, no consistent differences in objective color measurements were observed in the packaged pork products derived from those carcasses throughout the 10 day display period with the exception of less yellow hue (d 0 and d 10) and color saturation (d 10 and tendency d 0) in the 30% fat, ground product of the HS treatment. The b* measurement is associated with oxidative changes in meat color as metmyoglobin formation occurs resulting in more brown and green hues (MacDougall, 2002). However, visual assessment of brown color has been shown to inversely correlate to yellow (O'Sullivan et al., 2003) thus instrumental b* may have limited usefulness in representing the visual color attributes perceived by assessors. The lack of sustained color difference over time highlights the importance of studying muscle, processing, and time related changes in color in addition to initial color parameters.

Pork color appears to be fairly robust to different Zn supplementation levels. Similar to the present results, pork color was not impacted by dietary reduction of trace minerals preharvest (Gowanlock et al., 2013; Shelton et al., 2004). There has been a report of TM deletion negatively impacting water holding capacity and concomitantly affecting some color attributes of pork loin over time (Ma et al., 2012); however, the study was not designed to determine the direct effect of Zn deletion or supplementation. Over a 5 day display period, Zn supplementation improved color and MRA of pork from ractopamine fed pigs (Paulk et al., 2014). Zinc's amelioration of undesirable color shifts attributed to metabolic modification suggests a possible role of Zn in maintaining pork color quality under certain physiological conditions. However, in the present HS

model, Zn failed to provide any benefit to pork color. Loin firmness scores observed in this study were comparable to those reported by Guo et al. (2006a; 2006b).

Across treatments, the *longissimus thoracis* pH values were low relative to values reported in other studies (Shelton et al., 2004; Guo et al., 2006a; 2006b; Kellner et al., 2016). The low pH may have been the consequence of these pigs not having a lengthy feed withdrawal prior to slaughter, use of electrical stunning, and a modest carcass chilling rate at the research facilities. In agreement with the present study, previous data has failed to show an effect of Zn supplementation on ultimate loin pH (Shelton et al., 2004; Gowanlock et al., 2013; Paulk et al., 2014). The small but significant increase in pH observed in the cyclic HS carcasses on day 64 was unanticipated as studies have shown a negative effect of prolonged heat exposure on carcass pH (Hao et al., 2014; Cui et al., 2018; Simonetti et al., 2018) or no pH difference at all (Kellner et al., 2016). It is worth noting that the pigs of the present study lacked a prolonged feed withdrawal and transport duration prior to arrival at the abattoir (≤ 60 min) while the pigs in the studies which had decreased or unaffected pH had at least a 1 – 14 hour transport or 10 hour feed withdrawal. Potentially, a high glycolytic potential due to minimal preharvest handling and sustained feed intake ante mortem by the day 64 TN animals and day 71 HS animals but not by the day 64 HS animals which were undergoing an acute heat insult after 45 days of heat is responsible for the present observations. However, HS has been shown to increase ultimate pH values in ruminants (Kadim et al., 2004) and poultry (Zeferino et al., 2016) so further investigation of chronic, cyclic heat impact on glycolytic muscle metabolism and rate of glycogen depletion/repletion is needed to clarify these results. Further, the present observations are limited to the *longissimus thoracis*; investigation of HS effects on pH in other muscle metabolic types would be valuable since measurements on the loin are not necessarily strongly correlated to quality traits of other cuts (Arkfeld et al., 2016).

The *longissimus thoracis* samples in the present study yielded greater drip loss values than those reported by others employing similar methodology (Guo et al., 2006a; 2006b) but the higher values may be due in part to the overall low pH values also measured in the present study. Corroborating with the higher pH observed among the HS carcasses, a 19% reduction in drip loss relative to TN carcasses was observed. Between the thermal treatments, the stronger correlation between pH and drip loss was observed among the HS subset albeit weaker than the r = -0.52 reported by Yang et al. (2014). Few studies have investigated potential effects of HS on pork drip loss; although both Simonetti et al. (2018) and Cui et al. (2018) reported a decrease in pH of HS carcasses, only Simonetti et al. (2018) also observed a negative effect of HS on water holding capacity. Together, the results of the present and previous studies suggest Zn does not affect drip loss either via supplementation during HS or via either reduction (Gowanlock et al., 2013) or supplementation (Shelton et al., 2004) under TN conditions.

In the present study, water loss measured as purge increased over the 10-day display period comparable to the pattern observed by Joo et al. (2002). The initial improvement in HS *longissimus thoracis* water holding capacity indicated by the lower drip loss values was not evident after further processing and time postmortem. Early (≤ 48 h) postmortem water holding capacity may be augmented by accelerated degradation of intermyofibrillar proteins (Melody et al., 2004). Degradative proteolysis of skeletal muscle is believed to increase under HS (Rhoads et al., 2013; Wang et al., 2016) which may translate into accelerated rates early postmortem; however, further research is needed to demonstrate this potential effect.

Joo et al. (2000; 2002) also reported an inverse relationship between lipid content and drip loss in loin samples. Analysis of the lipid content and drip loss data of the present study failed to exhibit a significant relationship ($P > 0.05$) between these variables in all pigs or in the subset of

HS, TN, 50 mg kg-1 Zn, 130 mg kg-1 Zn, or inorganic Zn supplemented animals. Yet curiously, among the organic Zn supplemented pigs a significant inverse relationship was observed (r = -0.50, P = 0.014) indicating greater lipid content was associated with less drip loss. Furthermore, a tendency for significant correlation between chop purge on day 3 and lipid content (r = -0.37, P = 0.085) was observed. However, among this subset of animals there was no evidence of a relationship between lipid content and pH despite a negative correlation between pH and drip loss (r = -0.35, P = 0.029). Whether or not organic Zn supplementation has a causal effect of promoting improved water holding capacity independent of pH through increased intramuscular adiposity remains an opportunity for further investigation.

2.6 Conclusions

In summary, cyclic heat stress affected carcass composition and quality but Zn level and source imparted negligible benefits. The cyclic heat decreased carcass weight and yield but did not consistently reduce fat deposition across all depots. Perhaps as a consequence of lower energy status among chronically stressed animals antemortem, fresh loin quality showed minor improvements in pH, water holding capacity, and initial color due to cyclic heat although there were few consistent differences in retail displayed processed meat color from heat stressed carcasses. Future research should assess how long-term exposure to heat stress affects glycolytic potential and enzyme activity in muscle, and whether shifts in muscle fiber type contribute to this phenomenon. Furthermore, investigating whether changes caused by heat stress affect pork tenderness and consumer acceptability of the pork products would be beneficial. The effects of Zn source on chop color differed by environment and Zn level but greater amounts of Zn failed to impart any benefit to oxidative stability and color. The Zn supplementation strategies evaluated in this study could be implemented without causing negative impacts on pork quality.

Shifts in the fatty acid profile were attributed to heat stress but practical differences in displayed product shelf-life and belly firmness were undetectable. Further research on the responses to heat magnitude, duration, frequency of recurring exposure, and the combination of heat with other production and meat processing stressors would be edifying. When pigs are exposed to additional antemortem stress factors such as crowding, health challenge, thermal and social shipping stress, and highly unsaturated and/or oxidized dietary ingredients, the effects of heat stress may be exacerbated. The oxidative stability observations of the present study were limited to fresh pork product under simulated retail display conditions and may not reflect oxidative stability of cured and processed pork under different storage conditions.

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Table 2.1. Diet composition of grow-finish diets (as-fed basis).[1]

	Study Phase 1	Study Phase 2	Study Phase 3
Study Days:	0 - 21	21 - 42	42 - 71
Approximate Body Weight:	72 – 91 kg	91 – 109 kg	109 – 129 kg
Ingredient, %			
Corn	71.475	74.815	77.865
Soybean meal (47.7% Crude protein)	12.630	9.480	6.640
Corn DDGS[2], 6 - 9% oil	10.000	10.000	10.000
Swine grease	3.000	3.000	3.000
Limestone	1.290	1.210	1.140
Monocalcium phosphate	0.250	0.190	0.120
Sodium chloride	0.300	0.300	0.300
L-Lysine HCl	0.380	0.370	0.350
DL-Methionine	0.050	0.030	0.010
L-Threonine	0.100	0.100	0.095
L-Tryptophan	0.025	0.030	0.030
Phytase[3]	0.100	0.100	0.100
Selenium premix[4]	0.050	0.050	0.050
Vitamin premix	0.150[5]	0.125[6]	0.100[7]
Trace mineral (**TM**) premix[8]	0.200	0.200	0.200
Total	100.000	100.000	100.000
Calculated Analysis			
*Standardized ileal digestible (**SID**) amino acids, %*			
Lysine	0.810	0.725	0.641
Threonine:Lysine	64.1	65.7	67.7
Tryptophan:Lysine	18.1	18.5	18.5
Methionine:Lysine	33.7	33.1	32.5
Methionine & Cysteine:Lysine	60.6	61.2	62.5
Valine:Lysine	70.1	71.3	73.6
Isoleucine:Lysine	59.0	58.7	59.1
Total lysine, %	0.94	0.85	0.76
Crude protein, %	15.1	13.8	12.7
Fat, %	6.5	6.6	6.6
Metabolizable energy, kcal kg^{-1}	3,446	3,454	3,462
Net energy, kcal kg^{-1} [9]	2,636	2,658	2,679
SID Lysine:Met. energy, g $Mcal^{-1}$	2.35	2.10	1.85
Ca, %	0.60	0.55	0.50
P, %	0.41	0.38	0.36
Available P, %	0.25	0.23	0.21
Basal Zn, mg kg^{-1} [10]	23.1	22.1	21.2
Total Iodine Value Product[11]	70.10	71.32	72.44

[1] All diets were fed in meal form and formulated to be fed in 3 phases for body weight of 68.0 to 88.5 kg (Study Phase 1; d 0 – 21), 88.5 to 108.9 kg (Study Phase 2; d 21 – 42), and 108.9 to 129.3 kg (Study Phase 3; d 42 – 65).

[2] Distillers' dried grains with solubles.

[3] Phyzyme® (Danisco Animal Nutrition, Marlborough, UK) providing 600 phytase units (FTU)/kg.

[4] Provided 0.3 mg of Se per kg of diet.

[5] Provided per kg of Phase 1 diet: 3,968 IU vitamin A, 397 IU vitamin D_3, 26.5 IU vitamin E, 1.3 mg menadione (vitamin K), 0.02 vitamin B_{12}, 5.3 mg riboflavin, 13.2 mg pantothenic acid, and 19.8 mg niacin.

[6] Provided per kg of Phase 2 diet: 3,307 IU vitamin A, 331 IU vitamin D_3, 22.0 IU vitamin E, 1.1 mg vitamin K, 0.02 vitamin B_{12}, 4.4 mg riboflavin, 11.0 mg pantothenic acid, and 16.5 mg niacin.

[7] Provided per kg of Phase 3 diet: 2,646 IU vitamin A, 265 IU vitamin D_3, 17.6 IU vitamin E, 0.9 mg vitamin K, 0.02 vitamin B_{12}, 3.5 mg riboflavin, 8.8 mg pantothenic acid, and 13.2 mg niacin.

Table 2.1 continued

[8] Supplemental Zn treatments were added to a fine-ground corn premix to facilitate a constant TM premix inclusion rate. For the diets which contained 50 mg kg^{-1} of available Zn from ZnO, a mineral premix containing ZnO was added at 0.052% of the 0.20%. To this basal premix, an additional 80 mg kg^{-1} of available Zn from ZnO for the 130 mg kg^{-1} inorganic source treatment and from Availa®Zn 120 (12% Zn; Zinpro Corporation, Eden Prairie, MN) for the 130 mg kg^{-1} organic source treatment was added. For the diet containing 50 mg kg^{-1} of available Zn from an organic source, a separate TM premix was made solely with Zn from AvailaZn®. For all treatments, the TM premix also supplied per kg of diet: 50 mg Fe, 6.2 mg Mn, 4.66 mg Cu, and 0.19 mg I.

[9] Dietary net energy values derived from feed ingredient energy values (NRC, 2012).

[10] Basal dietary Zn contributions from corn, soybean, and DDGS estimated from ingredient Zn values (NRC, 2012).

[11]Total Iodine Value Product (IVP) is the summation of IVP for all ingredients in the diet where IVP = (IV of ingredient fat) × (% fat in the ingredient) × (0.1), (NRC, 2012).

Table 2.2. Average dietary analysis of diets fed to finishing pigs fed varying zinc level & source under environmental heat stress (as-fed basis).[1]

Phase:	Phase 1	Phase 2	Phase 3
Study Days:	0 - 21	21 - 42	42 - 71
Approximate Body Weight:	72 – 91 kg	91 – 109 kg	109 – 129 kg
Dry Matter, %	87.7	87.2	87.6
Crude Protein, %	14.16	13.17	12.09
Total lysine, %	0.99	0.86	0.82
Total tryptophan, %	0.19	0.17	0.15
Total threonine, %	0.56	0.50	0.48
Total isoleucine, %	0.60	0.50	0.47
Total valine, %	0.70	0.63	0.59
Total methionine, %	0.30	0.27	0.25
Total cysteine, %	0.26	0.24	0.24
Total histidine, %	0.42	0.39	0.39
Total arginine, %	0.82	0.70	0.65
Total leucine, %	1.35	1.23	1.22
Total phenylalanine, %	0.75	0.64	0.61
Crude Fat, %	6.08	8.23	6.92
Crude Fiber, %	2.29	2.35	2.25
Energy, kcal/g	3.96	3.92	3.96
Ash, %	3.98	3.58	3.29
Calcium, %	0.58	0.56	0.51
Phosphorus, %	0.43	0.40	0.37
Zinc (mg kg^{-1})	91 - 175	83 - 183	97 - 202

[1]Dietary analysis performed on feed samples from 4 dietary treatments within each phase.

Table 2.3. Dietary analysis of study phase 1 diets fed to finishing pigs fed varying zinc level & source under environmental heat stress (as-fed basis).[1]

Diets	Phase 1 (Study days 0 – 21; 72 - 91 kg)			
Zinc Level:	50 mg kg^{-1}		130 mg kg^{-1}	
Zinc Source:[2]	Inorganic	Organic	Inorganic	Organic
Dry Matter, %	87.39	88.07	87.62	87.52
Crude Protein, %	14.63	13.69	14.25	14.06
Total lysine, %	0.97	0.91	1.16	0.92
Total tryptophan, %	0.19	0.17	0.20	0.19
Total threonine, %	0.57	0.52	0.59	0.54
Total isoleucine, %	0.63	0.56	0.65	0.56
Total valine, %	0.73	0.67	0.74	0.67
Total methionine, %	0.32	0.29	0.29	0.28
Total cysteine, %	0.28	0.25	0.27	0.24
Total histidine, %	0.42	0.38	0.46	0.41
Total arginine, %	0.84	0.76	0.90	0.76
Total leucine, %	1.40	1.29	1.39	1.31
Total phenylalanine, %	0.77	0.69	0.81	0.72
Crude Fat, %	6.35	6.08	6.23	5.65
Crude Fiber, %	2.25	2.22	2.39	2.31
Energy, kcal/g	3.96	3.99	3.95	3.93
Ash, %	4.17	3.97	3.94	3.85
Calcium, %	0.52	0.61	0.53	0.66
Phosphorus, %	0.44	0.43	0.43	0.43
Zinc (mg kg^{-1})	112	91	174	174

[1]Dietary analysis performed on feed samples from 4 dietary treatments within each phase.
[2]Added Zn source was either 100% inorganic from ZnO or predominantly organic from Availa®Zn (Zinpro Corp, Eden Prairie, MN) consisting of 100% Zn from Availa®Zn at 50 mg kg^{-1} level or 62% Zn from Availa®Zn and 38% from ZnO at the 130 mg kg^{-1} level.

Table 2.4. Dietary analysis of study phase 2 diets fed to finishing pigs fed varying zinc level & source under environmental heat stress (as-fed basis).[1]

Diets	Phase 2 (Study days 21 – 42; 91 - 109 kg)			
Zinc Level:	50 mg kg^{-1}		130 mg kg^{-1}	
Zinc Source:[2]	Inorganic	Organic	Inorganic	Organic
Dry Matter, %	87.39	88.07	87.62	87.52
Crude Protein, %	14.63	13.69	14.25	14.06
Total lysine, %	0.97	0.91	1.16	0.92
Total tryptophan, %	0.19	0.17	0.20	0.19
Total threonine, %	0.57	0.52	0.59	0.54
Total isoleucine, %	0.63	0.56	0.65	0.56
Total valine, %	0.73	0.67	0.74	0.67
Total methionine, %	0.32	0.29	0.29	0.28
Total cysteine, %	0.28	0.25	0.27	0.24
Total histidine, %	0.42	0.38	0.46	0.41
Total arginine, %	0.84	0.76	0.90	0.76
Total leucine, %	1.40	1.29	1.39	1.31
Total phenylalanine, %	0.77	0.69	0.81	0.72
Crude Fat, %	6.35	6.08	6.23	5.65
Crude Fiber, %	2.25	2.22	2.39	2.31
Energy, kcal/g	3.95	3.91	3.92	3.92
Ash, %	3.62	3.63	3.52	3.56
Calcium, %	0.52	0.61	0.53	0.66
Phosphorus, %	0.44	0.43	0.43	0.43
Zinc (mg kg^{-1})	100	83	174	182

[1]Dietary analysis performed on feed samples from 4 dietary treatments within each phase.
[2]Added Zn source was either 100% inorganic from ZnO or predominantly organic from Availa®Zn (Zinpro Corp, Eden Prairie, MN) consisting of 100% Zn from Availa®Zn at 50 mg kg^{-1} level or 62% Zn from Availa®Zn and 38% from ZnO at the 130 mg kg^{-1} level.

Table 2.5. Dietary analysis of study phase 3 diets fed to finishing pigs fed varying zinc level & source under environmental heat stress (as-fed basis).[1]

Diets	Phase 3 (Study days 42 – 71; 109 - 129 kg)			
Zinc Level:	50 mg kg^{-1}		130 mg kg^{-1}	
Zinc Source:[2]	Inorganic	Organic	Inorganic	Organic
Dry Matter, %	87.61	87.56	87.53	87.63
Crude Protein, %	11.69	12.06	12.69	11.94
Total lysine, %	0.76	0.82	0.87	0.83
Total tryptophan, %	0.13	0.16	0.15	0.14
Total threonine, %	0.45	0.47	0.50	0.48
Total isoleucine, %	0.47	0.46	0.45	0.48
Total valine, %	0.59	0.58	0.57	0.61
Total methionine, %	0.31	0.25	0.21	0.23
Total cysteine, %	0.24	0.26	0.22	0.23
Total histidine, %	0.40	0.38	0.37	0.39
Total arginine, %	0.64	0.63	0.62	0.69
Total leucine, %	1.25	1.20	1.16	1.26
Total phenylalanine, %	0.66	0.56	0.61	0.59
Crude Fat, %	7.02	6.49	5.99	8.19
Crude Fiber, %	2.30	2.24	2.20	2.27
Energy, kcal/g	3.96	3.95	3.96	3.96
Ash, %	3.36	3.24	3.27	3.30
Calcium, %	0.41	0.58	0.57	0.50
Phosphorus, %	0.37	0.37	0.35	0.39
Zinc (mg kg^{-1})	106	97	187	202

[1]Dietary analysis performed on feed samples from 4 dietary treatments within each phase.
[2]Added Zn source was either 100% inorganic from ZnO or predominantly organic from Availa®Zn (Zinpro Corp, Eden Prairie, MN) consisting of 100% Zn from Availa®Zn at 50 mg kg^{-1} level or 62% Zn from Availa®Zn and 38% from ZnO at the 130 mg kg^{-1} level.

Table 2.6. Analyzed total dietary zinc (mg kg^{-1}) concentration in diets fed to finishing pigs fed varying zinc level & source under environmental heat stress (as-fed basis).[1]

Phase:	Phase 1	Phase 2	Phase 3
Study Days:	0 - 21	21 - 42	42 - 71
Approximate Body Weight:	72 – 91 kg	91 – 109 kg	109 – 129 kg
50 mg kg^{-1} Added Zn from ZnO			
Dry matter, %	88.9	88.4	89.2
Zinc, mg kg^{-1}	114	101	108
50 mg kg^{-1} Added Zn from Availa®Zn			
Dry matter, %	89.6	89.3	89.3
Zinc, mg kg^{-1}	92	85	99
130 mg kg^{-1} Added Zn from ZnO			
Dry matter, %	89.3	89.5	89.4
Zinc, mg kg^{-1}	177	179	191
130 mg kg^{-1} Added Zn from ZnO and Availa®Zn[2]			
Dry matter, %	89.8	89.5	89.5
Zinc, mg kg^{-1}	178	187	206

[1]Analysis performed at Purdue University.

[2]Availa®Zinc (Zinpro Corporation, Eden Prarie, MN) supplied 80 mg kg^{-1} of Zn in addition to 50 mg kg^{-1} supplied from ZnO.

Table 2.7. Average fatty acid profile of diets fed over 3 phases to finishing pigs fed varying zinc level & source under environmental heat stress.[1]

Diet Phase:	Phase 1	Phase 2	Phase 3
Study Days:	0 - 21	21 - 42	42 - 71
Approximate Body Weight:	72.2–91.2 kg	91.2–109.4 kg	109.4–129.3 kg
Diet treatments per phase, n=	*4*	*4*	4
Fatty acid			
C14:0, %	0.56	0.50	0.51
C16:0, %	15.38	14.09	14.27
C16:1n-7, %	0.76	0.68	0.68
C16:2n-4, %	0.19	0.18	0.18
C16:3n-4, %	0.12	0.09	0.06
C18:0, %	6.90	6.01	6.31
C18:1n-7, %	1.32	1.21	1.20
C18:1n-9, %	26.68	25.42	25.64
C18:2 ^{9,11}, %	0.07	0.05	0.05
C18:2 ^{10,12}, %	0.01	0.01	0.01
C18:2n-6, %	30.41	29.74	29.97
C18:3n-3, %	1.04	0.95	0.92
C18:3n-6, %	0.01	0.01	0.01
C18:3n-4, %	0.04	0.02	0.03
C18:4n-3, %	0.32	0.30	0.30
C20:0, %	0.45	0.42	0.36
C20:1n-9, %	0.01	0.01	0.01
C20:3n-6, %	0.56	0.69	0.70
C20:4n-6, %	0.11	0.11	0.11
C20:5n-3, %	0.02	0.01	0.01
C22:0, %	0.11	0.11	0.09
C22:1, %	0.02	0.01	0.01
C22:4n-6, %	0.07	0.05	0.06
C22:5n-3, %	0.01	0.01	0.01
C22:5n-6, %	0.20	0.28	0.39
C22:6n-3, %	0.02	0.02	0.02
C24:0, %	0.56	0.25	0.25
Σ UFA[2]	61.98	59.85	60.37
Σ SFA[3]	23.96	21.39	21.79
Σ MUFA[4]	28.78	27.33	27.54
Σ PUFA[5]	33.20	32.53	32.82
Σ n-3 PUFA[6]	1.41	1.29	1.26
Σ n-6 PUFA[7]	31.36	30.88	31.24
n-6:n-3 ratio[8]	22.26	23.88	24.86
SFA:UFA ratio[9]	0.39	0.36	0.36
UFA:SFA ratio[10]	2.59	2.80	2.78
Iodine value, g/100g[11]	81.75	79.32	80.21

[1]Feed samples analyzed in duplicate for fatty acid profile via gas-liquid chromatography.

[2]Total UFA calculated as [C16:1n-7] + [C16:2n-4] + [C16:3n-4] + [C18:1n-7] + [C18:1n-9] + [C18:2^{9,11}] + [C18:2^{10,12}] + [C18:2n-6] + [C18:3n-3] + [C18:3n-6] + [C18:3n-4] + [C18:4n-3] + [C20:1n-9] + [C20:3n-6] + [C20:4n-6] + [C20:5n-3] + [C22:1] + [C22:4n-6] + [C22:5n-3] + [C22:5n-6] + [C22:6n-3].

[3]Total SFA calculated as [C14:0] + [C16:0] + [C18:0] + [C20:0] + [C22:0] + [C24:0].

[4]Total MUFA calculated as [C16:1n-7] + [C18:1n-7] + [C18:1n-9] + [C20:1n-9] + [C22:1].

[5]Total PUFA calculated as [C16:2n-4] + [C16:3n-4] + [C18:2^{9,11}] + [C18:2^{10,12}] + [C18:2n-6] + [C18:3n-3] + [C18:3n-6] + [C18:3n-4] + [C18:4n-3] + [C20:3n-6] + [C20:4n-6] + [C20:5n-3] + [C22:4n-6] + [C22:5n-3] + [C22:5n-6] + [C22:6n-3].

Table 2.7 continued

[6]Total n-3 PUFA calculated as [C18:3n-3] + [C18:4n-3] + [C20:5n-3] + [C22:5n-3] + [C22:6n-3].
[7]Total n-6 PUFA calculated [C18:2n-6] + [C18:3n-6] + [C20:3n-6] + [C20:4n-6] + [C22:4n-6] + [C22:5n-6].
[8]n-6:n-3 ratio calculated as Total n-6 PUFA ÷ Total n-3 PUFA.
[9]SFA:UFA ratio calculated as total SFA ÷ (total MUFA + total PUFA).
[10]UFA:SFA ratio calculated as (total MUFA + total PUFA) ÷ total SFA.
[11]Iodine value (IV) calculated as [C16:1] × 0.950 + [C18:1] × 0.860 + [C18:2] × 1.732 + [C18:3] × 2.615 + [C20:1] × 0.785 + [C20:4] × 3.201 + [C20:5] × 4.027 + [C22:1] × 0.723 + [C22:5] × 3.697 + [C22:6] × 4.463; equation 3-4, NRC (2012).

Table 2.8. Fatty acid analysis of study phase 1 diets fed to finishing pigs fed varying zinc level & source under environmental heat stress.[1]

	Phase 1 (Study Day 0 – 21; 72.2 – 91.2 kg)			
Dietary Treatment				
Zinc Level:	50 mg kg^{-1}		130 mg kg^{-1}	
Zinc Source:[2]	Inorganic	Organic	Inorganic	Organic
Fatty acid				
C14:0, %	0.57	0.60	0.52	0.57
C16:0, %	15.68	16.07	14.41	15.34
C16:1n-7, %	0.76	0.80	0.72	0.75
C16:2n-4, %	0.20	0.20	0.18	0.19
C16:3n-4, %	0.12	0.11	0.10	0.13
C18:0, %	7.21	7.19	6.30	6.90
C18:1n-7, %	1.33	1.37	1.25	1.32
C18:1n-9, %	26.96	27.77	25.36	26.63
C18:2 ^{9,11}, %	0.08	0.06	0.09	0.06
C18:2 ^{10,12}, %	0.01	0.00	0.01	0.00
C18:2n-6, %	30.67	31.32	28.91	30.74
C18:3n-3, %	1.02	1.07	0.98	1.08
C18:3n-6, %	0.01	0.01	0.01	0.01
C18:3n-4, %	0.07	0.05	0.02	0.03
C18:4n-3, %	0.34	0.32	0.31	0.32
C20:0, %	0.43	0.47	0.46	0.43
C20:1n-9, %	0.00	0.01	0.01	0.01
C20:3n-6, %	0.60	0.28	0.74	0.63
C20:4n-6, %	0.12	0.12	0.06	0.12
C20:5n-3, %	0.06	0.01	0.01	0.01
C22:0, %	0.11	0.10	0.11	0.12
C22:1, %	0.03	0.02	0.02	0.00
C22:4n-6, %	0.04	0.08	0.08	0.08
C22:5n-3, %	0.01	0.02	0.01	0.01
C22:5n-6, %	0.22	0.21	0.22	0.16
C22:6n-3, %	0.02	0.01	0.02	0.02
C24:0, %	0.10	0.26	0.70	1.19
Σ UFA[3]	62.69	63.84	59.10	62.30
Σ SFA[4]	24.09	24.69	22.50	24.55
Σ MUFA[5]	29.09	29.97	27.35	28.71
Σ PUFA[6]	33.60	33.87	31.75	33.59
Σ n-3 PUFA[7]	1.46	1.42	1.33	1.43
Σ n-6 PUFA[8]	31.67	32.03	30.02	31.74
n-6:n-3 ratio[9]	21.74	22.54	22.61	22.14
SFA:UFA ratio[10]	0.38	0.39	0.38	0.39
UFA:SFA ratio[11]	2.60	2.59	2.63	2.54
Iodine value, g/100g[12]	82.82	84.42	77.61	82.14

[1]Feed samples analyzed in duplicate for fatty acid profile via gas-liquid chromatography.

[2] Added Zn source was either 100% inorganic from ZnO or predominantly organic from Availa®Zn (Zinpro Corp, Eden Prairie, MN) consisting of 100% Zn from Availa®Zn at 50 mg kg-1 level or 62% Zn from Availa®Zn and 38% from ZnO at the 130 mg kg-1 level.

[3]Total UFA calculated as [C16:1n-7] + [C16:2n-4] + [C16:3n-4] + [C18:1n-7] + [C18:1n-9] + [C18:2^{9,11}] + [C18:2^{10,12}] + [C18:2n-6] + [C18:3n-3] + [C18:3n-6] + [C18:3n-4] + [C18:4n-3] + [C20:1n-9] + [C20:3n-6] + [C20:4n-6] + [C20:5n-3] + [C22:1] + [C22:4n-6] + [C22:5n-3] + [C22:5n-6] + [C22:6n-3].

[4]Total SFA calculated as [C14:0] + [C16:0] + [C18:0] + [C20:0] + [C22:0] + [C24:0].

[5]Total MUFA calculated as [C16:1n-7] + [C18:1n-7] + [C18:1n-9] + [C20:1n-9] + [C22:1].

Table 2.8 continued

[6]Total PUFA calculated as [C16:2n-4] + [C16:3n-4] + [C18:2[9,11]] + [C18:2[10,12]] + [C18:2n-6] + [C18:3n-3] + [C18:3n-6] + [C18:3n-4] + [C18:4n-3] + [C20:3n-6] + [C20:4n-6] + [C20:5n-3] + [C22:4n-6] + [C22:5n-3] + [C22:5n-6] + [C22:6n-3].
[7]Total n-3 PUFA calculated as [C18:3n-3] + [C18:4n-3] + [C20:5n-3] + [C22:5n-3] + [C22:6n-3].
[8]Total n-6 PUFA calculated [C18:2n-6] + [C18:3n-6] + [C20:3n-6] + [C20:4n-6] + [C22:4n-6] + [C22:5n-6].
[9]n-6:n-3 ratio calculated as Total n-6 PUFA ÷ Total n-3 PUFA.
[10]SFA:UFA ratio calculated as total SFA ÷ (total MUFA + total PUFA).
[11]UFA:SFA ratio calculated as (total MUFA + total PUFA) ÷ total SFA.
[12]Iodine value (IV) calculated as [C16:1] × 0.950 + [C18:1] × 0.860 + [C18:2] × 1.732 + [C18:3] × 2.615 + [C20:1] × 0.785 + [C20:4] × 3.201 + [C20:5] × 4.027 + [C22:1] × 0.723 + [C22:5] × 3.697 + [C22:6] × 4.463; equation 3-4, NRC (2012).

Table 2.9. Fatty acid analysis of study phase 2 diets fed to finishing pigs fed varying zinc level & source under environmental heat stress.[1]

	Phase 2 (Study Day 21 - 42; 91 – 109 kg)			
Dietary Treatment				
Zinc Level:	50 mg kg^{-1}		130 mg kg^{-1}	
Zinc Source:[2]	Inorganic	Organic	Inorganic	Organic
Fatty acid				
C14:0, %	0.52	0.52	0.49	0.49
C16:0, %	14.09	14.05	14.26	13.95
C16:1n-7, %	0.69	0.67	0.68	0.68
C16:2n-4, %	0.18	0.19	0.18	0.17
C16:3n-4, %	0.11	0.09	0.11	0.06
C18:0, %	6.11	6.04	6.09	5.82
C18:1n-7, %	1.22	1.21	1.22	1.20
C18:1n-9, %	25.06	25.46	25.89	25.25
C18:2 ^{9,11}, %	0.05	0.07	0.04	0.04
C18:2 ^{10,12}, %	0.00	0.01	0.01	0.01
C18:2n-6, %	28.95	29.65	30.63	29.74
C18:3n-3, %	0.97	0.95	0.98	0.91
C18:3n-6, %	0.02	0.01	0.00	0.02
C18:3n-4, %	0.02	0.02	0.00	0.04
C18:4n-3, %	0.30	0.31	0.27	0.30
C20:0, %	0.41	0.43	0.42	0.43
C20:1n-9, %	0.01	0.01	0.01	0.01
C20:3n-6, %	0.78	0.75	0.45	0.79
C20:4n-6, %	0.12	0.10	0.11	0.12
C20:5n-3, %	0.01	0.01	0.01	0.02
C22:0, %	0.10	0.11	0.15	0.09
C22:1, %	0.01	0.02	0.00	0.01
C22:4n-6, %	0.02	0.03	0.09	0.04
C22:5n-3, %	0.01	0.01	0.01	0.01
C22:5n-6, %	0.29	0.28	0.24	0.32
C22:6n-3, %	0.01	0.01	0.03	0.02
C24:0, %	0.26	0.24	0.24	0.24
Σ UFA[3]	58.84	59.86	60.96	59.75
Σ SFA[4]	21.49	21.39	21.65	21.02
Σ MUFA[5]	26.99	27.37	27.80	27.15
Σ PUFA[6]	31.85	32.49	33.16	32.60
Σ n-3 PUFA[7]	1.31	1.29	1.30	1.27
Σ n-6 PUFA[8]	30.18	30.82	31.52	31.02
n-6:n-3 ratio[9]	23.09	23.85	24.19	24.40
SFA:UFA ratio[10]	0.37	0.36	0.36	0.35
UFA:SFA ratio[11]	2.74	2.80	2.82	2.84
Iodine value, g/100g[12]	77.74	79.12	81.12	79.29

[1]Feed samples analyzed in duplicate for fatty acid profile via gas-liquid chromatography.

[2]Added Zn source was either 100% inorganic from ZnO or predominantly organic from Availa®Zn (Zinpro Corp, Eden Prairie, MN) consisting of 100% Zn from Availa®Zn at 50 mg kg^{-1} level or 62% Zn from Availa®Zn and 38% from ZnO at the 130 mg kg^{-1} level.

[3]Total UFA calculated as [C16:1n-7] + [C16:2n-4] + [C16:3n-4] + [C18:1n-7] + [C18:1n-9] + [C18:2^{9,11}] + [C18:2^{10,12}] + [C18:2n-6] + [C18:3n-3] + [C18:3n-6] + [C18:3n-4] + [C18:4n-3] + [C20:1n-9] + [C20:3n-6] + [C20:4n-6] + [C20:5n-3] + [C22:1] + [C22:4n-6] + [C22:5n-3] + [C22:5n-6] + [C22:6n-3].

[4]Total SFA calculated as [C14:0] + [C16:0] + [C18:0] + [C20:0] + [C22:0] + [C24:0].

[5]Total MUFA calculated as [C16:1n-7] + [C18:1n-7] + [C18:1n-9] + [C20:1n-9] + [C22:1].

Table 2.9 continued

[6]Total PUFA calculated as [C16:2n-4] + [C16:3n-4] + [C18:2[9,11]] + [C18:2[10,12]] + [C18:2n-6] + [C18:3n-3] + [C18:3n-6] + [C18:3n-4] + [C18:4n-3] + [C20:3n-6] + [C20:4n-6] + [C20:5n-3] + [C22:4n-6] + [C22:5n-3] + [C22:5n-6] + [C22:6n-3].
[7]Total n-3 PUFA calculated as [C18:3n-3] + [C18:4n-3] + [C20:5n-3] + [C22:5n-3] + [C22:6n-3].
[8]Total n-6 PUFA calculated [C18:2n-6] + [C18:3n-6] + [C20:3n-6] + [C20:4n-6] + [C22:4n-6] + [C22:5n-6].
[9]n-6:n-3 ratio calculated as Total n-6 PUFA ÷ Total n-3 PUFA.
[10]SFA:UFA ratio calculated as total SFA ÷ (total MUFA + total PUFA).
[11]UFA:SFA ratio calculated as (total MUFA + total PUFA) ÷ total SFA.
[12]Iodine value (IV) calculated as [C16:1] × 0.950 + [C18:1] × 0.860 + [C18:2] × 1.732 + [C18:3] × 2.615 + [C20:1] × 0.785 + [C20:4] × 3.201 + [C20:5] × 4.027 + [C22:1] × 0.723 + [C22:5] × 3.697 + [C22:6] × 4.463; equation 3-4, NRC (2012).

Table 2.10. Fatty acid analysis of study phase 3 diets fed to finishing pigs fed varying zinc level & source under environmental heat stress.[1]

	Phase 3 (Study Day 42 – 71; 109 – 129 kg)			
Dietary Treatment				
Zinc Level:	50 mg kg^{-1}		130 mg kg^{-1}	
Zinc Source:[2]	Inorganic	Organic	Inorganic	Organic
Fatty acid				
C14:0, %	0.51	0.49	0.55	0.49
C16:0, %	14.60	13.08	15.41	13.99
C16:1n-7, %	0.69	0.65	0.73	0.66
C16:2n-4, %	0.19	0.16	0.21	0.16
C16:3n-4, %	0.05	0.05	0.06	0.06
C18:0, %	6.26	5.49	7.48	6.03
C18:1n-7, %	1.23	1.16	1.22	1.19
C18:1n-9, %	26.14	24.58	26.88	24.95
C18:2 ^{9,11}, %	0.05	0.07	0.07	0.01
C18:2 ^{10,12}, %	0.01	0.01	0.00	0.01
C18:2n-6, %	31.17	28.72	30.87	29.10
C18:3n-3, %	0.96	0.87	0.94	0.90
C18:3n-6, %	0.01	0.01	0.02	0.02
C18:3n-4, %	0.05	0.02	0.03	0.02
C18:4n-3, %	0.30	0.29	0.31	0.29
C20:0, %	0.43	0.36	0.45	0.21
C20:1n-9, %	0.01	0.01	0.01	0.01
C20:3n-6, %	0.69	0.74	0.59	0.80
C20:4n-6, %	0.12	0.09	0.12	0.10
C20:5n-3, %	0.01	0.02	0.01	0.00
C22:0, %	0.11	0.08	0.12	0.03
C22:1, %	0.01	0.02	0.01	0.02
C22:4n-6, %	0.06	0.06	0.06	0.06
C22:5n-3, %	0.02	0.01	0.01	0.01
C22:5n-6, %	0.26	0.81	0.19	0.30
C22:6n-3, %	0.02	0.01	0.02	0.03
C24:0, %	0.22	0.47	0.09	0.24
Σ UFA[3]	62.04	58.36	62.36	58.70
Σ SFA[4]	22.12	19.95	24.10	20.99
Σ MUFA[5]	28.08	26.42	28.84	26.83
Σ PUFA[6]	33.96	31.94	33.52	31.87
Σ n-3 PUFA[7]	1.30	1.20	1.30	1.23
Σ n-6 PUFA[8]	32.31	30.43	31.84	30.38
n-6:n-3 ratio[9]	24.78	25.44	24.54	24.70
SFA:UFA ratio[10]	0.36	0.34	0.39	0.36
UFA:SFA ratio[11]	2.80	2.93	2.59	2.80
Iodine value, g/100g[12]	82.46	78.43	82.31	77.63

[1]Feed samples analyzed in duplicate for fatty acid profile via gas-liquid chromatography.

[2]Added Zn source was either 100% inorganic from ZnO or predominantly organic from Availa®Zn (Zinpro Corp, Eden Prairie, MN) consisting of 100% Zn from Availa®Zn at 50 mg kg^{-1} level or 62% Zn from Availa®Zn and 38% from ZnO at the 130 mg kg^{-1} level.

[3]Total UFA calculated as [C16:1n-7] + [C16:2n-4] + [C16:3n-4] + [C18:1n-7] + [C18:1n-9] + [C18:2^{9,11}] + [C18:2^{10,12}] + [C18:2n-6] + [C18:3n-3] + [C18:3n-6] + [C18:3n-4] + [C18:4n-3] + [C20:1n-9] + [C20:3n-6] + [C20:4n-6] + [C20:5n-3] + [C22:1] + [C22:4n-6] + [C22:5n-3] + [C22:5n-6] + [C22:6n-3].

[4]Total SFA calculated as [C14:0] + [C16:0] + [C18:0] + [C20:0] + [C22:0] + [C24:0].

[5]Total MUFA calculated as [C16:1n-7] + [C18:1n-7] + [C18:1n-9] + [C20:1n-9] + [C22:1].

Table 2.10 continued

[6]Total PUFA calculated as [C16:2n-4] + [C16:3n-4] + [C18:2^{9,11}] + [C18:2^{10,12}] + [C18:2n-6] + [C18:3n-3] + [C18:3n-6] + [C18:3n-4] + [C18:4n-3] + [C20:3n-6] + [C20:4n-6] + [C20:5n-3] + [C22:4n-6] + [C22:5n-3] + [C22:5n-6] + [C22:6n-3].
[7]Total n-3 PUFA calculated as [C18:3n-3] + [C18:4n-3] + [C20:5n-3] + [C22:5n-3] + [C22:6n-3].
[8]Total n-6 PUFA calculated [C18:2n-6] + [C18:3n-6] + [C20:3n-6] + [C20:4n-6] + [C22:4n-6] + [C22:5n-6].
[9]n-6:n-3 ratio calculated as Total n-6 PUFA ÷ Total n-3 PUFA.
[10]SFA:UFA ratio calculated as total SFA ÷ (total MUFA + total PUFA).
[11]UFA:SFA ratio calculated as (total MUFA + total PUFA) ÷ total SFA.
[12]Iodine value (IV) calculated as [C16:1] × 0.950 + [C18:1] × 0.860 + [C18:2] × 1.732 + [C18:3] × 2.615 + [C20:1] × 0.785 + [C20:4] × 3.201 + [C20:5] × 4.027 + [C22:1] × 0.723 + [C22:5] × 3.697 + [C22:6] × 4.463; equation 3-4, NRC (2012).

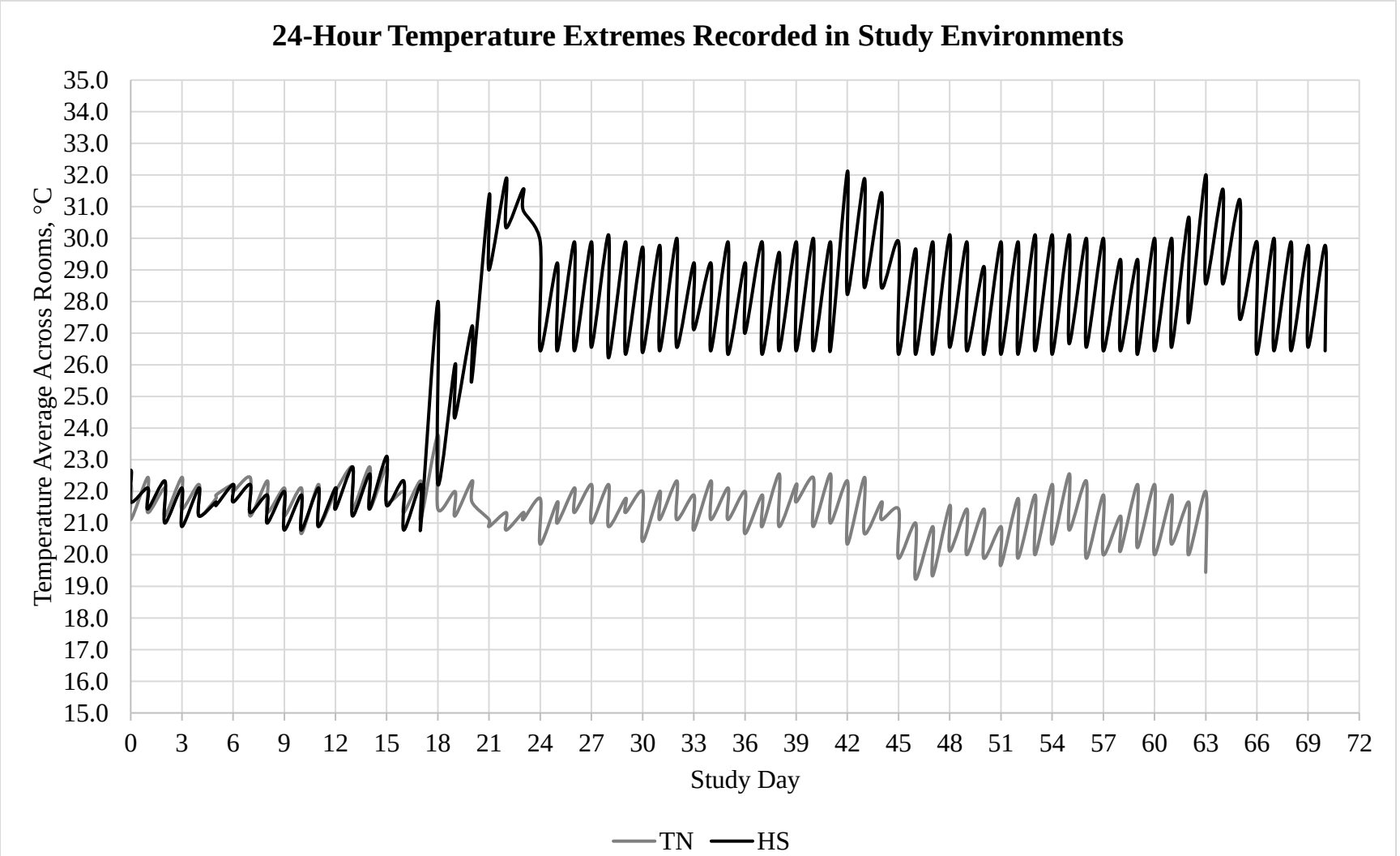

Figure 2.1. Environmental temperature extremes (24-hour high and 24-hour low) recorded throughout the study from day 0 to day 63 (thermoneutral rooms, n = 5) or to day 71 (heat stress rooms, n = 5) by an AcuRite thermometer (Chaney Instrument Co., Lake Geneva, WI) placed beside each room's EasyLog Data Logger (Lascar Electronics, Erie, PA).

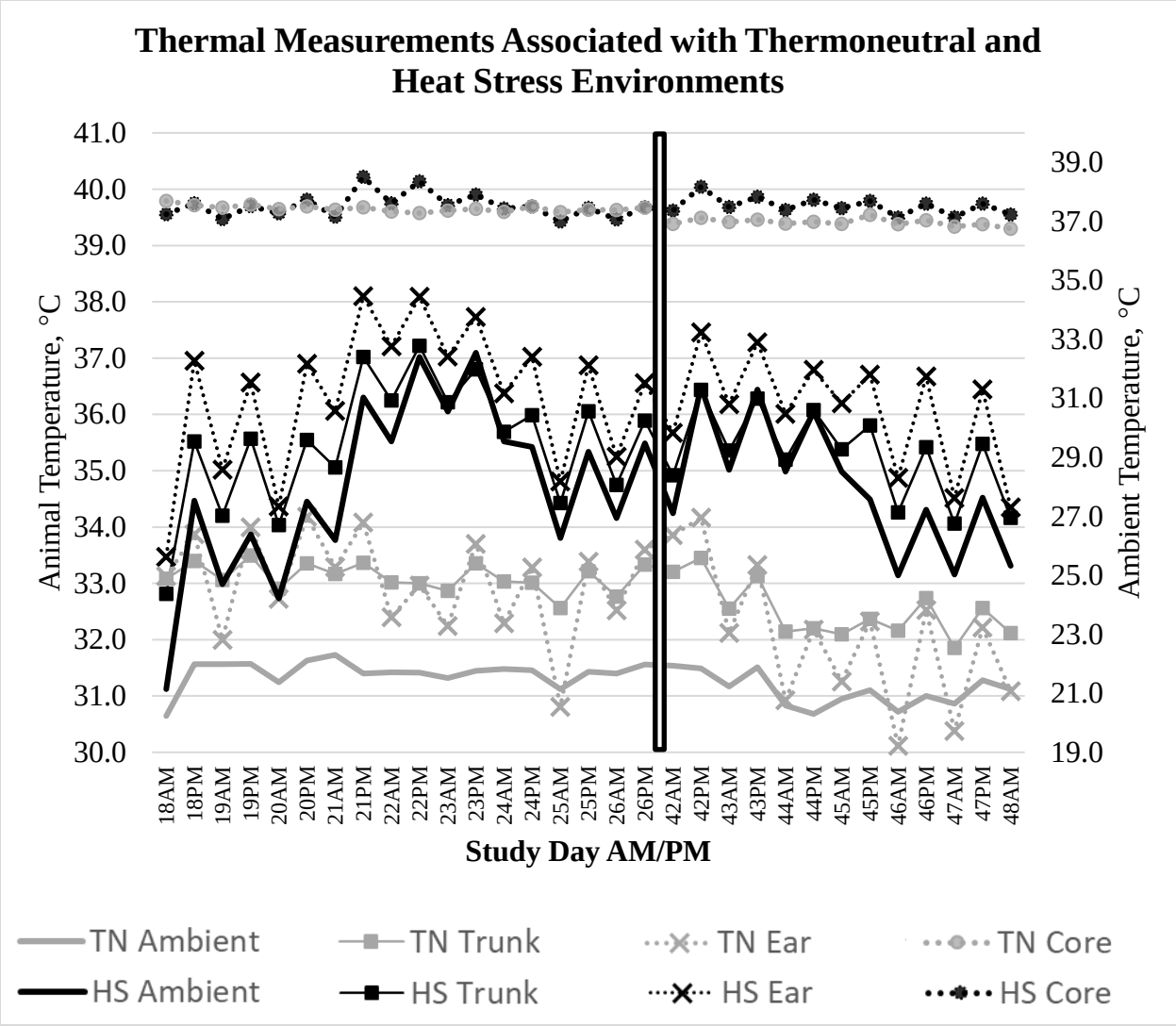

Figure 2.2. Thermal data at AM and PM timepoints shows ambient temperature is greater under HS environment and causes elevation of core body temperature despite ear skin temperatures being consistently greater than trunk skin temperatures among pigs supplemented with varying zinc level & source.

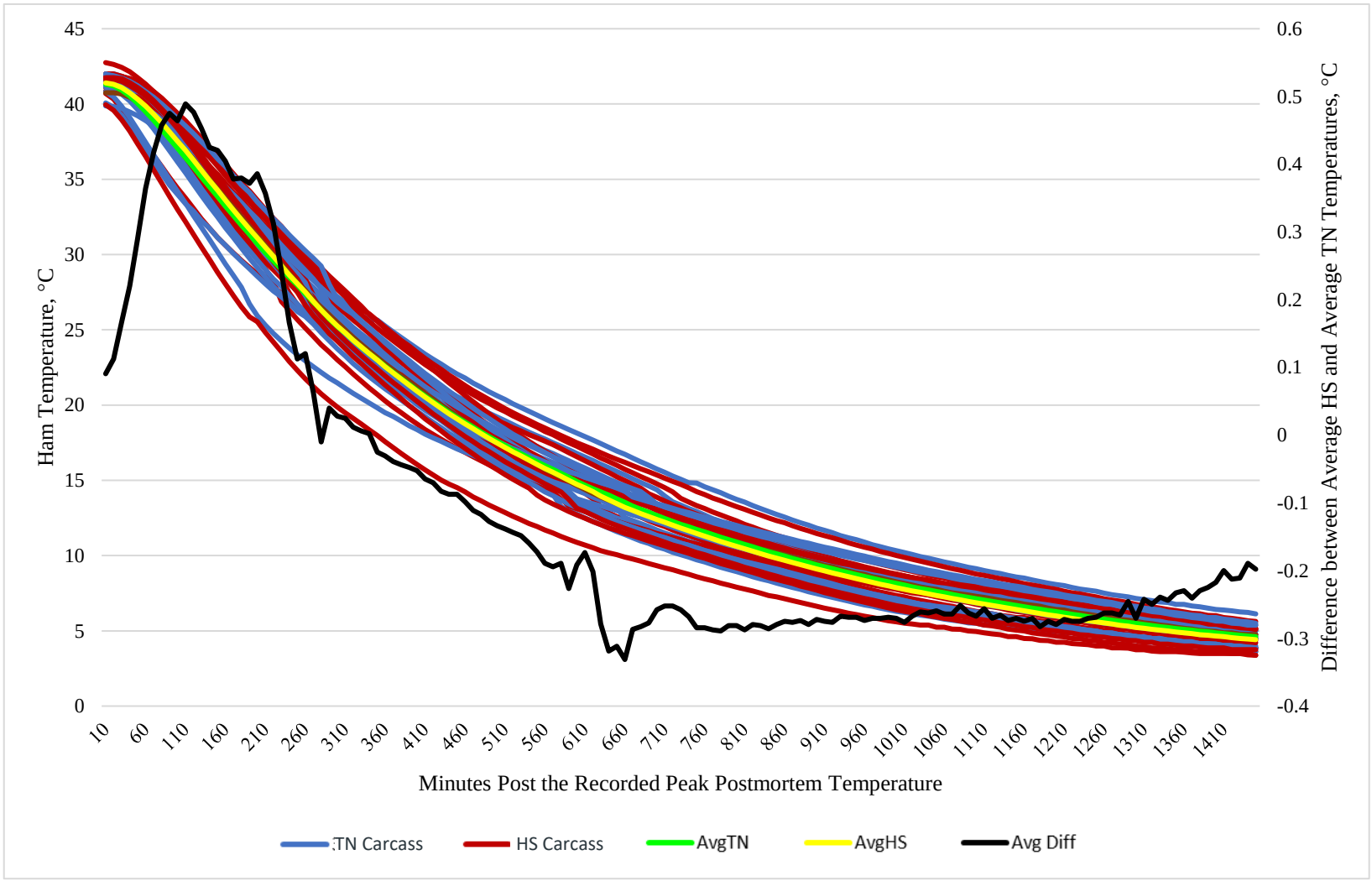

Figure 2.3. Ten-minute average deep ham temperatures recorded postmortem in subset of carcasses from antemortem thermoneutral (TN; n = 16) or heat stress (HS; n = 16) environments and supplemented with varying zinc level & source.

Table 2.11. Least square means for carcass composition of finishing pigs fed varying zinc level & source for 64 days under environmental heat stress or thermoneutral conditions.

Temperature:[1]	TN	TN	TN	TN	HS	HS	HS	HS	
Added Zn level, mg kg^{-1}:	50	50	130	130	50	50	130	130	
Dietary Zn Source:	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic	SEM[2]
n=	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	
Live body weight, kg	123.83	125.96	126.42	128.50	123.79	122.61	122.70	122.74	2.880
Hot carcass weight, kg	97.89	98.52	100.20	100.70	96.25	95.57	96.43	96.12	2.309
Dressing percent, %	79.1	78.3	79.3	78.4	77.9	77.9	78.7	78.4	0.58
Percent lean, %	48.7	49.6	49.0	48.5	50.1	48.9	50.0	48.7	0.79
Backfat, mm									
10^{th} rib	21.8	20.1	20.1	22.6	19.8	20.6	22.4	22.1	1.19
Last rib	30.7	29.0	31.0	31.0	26.9	30.2	27.9	28.7	1.68
Last lumbar	23.4	21.6	23.9	23.4	20.6	23.1	22.4	20.8	1.55
Leaf fat, kg	1.77	1.63	1.50	1.59	1.36	1.50	1.41	1.54	0.141
Loin eye area, cm^2	52.90	54.19	54.19	54.19	52.26	51.61	53.55	50.32	2.129
Backfat,[3] mm									
10^{th} rib	21.6	19.8	19.6	21.8	20.1	21.1	22.6	22.4	1.12
Last rib	30.7	28.7	30.5	30.5	27.2	30.7	28.2	29.0	1.65
Last lumbar	23.4	21.6	23.6	23.1	20.8	23.6	22.6	21.1	1.68
Leaf fat,[3] kg	1.77	1.59	1.41	1.50	1.41	1.54	1.41	1.59	0.132
Loin eye area,[3] cm^2	52.58	53.55	53.23	53.35	52.77	52.58	53.74	50.97	1.813

[1]Environmental treatments consisted of thermoneutral (TN) or heat stress (HS) conditions.
[2]SEM = standard error of the means.
[3]Adjusted for individual hot carcass weight (HCW) using HCW as a covariate.

Table 2.12. Statistical analysis of the carcass composition of finishing pigs fed different zinc levels & sources for 64 days under environmental heat stress or thermoneutral conditions.

	Probability, $P <$						
	Temp	Zn Level	Zn Source	Temp × Level	Temp × Source	Level × Source	Temp × Level × Source
Live body weight, kg	0.039	0.496	0.609	0.322	0.387	0.841	0.841
Hot carcass weight, kg	0.011	0.300	0.983	0.449	0.673	0.963	0.904
Dressing percent, %	0.088	0.243	0.126	0.454	0.238	0.794	0.795
Percent lean, %	0.377	0.630	0.368	0.821	0.189	0.521	0.553
Backfat, mm							
10^{th} rib	0.938	0.167	0.699	0.355	0.938	0.355	0.125
Last rib	0.032	0.583	0.559	0.387	0.119	0.818	0.199
Last lumbar	0.101	0.642	0.809	0.383	0.344	0.383	0.109
Leaf fat, kg	0.054	0.391	0.424	0.195	0.288	0.359	0.594
Loin eye area, cm^2	0.062	0.673	0.643	0.511	0.217	0.395	0.705
Backfat[1], mm							
10^{th} rib[1]	0.342	0.273	0.689	0.219	0.948	0.338	0.098
Last rib[1]	0.182	0.824	0.547	0.518	0.078	0.798	0.168
Last lumbar[1]	0.292	0.810	0.803	0.476	0.295	0.373	0.100
Leaf fat[1], kg	0.311	0.198	0.404	0.100	0.201	0.343	0.543
Loin eye area[1], cm^2	0.490	0.948	0.619	0.749	0.273	0.359	0.647

[1]Adjusted for individual hot carcass weight (HCW) using HCW as a covariate.

Table 2.13. Least square means for carcass quality of finishing pigs fed varying zinc level & source for 64 days under environmental heat stress or thermoneutral conditions.

Temperature:[1]	TN	TN	TN	TN	HS	HS	HS	HS	
Added Zn level, mg kg^{-1}:	50	50	130	130	50	50	130	130	
Dietary Zn Source:	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic	SEM[2]
n=	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	
Loin color score	2.5	2.4	2.4	2.4	2.7	2.7	2.4	2.8	0.15
Loin marbling score	1.6	1.6	1.5	1.5	1.7	1.5	1.4	1.5	0.19
Loin firmness score	2.4	2.2	2.3	2.3	2.7	2.4	2.4	2.8	0.17
24-hour loin pH	5.41	5.44	5.44	5.46	5.48	5.47	5.47	5.49	0.021
Loin drip loss, %	5.78	5.30	4.74	5.10	3.79	4.69	4.50	3.98	0.824
Chop purge, %									
Display day 3	3.10	3.17	3.70	3.35	2.50	3.25	3.30	3.06	0.589
Display day 7	3.65	4.21	4.44	3.99	3.55	4.30	3.98	5.01	0.979
Display day 10	4.17	4.61	5.65	4.68	4.51	4.42	5.34	5.26	0.807
Belly weight, kg	6.70	6.70	6.86	6.89	6.57	6.40	6.62	6.70	0.334
Belly temperature, °C	4.6	4.5	4.6	4.6	4.5	4.6	4.2	4.4	0.41
Belly length, cm	63.3	64.0	63.1	64.0	64.5	62.8	62.7	63.9	1.29
Belly avg. width, cm	26.8	26.6	27.3	27.1	26.6	26.9	26.8	26.8	0.86
Belly avg. thickness, cm	3.6	3.5	3.5	3.5	3.5	3.3	3.6	3.6	0.18
Fresh belly flex, cm									
Vertical	54.4	54.9	55.1	55.1	57.2	54.6	53.8	54.1	1.75
Lateral	18.0	15.7	15.2	15.2	13.5	16.5	15.5	17.3	1.83
Vertical[3]	54.4	54.4	55.4	54.6	56.4	55.1	54.4	53.8	1.85
Lateral[4]	18.0	16.0	15.2	15.2	13.5	17.0	15.2	17.0	1.83
Center slice lean, %	44.5	45.3	43.2	43.7	46.8	44.5	45.3	42.6	2.54

[1]Environmental treatments consisted of thermoneutral (TN) or heat stress (HS) conditions.
[2]SEM = standard error of the means.
[3]Adjusted for individual belly length using length as a covariate ($P < 0.001$).
[4]Adjusted for individual belly thickness using thickness as a covariate ($P = 0.001$).

Table 2.14. Statistical analysis of carcass quality of finishing pigs fed different dietary zinc levels & sources for 64 days under environmental heat stress or thermoneutral conditions.

	Probability, $P <$						
	Temp	Zn Level	Zn Source	Temp × Level	Temp × Source	Level × Source	Temp × Level × Source
Loin color score	0.024	0.546	0.278	0.717	0.186	0.186	0.546
Loin marbling score	0.606	0.354	0.918	0.918	0.918	0.471	0.471
Loin firmness score	0.030	0.825	0.825	0.825	0.509	0.081	0.379
24-hour loin pH	0.001	0.157	0.249	0.424	0.374	0.859	0.374
Loin drip loss, %	0.034	0.497	0.886	0.497	0.783	0.748	0.221
Chop purge, %							
Display day 3	0.379	0.309	0.864	0.898	0.554	0.301	0.679
Display day 7	0.827	0.488	0.443	0.814	0.499	0.767	0.604
Display day 10	0.839	0.134	0.745	0.953	0.867	0.508	0.503
Belly weight, kg	0.169	0.247	0.915	0.982	0.850	0.641	0.710
Belly temperature, °C	0.054	0.168	0.652	0.075	0.168	0.548	0.970
Belly length, cm	0.855	0.714	0.680	0.855	0.437	0.254	0.337
Belly avg. width, cm	0.527	0.303	0.986	0.413	0.478	0.810	0.793
Belly avg. thickness, cm	0.844	0.386	0.479	0.248	0.832	0.291	0.677
Fresh belly flex, cm							
Vertical	0.868	0.360	0.505	0.117	0.405	0.454	0.280
Lateral	0.578	0.811	0.427	0.050	0.029	0.750	0.268
Vertical[1]	0.691	0.377	0.201	0.054	0.703	0.940	0.587
Lateral[2]	0.604	0.583	0.282	0.095	0.018	0.976	0.190
Center slice lean, %	0.591	0.178	0.422	0.912	0.179	0.861	0.981

[1]Adjusted for individual belly length using length as a covariate ($P < 0.001$).

[2]Adjusted for individual belly thickness using thickness as a covariate ($P = 0.001$).

Table 2.15. Least square means for carcass composition of finishing pigs fed varying zinc level & source for either 64 days under thermoneutral or 71 days under heat stress environments.

Temperature:[1]	TN	TN	TN	TN	HS	HS	HS	HS	
Added Zn level, mg kg^{-1}:	50	50	130	130	50	50	130	130	
Dietary Zn Source:	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic	SEM[2]
n=	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	
Live body weight, kg	124.01	126.14	126.60	128.68	123.69	123.79	129.55	126.10	2.504
Hot carcass weight, kg	97.93	98.57	100.29	100.74	95.80	96.89	101.29	97.39	1.996
Dressing percent, %	79.0	78.1	79.1	78.3	77.5	78.3	78.2	77.3	0.46
Percent lean, %	48.7	49.6	49.0	48.5	48.2	48.2	48.9	47.1	0.89
Backfat, mm									
10th rib	21.8	20.1	20.1	22.6	19.8	21.6	20.1	21.6	1.22
Last rib	31.0	29.2	31.2	31.5	33.5	31.0	32.5	33.0	1.60
Last lumbar	23.1	21.6	23.6	23.4	22.9	19.8	21.6	21.1	1.32
Leaf fat, kg	1.77	1.63	1.50	1.59	1.41	1.36	1.50	1.54	0.141
Loin eye area, cm^2	53.03	54.26	54.58	54.90	53.29	51.23	56.65	51.48	1.684
Backfat,[3] mm									
10th rib	21.8	20.1	19.6	21.8	20.6	22.1	19.3	21.8	1.14
Last rib	31.2	29.2	31.0	30.7	34.3	31.5	31.8	33.3	1.50
Last lumbar	23.4	21.6	23.1	22.6	23.6	20.3	20.8	21.3	1.22
Leaf fat,[3] kg	1.77	1.63	1.45	1.54	1.45	1.41	1.45	1.54	0.132
Loin eye area,[3] cm^2	53.16	54.26	54.26	54.45	53.87	51.55	56.13	51.74	1.594

[1]Environmental treatments consisted of thermoneutral (TN) or heat stress (HS) conditions.
[2]SEM = standard error of the means.
[3]Adjusted for individual hot carcass weight (HCW) using HCW as a covariate.

Table 2.16. Statistical analysis of carcass composition of finishing pigs fed different dietary zinc levels & sources for either 64 days under thermoneutral or 71 days under heat stress environments.

	Probability, $P <$						
	Temp	Zn Level	Zn Source	Temp × Level	Temp × Source	Level × Source	Temp × Level × Source
Live body weight, kg	0.674	0.017	0.871	0.580	0.169	0.519	0.519
Hot carcass weight, kg	0.142	0.014	0.686	0.708	0.351	0.216	0.250
Dressing percent, %	0.012	0.982	0.130	0.685	0.216	0.185	0.184
Percent lean, %	0.122	0.593	0.546	0.869	0.308	0.165	0.860
Backfat, mm							
10^{th} rib	0.668	0.785	0.201	0.845	0.414	0.229	0.201
Last rib	0.091	0.361	0.342	0.766	0.951	0.232	0.795
Last lumbar	0.064	0.522	0.112	0.522	0.683	0.289	0.716
Leaf fat, kg	0.047	0.907	0.860	0.047	0.930	0.381	0.578
Loin eye area, cm^2	0.278	0.131	0.128	0.690	0.022	0.284	0.564
Backfat,[1] mm							
10^{th} rib	0.927	0.527	0.129	0.727	0.223	0.084	0.347
Last rib	0.033	0.863	0.393	0.676	0.828	0.114	0.541
Last lumbar	0.150	0.822	0.123	0.415	0.924	0.120	0.425
Leaf fat,[1] kg	0.112	0.410	0.952	0.055	0.718	0.215	0.821
Loin eye area,[1] cm^2	0.452	0.353	0.157	0.753	0.039	0.430	0.747

[1]Adjusted for individual hot carcass weight (HCW) using HCW as a covariate.

Table 2.17. Least square means for carcass quality of finishing pigs fed varying zinc level & source for either 64 days under thermoneutral or 71 days under heat stress environments.

Temperature:[1]	TN	TN	TN	TN	HS	HS	HS	HS	
Added Zn level, mg kg^{-1}:	50	50	130	130	50	50	130	130	
Dietary Zn Source:	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic	SEM[2]
n=	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	
Loin color score	2.5	2.4	2.4	2.5	2.6	2.4	2.5	2.7	0.16
Loin marbling score	1.6	1.6	1.5	1.5	1.5	1.2	1.2	1.4	0.20
Loin firmness score	2.4	2.2	2.3	2.3	2.6	2.3	2.5	2.5	0.18
24-hour loin pH	5.41	5.44	5.44	5.46	5.42	5.42	5.43	5.42	0.019

[1]Environmental treatments consisted of thermoneutral (TN) or heat stress (HS) conditions.
[2]SEM = standard error of the means.

Table 2.18. Statistical analysis of carcass quality of finishing pigs fed different dietary zinc levels & sources for either 64 days under thermoneutral or 71 days under heat stress environments.

	Probability, *P* <						
	Temp	Zn Level	Zn Source	Temp × Level	Temp × Source	Level × Source	Temp × Level × Source
Loin color score	0.521	0.830	1.000	0.668	0.830	0.286	0.668
Loin marbling score	0.088	0.525	0.785	0.928	0.785	0.414	0.414
Loin firmness score	0.235	0.842	0.427	0.842	1.000	0.321	0.842
24-hour loin pH	0.247	0.300	0.476	0.319	0.247	0.668	0.822

Table 2.19. Chemical analysis of pork loin chops derived from finishing pigs fed varying zinc level & source under environmental heat stress or thermoneutral conditions (least square means).

Temperature:[1]	TN	TN	TN	TN	HS	HS	HS	HS	
Added Zn level, mg kg^{-1}:	50	50	130	130	50	50	130	130	
Dietary Zn Source:	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic	SEM[2]
n=	*6*	*6*	*6*	*6*	*6*	*6*	*6*	*6*	
Moisture, %	73.33	73.17	73.33	73.33	74.00	73.33	73.67	72.83	0.318
Crude protein, %	21.57	21.13	21.59	22.00	21.12	21.61	21.63	20.88	0.355
Ether extract, %	2.10	2.41	2.09	2.33	2.13	2.20	2.58	3.20	0.390

[1]Environmental treatments consisted of thermoneutral (TN) or heat stress (HS) conditions.
[2]SEM = standard error of the means.

Table 2.20. Statistical analysis of chemical properties of loin chops from finishing pigs fed different dietary zinc levels & sources under environmental heat stress or thermoneutral conditions.

	Probability, *P* <						
	Temp	Zn Level	Zn Source	Temp × Level	Temp × Source	Level × Source	Temp × Level × Source
Moisture, %	0.464	0.464	0.073	0.274	0.148	1.000	0.714
Crude protein, %	0.261	0.462	0.743	0.230	0.787	0.673	0.028
Ether extract, %	0.181	0.121	0.159	0.080	0.869	0.583	0.473

Table 2.21. Chemical analysis of pork sausage derived from finishing pigs fed varying zinc level & source under environmental heat stress or thermoneutral conditions (least square means).

Temperature:[1]	TN	TN	TN	TN	HS	HS	HS	HS	
Added Zn level, mg kg^{-1}:	50	50	130	130	50	50	130	130	
Dietary Zn Source:	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic	SEM[2]
n=	*3*	*3*	*3*	*3*	*3*	*3*	*3*	*3*	
Moisture, %	58.67	60.67	58.00	59.33	61.00	60.67	61.00	61.67	1.845
Crude protein, %	15.23	16.32	15.78	16.20	16.21	15.95	15.75	15.54	0.768
Ether extract, %	17.30	16.76	19.85	16.78	18.86	17.11	16.38	16.50	2.316

[1]Environmental treatments consisted of thermoneutral (TN) or heat stress (HS) conditions.
[2]SEM = standard error of the means.

Table 2.22. Statistical analysis of chemical properties of sausage from finishing pigs fed different dietary zinc levels & sources under environmental heat stress or thermoneutral conditions.

	Probability, *P* <						
	Temp	Zn Level	Zn Source	Temp × Level	Temp × Source	Level × Source	Temp × Level × Source
Moisture, %	0.030	0.758	0.268	0.361	0.361	0.918	0.608
Crude protein, %	0.959	0.749	0.448	0.351	0.160	0.644	0.593
Ether extract, %	0.752	0.929	0.375	0.340	0.736	0.908	0.454

Table 2.23. Fatty acid analysis of pork sausage derived from finishing pigs fed varying zinc level & source under environmental heat stress or thermoneutral conditions (least square means).

Temperature:[1]	TN	TN	TN	TN	HS	HS	HS	HS	
Added Zn level, mg kg^{-1}:	50	50	130	130	50	50	130	130	
Dietary Zn Source:	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic	SEM[2]
n=	*10*	*10*	*9*	*10*	*8*	*10*	*10*	*9*	
Fatty acid									
C14:0, %	1.27	1.34	1.25	1.30	1.30	1.25	1.35	1.35	0.045
C16:0, %	22.33	22.44	22.01	22.40	22.36	22.18	22.62	22.90	0.401
C16:1n-7, %	2.46	2.48	2.57	2.50	2.45	2.44	2.48	2.36	0.139
C16:2n-4, %	0.29	0.27	0.26	0.26	0.32	0.28	0.24	0.29	0.015
C16:3n-4, %	0.29	0.27	0.28	0.27	0.31	0.28	0.25	0.27	0.014
C18:0, %	10.28	10.28	9.86	10.32	10.62	10.34	10.52	11.20	0.482
C18:1n-7, %	3.31	3.42	3.57	3.41	3.24	3.51	3.19	3.27	0.155
C18:1n-9, %	41.28	40.48	41.59	41.14	39.53	40.78	40.04	39.63	0.560
C18:2 ^{9,11}, %	0.12	0.12	0.13	0.12	0.12	0.11	0.12	0.11	0.007
C18:2 ^{10,12}, %	0.04	0.03	0.04	0.04	0.03	0.04	0.04	0.03	0.004
C18:2n-6, %	12.90	13.55	13.09	12.89	14.30	13.32	13.57	13.28	0.468
C18:3n-3, %	0.42	0.42	0.42	0.40	0.47	0.45	0.44	0.44	0.020
C18:3n-6, %	0.03	0.03	0.03	0.02	0.03	0.03	0.03	0.03	0.003
C18:3n-4, %	0.08	0.09	0.08	0.09	0.08	0.08	0.07	0.08	0.011
C18:4n-3, %	0.16	0.17	0.16	0.17	0.16	0.16	0.17	0.17	0.012
C20:0, %	0.86	0.82	0.88	0.88	0.79	0.84	0.85	0.85	0.035
C20:1n-9, %	0.06	0.06	0.06	0.06	0.05	0.10	0.05	0.05	0.016
C20:3n-6, %	0.68	0.69	0.71	0.70	0.69	0.70	0.70	0.68	0.027
C20:4n-6, %	0.38	0.41	0.36	0.37	0.44	0.41	0.40	0.40	0.040
C20:5n-3, %	0.03	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.005
C22:0, %	0.007	0.005	0.003	0.007	0.005	0.004	0.006	0.007	0.002
C22:1, %	0.02	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.004
C22:4n-6, %	0.15	0.15	0.14	0.14	0.17	0.15	0.15	0.16	0.010
C22:5n-3, %	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.002
C22:5n-6, %	0.000	0.003	0.000	0.002	0.011	0.000	0.001	0.000	0.004
C22:6n-3, %	0.08	0.08	0.08	0.09	0.09	0.09	0.09	0.09	0.007
C24:0, %	0.06	0.04	0.05	0.04	0.05	0.05	0.05	0.03	0.007
Σ UFA[3]	62.73	62.70	63.47	62.65	62.32	62.59	62.03	61.23	0.720

Table 2.23 continued

Σ SFA[4]	34.85	34.96	34.10	34.99	35.19	34.71	35.44	36.39	0.813
Σ MUFA[5]	47.14	46.46	47.78	47.14	45.23	46.93	45.80	45.30	0.659
Σ PUFA[6]	15.66	16.31	15.79	15.57	17.24	16.11	16.30	16.04	0.538
Σ n-3 PUFA[7]	0.71	0.70	0.69	0.68	0.76	0.72	0.74	0.74	0.020
Σ n-6 PUFA[8]	14.13	14.82	14.32	14.11	15.63	14.60	14.85	14.54	0.508
n-6:n-3 ratio[9]	20.10	21.62	20.71	20.90	20.66	20.29	20.34	19.94	0.757
SFA:UFA ratio[10]	0.557	0.561	0.539	0.561	0.566	0.561	0.573	0.596	0.0202
Iodine value, g/100g[11]	66.40	67.01	67.13	66.27	67.47	66.45	66.53	65.45	0.952

[1]Environmental treatments consisted of thermoneutral (TN) or heat stress (HS) conditions.

[2]SEM = standard error of the means.

[3]Total UFA calculated as [C16:1n-7] + [C16:2n-4] + [C16:3n-4] + [C18:1n-7] + [C18:1n-9] + [C18:2^{9,11}] + [C18:2^{10,12}] + [C18:2n-6] + [C18:3n-3] + [C18:3n-6] + [C18:3n-4] + [C18:4n-3] + [C20:1n-9] + [C20:3n-6] + [C20:4n-6] + [C20:5n-3] + [C22:1] + [C22:4n-6] + [C22:5n-3] + [C22:5n-6] + [C22:6n-3].

[4]Total SFA calculated as [C14:0] + [C16:0] + [C18:0] + [C20:0] + [C22:0] + [C24:0].

[5]Total MUFA calculated as [C16:1n-7] + [C18:1n-7] + [C18:1n-9] + [C20:1n-9] + [C22:1].

[6]Total PUFA calculated as [C16:2n-4] + [C16:3n-4] + [C18:2^{9,11}] + [C18:2^{10,12}] + [C18:2n-6] + [C18:3n-3] + [C18:3n-6] + [C18:3n-4] + [C18:4n-3] + [C20:3n-6] + [C20:4n-6] + [C20:5n-3] + [C22:4n-6] + [C22:5n-3] + [C22:5n-6] + [C22:6n-3].

[7]Total n-3 PUFA calculated as [C18:3n-3] + [C18:4n-3] + [C20:5n-3] + [C22:5n-3] + [C22:6n-3].

[8]Total n-6 PUFA calculated [C18:2n-6] + [C18:3n-6] + [C20:3n-6] + [C20:4n-6] + [C22:4n-6] + [C22:5n-6].

[9]n-6:n-3 ratio calculated as Total n-6 PUFA ÷ Total n-3 PUFA.

[10]SFA:UFA ratio calculated as total SFA ÷ (total MUFA + total PUFA).

[11]Iodine value (IV) calculated as [C16:1] × 0.950 + [C18:1] × 0.860 + [C18:2] × 1.732 + [C18:3] × 2.615 + [C20:1] × 0.785 + [C20:4] × 3.201 + [C20:5] × 4.027 + [C22:1] × 0.723 + [C22:5] × 3.697 + [C22:6] × 4.463; equation 3-4, NRC (2012).

Table 2.24. Statistical analysis of fatty acid profile of pork sausage from finishing pigs fed varying dietary zinc level & source under environmental heat stress or thermoneutral conditions.

	Probability, *P* <						
	Temp	Zn Level	Zn Source	Temp × Level	Temp × Source	Level × Source	Temp × Level × Source
Fatty acid							
C14:0, %	0.425	0.468	0.565	0.079	0.179	0.797	0.506
C16:0, %	0.209	0.383	0.393	0.061	0.569	0.288	0.794
C16:1n-7, %	0.282	0.762	0.451	0.494	0.748	0.436	0.981
C16:2n-4, %	0.180	0.005	0.831	0.481	0.373	0.011	0.087
C16:3n-4, %	0.695	0.006	0.217	0.086	0.362	0.068	0.314
C18:0, %	0.006	0.575	0.206	0.099	0.926	0.041	0.477
C18:1n-7, %	0.096	0.890	0.323	0.070	0.207	0.129	0.797
C18:1n-9, %	0.002	0.814	0.770	0.247	0.137	0.350	0.153
C18:2 ^{9,11}, %	0.418	0.764	0.055	0.677	0.323	0.823	0.982
C18:2 ^{10,12}, %	0.049	0.121	0.903	0.686	0.474	0.025	0.029
C18:2n-6, %	0.061	0.247	0.443	0.780	0.112	0.886	0.159
C18:3n-3, %	0.006	0.331	0.313	0.742	0.977	0.930	0.232
C18:3n-6, %	0.088	0.217	0.892	0.265	0.989	0.302	0.908
C18:3n-4, %	0.224	0.384	0.374	0.720	0.384	0.733	0.652
C18:4n-3, %	0.840	0.714	0.327	0.236	0.644	0.538	1.000
C20:0, %	0.184	0.072	0.981	0.922	0.272	0.938	0.211
C20:1n-9, %	0.488	0.418	0.305	0.224	0.299	0.292	0.300
C20:3n-6, %	0.924	0.544	0.920	0.487	0.842	0.368	0.985
C20:4n-6, %	0.025	0.047	0.886	0.896	0.233	0.928	0.380
C20:5n-3, %	0.397	0.731	0.364	0.260	0.731	0.612	0.343
C22:0, %	0.818	0.649	0.705	0.207	0.663	0.124	0.299
C22:1, %	0.038	0.646	0.858	0.224	0.474	0.817	0.596
C22:4n-6, %	0.016	0.332	0.755	0.565	0.931	0.323	0.231

Table 2.24 continued

C22:5n-3, %	0.064	0.274	0.011	0.729	0.720	0.693	0.247
C22:5n-6, %	0.404	0.190	0.404	0.286	0.047	0.286	0.201
C22:6n-3, %	0.091	0.835	0.978	0.900	0.384	0.350	0.408
C24:0, %	0.383	0.181	0.024	0.796	0.900	0.588	0.021
Σ UFA	0.007	0.427	0.260	0.059	0.790	0.129	0.821
Σ SFA	0.019	0.305	0.216	0.027	0.660	0.063	0.582
Σ MUFA	<0.001	0.847	0.933	0.085	0.070	0.119	0.112
Σ PUFA	0.054	0.188	0.422	0.746	0.137	0.996	0.157
Σ n-3 PUFA	0.001	0.419	0.260	0.770	0.817	0.471	0.441
Σ n-6 PUFA	0.056	0.238	0.452	0.777	0.117	0.877	0.165
n-6:n-3 ratio	0.211	0.646	0.580	0.744	0.146	0.421	0.444
SFA:UFA ratio	0.010	0.383	0.151	0.039	0.811	0.118	0.752
Iodine value, g/100g	0.586	0.249	0.165	0.254	0.273	0.360	0.402

Table 2.25. Hunter color scores of pork loin chops derived from finishing pigs fed varying zinc level & source under environmental heat stress or thermoneutral conditions (least square means).

Temperature:[1]	TN	TN	TN	TN	HS	HS	HS	HS	
Added Zn level, mg kg^{-1}:	50	50	130	130	50	50	130	130	
Dietary Zn Source:	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic	SEM[2]
n=	*10*	*10*	*10*	*10*	*10*	*10*	*10*	*10*	
*Hunter L**									
Display day 0[a]	55.45	56.30	56.36	57.37	56.07	56.52	54.72	55.68	0.932
Display day 3[b]	58.52	58.43	57.96	59.26	58.54	58.08	58.03	58.54	0.355
Display day 7[cd]	59.22	59.40	59.31	59.58	59.19	58.67	59.17	59.24	0.295
Display day 10[d]	59.30	59.30	58.79	59.35	59.63	58.97	59.12	59.75	0.409
*Hunter a**									
Display day 0[a]	7.35	7.66	7.34	7.21	7.14	6.81	7.37	7.08	0.333
Display day 3[b]	9.49	9.13	9.53	9.19	9.14	9.40	9.36	9.45	0.206
Display day 7[a]	7.65	7.73	7.09	7.61	7.32	7.80	7.75	7.61	0.434
Display day 10[c]	6.31	5.76	6.26	5.82	5.80	5.88	6.27	5.85	0.488
*Hunter b**									
Display day 0[a]	13.99	14.08	13.94	13.97	13.60	13.65	13.82	13.48	0.354
Display day 3[b]	15.76	15.86	16.11	15.84	15.69	15.80	16.07	15.98	0.183
Display day 7[b]	15.95	15.71	15.85	15.62	15.60	15.97	15.75	15.97	0.166
Display day 10[b]	16.14	15.75	15.96	15.70	15.47	15.85	15.58	15.78	0.175
Hue angle									
Display day 0[a]	62.44	61.61	62.21	62.72	62.33	63.58	62.17	62.32	0.811
Display day 3[b]	59.14	59.88	59.38	59.95	60.06	59.30	59.81	59.53	0.363
Display day 7[c]	64.60	63.80	66.21	64.27	65.21	64.13	64.18	64.93	1.263
Display day 10[d]	68.86	70.12	68.91	69.93	69.60	69.89	68.32	69.85	1.481
Chroma									
Display day 0[a]	15.82	16.05	15.76	15.72	15.37	15.27	15.69	15.23	0.434
Display day 3[b]	18.38	18.38	18.72	18.31	18.18	18.40	18.62	18.55	0.256
Display day 7[c]	17.72	17.52	17.43	17.41	17.30	17.80	17.62	17.76	0.288
Display day 10[d]	17.35	16.81	17.26	16.83	16.55	16.95	16.84	16.87	0.292

[a, b, c, d] Overall effect of display day was found to be statistically significant ($P < 0.001$); for each color parameter, days lacking a common superscript differ ($P < 0.05$).

[1]Environmental treatments consisted of thermoneutral (TN) or heat stress (HS) conditions.

[2]SEM = standard error of the means.

Table 2.26. Statistical analysis of hunter color scores of pork loin chops derived from finishing pigs fed varying zinc level & source under environmental heat stress or thermoneutral conditions.

	Probability, $P <$								
	Day × Main Effects	Initial Covar[1]	Temp	Level	Source	Temp × Level	Temp × Source	Level × Source	Temp × Level × Source
*Hunter L**	>0.100								
Display day 0[a]		n/a	0.272	0.928	0.151	0.069	0.844	0.762	0.876
Display day 3[b]		<0.001	0.299	0.799	0.177	0.727	0.217	0.014	0.650
Display day 7[cd]		<0.001	0.098	0.260	0.989	0.705	0.221	0.350	0.498
Display day 10[d]		<0.001	0.510	0.870	0.626	0.528	0.609	0.099	0.512
*Hunter a**	>0.100								
Display day 0[a]		n/a	0.183	0.964	0.611	0.267	0.360	0.642	0.582
Display day 3[b]		<0.001	0.987	0.484	0.504	0.742	0.053	0.780	0.701
Display day 7[a]		<0.001	0.616	0.582	0.245	0.253	0.758	0.821	0.195
Display day 10[c]		<0.001	0.502	0.352	0.008	0.381	0.189	0.423	0.220
*Hunter b**	>0.100								
Display day 0[a]		n/a	0.126	0.905	0.847	0.822	0.662	0.628	0.711
Display day 3[b]		<0.001	0.934	0.048	0.755	0.609	0.653	0.214	0.703
Display day 7[b]		<0.001	0.741	0.917	0.800	0.469	0.026	0.795	0.724
Display day 10[b]		<0.001	0.062	0.691	0.883	0.565	0.010	0.907	0.510
Hue angle	>0.100								
Display day 0[a]		n/a	0.532	0.812	0.636	0.318	0.450	0.912	0.285
Display day 3[b]		<0.001	0.733	0.771	0.776	0.733	0.020	0.755	0.513
Display day 7[c]		<0.001	0.850	0.414	0.177	0.306	0.286	0.761	0.197
Display day 10[d]		<0.001	0.911	0.290	0.004	0.394	0.740	0.470	0.282
Chroma	>0.100								
Display day 0[a]		n/a	0.112	0.920	0.746	0.549	0.505	0.571	0.937
Display day 3[b]		<0.001	0.946	0.182	0.703	0.612	0.387	0.302	0.858
Display day 7[c]		<0.001	0.555	0.852	0.525	0.321	0.195	0.793	0.410
Display day 10[d]		<0.001	0.057	0.797	0.308	0.601	0.010	0.646	0.386

[a, b, c, d] Overall effect of display day was found to be statistically significant ($P < 0.001$); for each color parameter, days lacking a common superscript differ ($P < 0.05$).

[1]The initial value of the day 0 individual package was included in the model as a covariate to determine whether the day 3, 7, and 10 values, adjusted for day 0 color, differ between treatments, and to account for variation in the subsequent measurements which comes from variation among initial day 0 values.

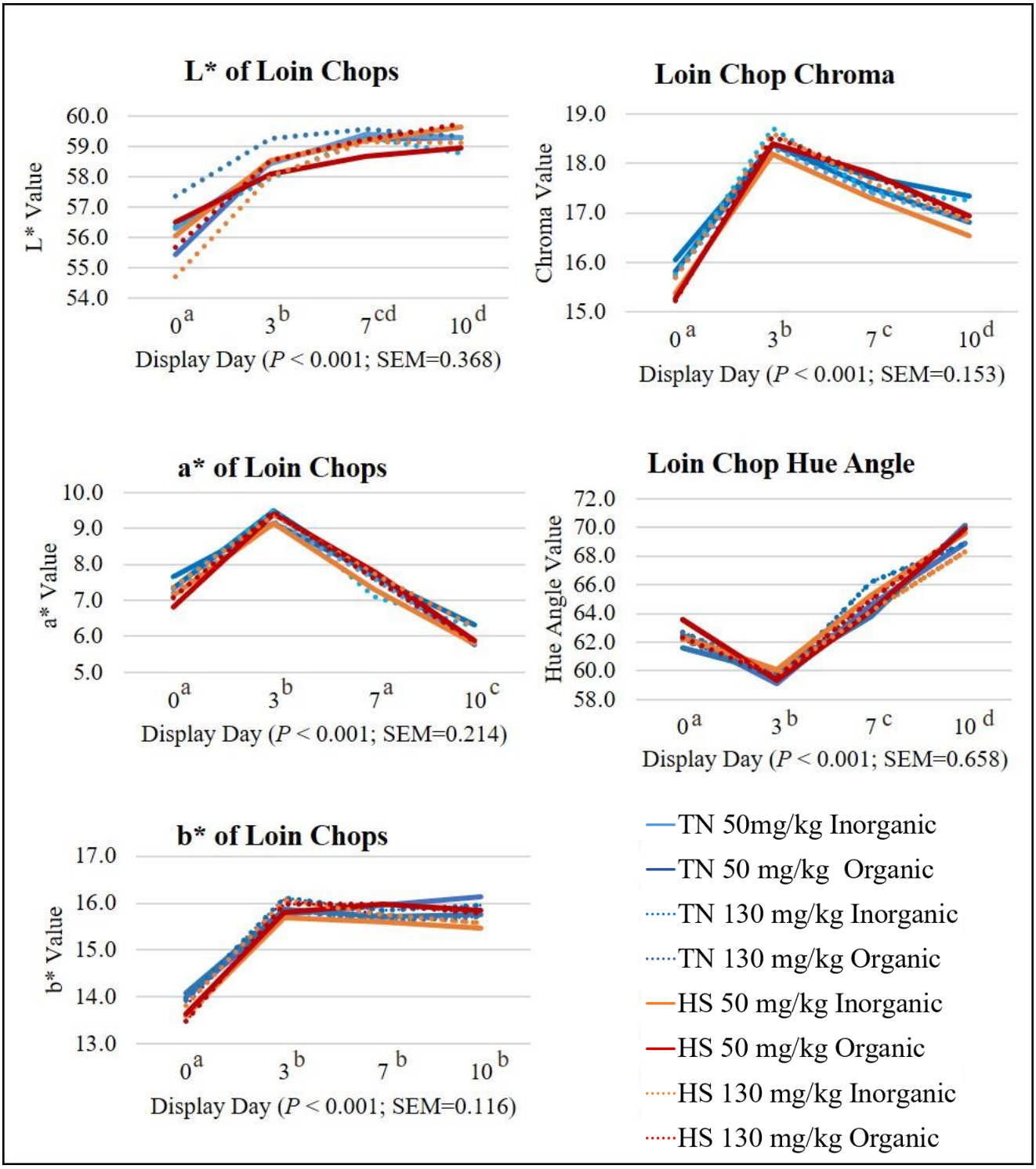

Figure 2.4. Color attributes over 10-day simulated retail display of pork loin chops originating from pigs receiving antemortem dietary Zn supplementation at either 50 or 130 mg kg-1 from inorganic or organic sources under thermoneutral (TN) or heat stress (HS) environments; days lacking common superscripts differ (P < 0.05).

Table 2.27. Hunter color scores of pork sausage patties derived from finishing pigs fed varying zinc level & source under environmental heat stress or thermoneutral conditions (least square means).

Temperature:[1]	TN	TN	TN	TN	HS	HS	HS	HS	
Added Zn level, mg kg^{-1}:	50	50	130	130	50	50	130	130	
Dietary Zn Source:	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic	SEM[2]
n=	*10*	*10*	*9*	*10*	*8*	*10*	*10*	*9*	
*Hunter L**									
Display day 0[a]	64.30	63.82	64.09	64.34	63.80	63.56	63.57	65.06	1.032
Display day 3[ab]	64.70	64.37	64.87	64.86	64.65	64.57	65.29	65.04	0.540
Display day 7[bc]	65.40	65.22	65.31	65.35	64.76	65.16	65.61	65.58	0.560
Display day 10[c]	65.62	65.20	65.49	65.57	65.32	65.14	65.53	65.35	0.406
*Hunter a**									
Display day 0[a]	12.61	12.39	12.84	12.42	12.19	12.77	12.39	12.04	0.541
Display day 3[b]	8.43	8.42	8.50	8.54	8.39	8.55	8.58	8.68	0.238
Display day 7[c]	4.95	5.14	4.93	4.98	4.97	5.23	5.69	5.22	0.420
Display day 10[d]	3.49	3.71	3.46	3.50	3.68	3.71	3.87	3.65	0.242
*Hunter b**									
Display day 0[a]	20.53	19.88	20.33	20.26	19.66	19.96	19.81	19.77	0.593
Display day 3[b]	16.90	16.65	16.77	16.91	16.74	16.57	16.63	16.80	0.249
Display day 7[c]	16.07	16.27	16.01	15.92	16.02	15.72	15.89	15.96	0.207
Display day 10[b]	16.56	16.64	16.64	16.75	16.22	16.05	16.18	16.51	0.194
Hue angle									
Display day 0[a]	58.43	58.04	57.75	58.62	58.17	57.45	58.03	58.61	0.800
Display day 3[b]	63.33	63.40	63.11	62.99	63.63	62.64	62.86	62.72	0.462
Display day 7[c]	72.81	72.40	72.94	72.50	73.13	71.73	70.53	71.89	1.275
Display day 10[d]	78.01	77.58	78.13	78.11	77.30	77.01	76.82	77.53	0.771
Chroma									
Display day 0[a]	24.09	23.44	24.06	23.79	23.16	23.72	23.38	23.17	0.731
Display day 3[b]	18.92	18.66	18.85	18.95	18.77	18.69	18.70	18.92	0.283
Display day 7[cd]	16.87	17.10	16.82	16.69	16.79	16.64	16.92	16.82	0.247
Display day 10[d]	16.94	17.07	17.02	17.11	16.65	16.49	16.65	16.93	0.195

[a, b, c, d] Overall effect of display day was found to be statistically significant ($P < 0.001$); for each color parameter, days lacking a common superscript differ ($P < 0.05$).

[1]Environmental treatments consisted of thermoneutral (TN) or heat stress (HS) conditions.

[2]SEM = standard error of the means.

Table 2.28. Statistical analysis of hunter color scores of pork sausage patties derived from finishing pigs fed varying zinc level & source under environmental heat stress or thermoneutral conditions.

	Probability, $P <$								
	Day × Main Effects	Initial Covar[1]	Temp	Level	Source	Temp × Level	Temp × Source	Level × Source	Temp × Level × Source
*Hunter L**	>0.100								
Display day 0[a]		n/a	0.790	0.451	0.622	0.641	0.474	0.241	0.637
Display day 3[ab]		<0.001	0.287	0.016	0.346	0.532	0.985	0.825	0.499
Display day 7[bc]		<0.001	0.844	0.106	0.770	0.134	0.530	0.796	0.429
Display day 10[c]		<0.001	0.380	0.279	0.249	0.780	0.979	0.428	0.415
*Hunter a**	>0.100								
Display day 0[a]		n/a	0.446	0.818	0.715	0.488	0.444	0.325	0.518
Display day 3[b]		<0.001	0.538	0.333	0.576	0.784	0.642	0.987	0.827
Display day 7[c]		<0.001	0.297	0.611	0.981	0.392	0.666	0.404	0.569
Display day 10[d]		<0.001	0.183	0.869	0.900	0.516	0.422	0.450	0.900
*Hunter b**	>0.100								
Display day 0[a]		n/a	0.007	0.824	0.483	0.720	0.132	0.713	0.150
Display day 3[b]		<0.001	0.303	0.578	0.820	0.985	0.845	0.123	0.937
Display day 7[c]		0.124	0.129	0.488	0.795	0.247	0.451	0.857	0.155
Display day 10[b]		0.330	<0.001	0.159	0.427	0.580	0.967	0.231	0.280
Hue angle	>0.100								
Display day 0[a]		n/a	0.772	0.642	0.865	0.574	0.756	0.200	0.991
Display day 3[b]		<0.001	0.390	0.240	0.287	0.953	0.337	0.566	0.364
Display day 7[c]		0.002	0.298	0.491	0.781	0.410	0.805	0.399	0.389
Display day 10[d]		<0.001	0.096	0.714	0.992	0.744	0.644	0.458	0.752
Chroma	>0.100								
Display day 0[a]		n/a	0.058	0.986	0.567	0.513	0.210	0.703	0.265
Display day 3[b]		<0.001	0.590	0.493	0.982	0.929	0.601	0.250	0.922
Display day 7[cd]		0.271	0.577	0.796	0.796	0.183	0.545	0.581	0.472
Display day 10[d]		0.074	0.002	0.193	0.430	0.473	0.805	0.373	0.283

[a, b, c, d] Overall effect of display day was found to be statistically significant ($P < 0.001$); for each color parameter, days lacking a common superscript differ ($P < 0.05$).

[1]The initial value of the day 0 individual package was included in the model as a covariate to determine whether the day 3, 7, and 10 values, adjusted for day 0 color, differ between treatments, and to account for variation in the subsequent measurements which comes from variation among initial day 0 values.

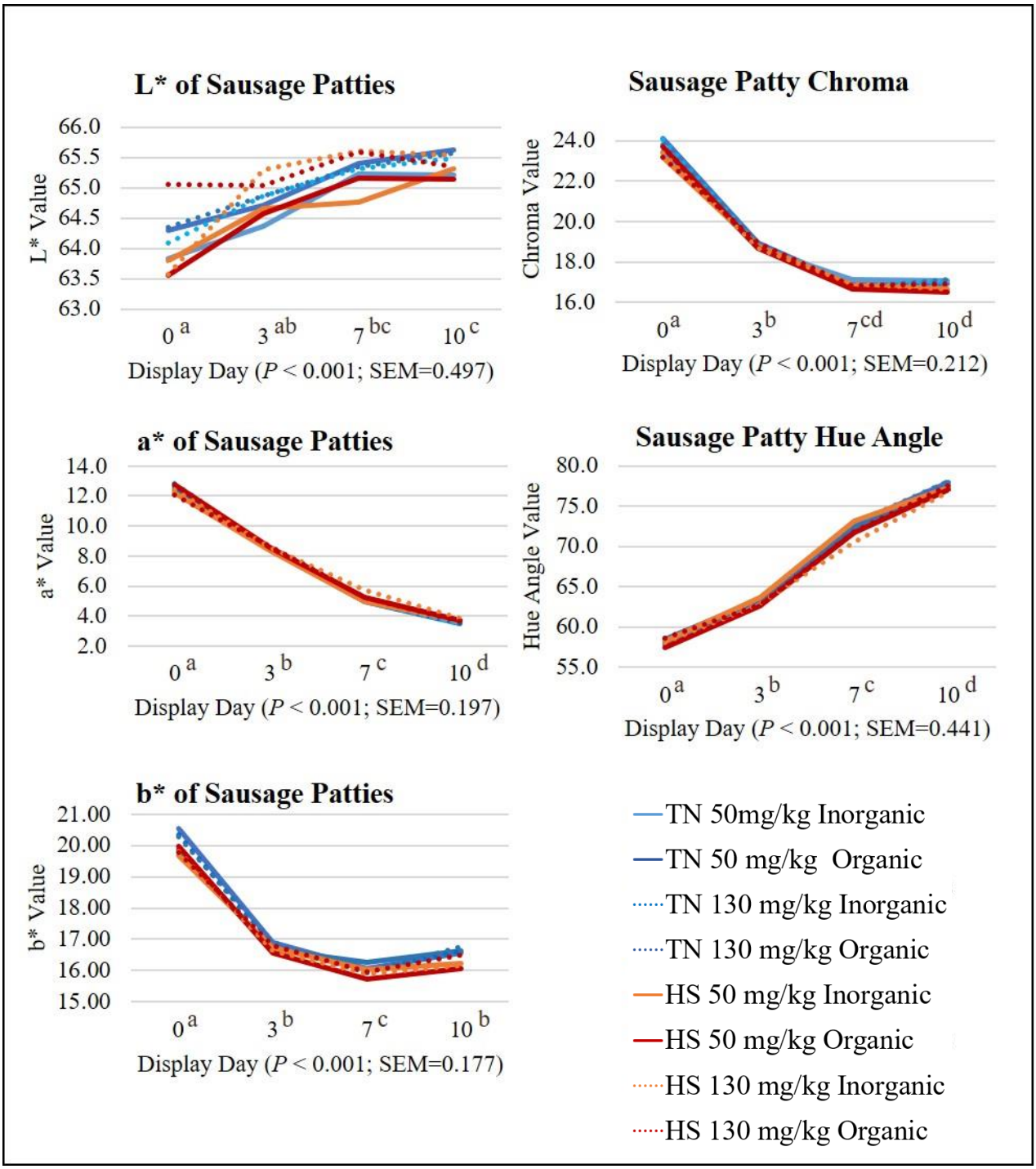

Figure 2.5. Color attributes over 10-day simulated retail display of 30% fat/70% lean fresh pork sausage (1.9% NaCl) made from picnic shoulder of pigs receiving antemortem dietary Zn supplementation at either 50 or 130 mg kg-1 from inorganic or organic sources under thermoneutral (TN) or heat stress (HS) environments; days lacking common superscripts differ (P < 0.05).

Table 2.29. Ten-day oxidative stability of pork loin chops derived from finishing pigs fed varying zinc level & source under environmental heat stress or thermoneutral conditions (least square means).

Temperature:[1]	TN	TN	TN	TN	HS	HS	HS	HS	
Added Zn level, mg kg^{-1}:	50	50	130	130	50	50	130	130	
Dietary Zn Source:	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic	SEM[2]
n=	*6*	*6*	*6*	*6*	*6*	*6*	*6*	*6*	
Malondialdehyde, mg kg^{-1}									
Display day 0	0.625	0.613	0.629	0.615	0.714	0.599	0.593	0.584	0.0580
Display day 3	0.720	0.725	0.661	0.668	0.763	0.642	0.636	0.745	0.0632
Display day 7	0.773	0.770	0.721	0.702	0.773	0.670	0.665	0.738	0.0666
Display day 10	0.756	0.734	0.773	0.745	0.809	0.707	0.706	0.749	0.0684

[1]Environmental treatments consisted of thermoneutral (TN) or heat stress (HS) conditions.
[2]SEM = standard error of the means.

Table 2.30. Statistical analysis of 10-day oxidative stability of pork loin chops from finishing pigs fed different dietary zinc levels & sources under environmental heat stress or thermoneutral conditions.

	Probability, *P* <						
	Temp	Zn Level	Zn Source	Temp × Level	Temp × Source	Level × Source	Temp × Level × Source
Malondialdehyde, mg kg^{-1}							
Display day 0[a]	0.941	0.235	0.174	0.193	0.369	0.339	0.325
Display day 3[b]	0.936	0.305	1.000	0.500	0.866	0.094	0.099
Display day 7 [bc]	0.424	0.283	0.722	0.587	0.957	0.281	0.196
Display day 10[c]	0.791	0.800	0.424	0.512	0.942	0.311	0.265

[a, b, c] Overall effect of display day was found to be statistically significant ($P < 0.001$); days lacking common superscript differ ($P < 0.05$).

Table 2.31. Ten-day oxidative stability of pork sausage derived from finishing pigs fed varying zinc level & source under environmental heat stress or thermoneutral conditions (least square means).

Temperature:[1]	TN	TN	TN	TN	HS	HS	HS	HS	
Added Zn level, mg kg^{-1}:	50	50	130	130	50	50	130	130	
Dietary Zn Source:	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic	Inorganic	Organic	SEM[2]
n=	*10*	*10*	*9*	*10*	*8*	*10*	*10*	*9*	
Malondialdehyde, mg kg^{-1}									
Display day 0	0.993	1.107	0.988	1.102	0.978	0.888	0.854	1.015	0.1146
Display day 3	1.408	1.523	1.394	1.393	1.280	1.402	1.373	1.405	0.1353
Display day 7	1.730	1.886	1.685	1.777	1.868	1.703	1.631	1.680	0.1345
Display day 10	2.203	2.154	1.994	2.076	2.108	2.176	2.011	2.153	0.1445

[1]Environmental treatments consisted of thermoneutral (TN) or heat stress (HS) conditions.
[2]SEM = standard error of the means.

Table 2.32. Statistical analysis of 10-day oxidative stability of pork sausage from finishing pigs fed different dietary zinc levels & sources under environmental heat stress or thermoneutral conditions.

	Probability, *P* <						
	Temp	Zn Level	Zn Source	Temp × Level	Temp × Source	Level × Source	Temp × Level × Source
Malondialdehyde, mg kg^{-1}							
Display day 0[a]	0.071	0.976	0.230	0.963	0.525	0.313	0.319
Display day 3[b]	0.465	0.891	0.451	0.498	0.907	0.559	0.943
Display day 7[c]	0.568	0.231	0.701	0.759	0.290	0.661	0.420
Display day 10[d]	0.956	0.253	0.494	0.635	0.618	0.562	0.873

[a, b, c, d] Overall effect of display day was found to be statistically significant ($P < 0.001$); days lacking common superscript differ ($P < 0.05$).

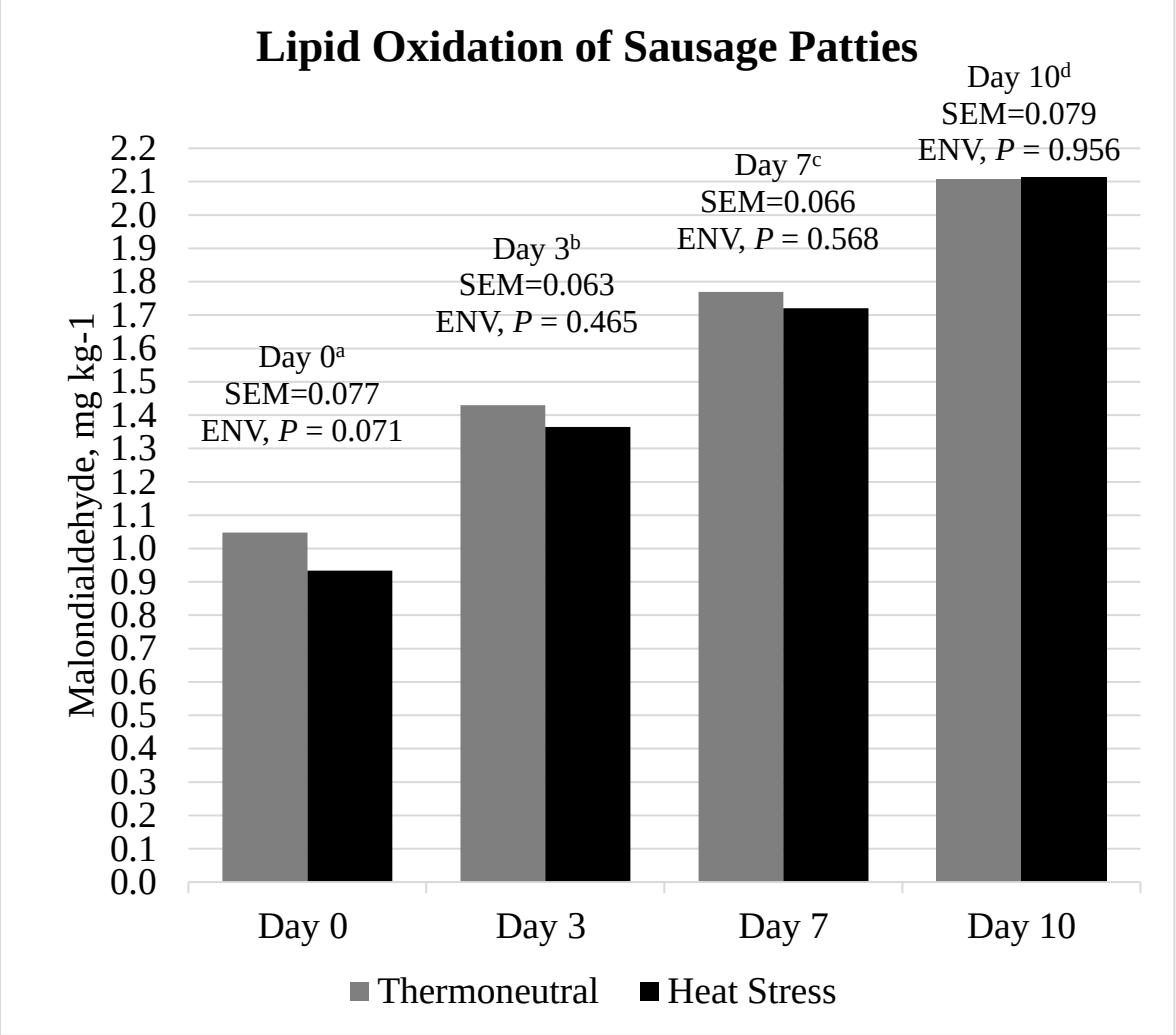

Figure 2.6. Ten-day oxidative stability of pork sausage patties derived from finishing pigs fed varying zinc level & source under environmental heat stress or thermoneutral conditions (ENV= main effect of environment). Overall effect of display day was found to be statistically significant ($P < 0.001$); days lacking common superscript letter differ ($P < 0.05$).

CHAPTER 3. THERMAL INDICATORS ASSOCIATED WITH INTESTINAL STERSS RESPONSE IN PIGS EXPOSED TO CHRONIC CYCLIC HEAT.

3.1 Abstract

Heat stress negatively impacts gut morphology and integrity but it is unclear how body and ambient temperature correspond to physiological changes in pigs receiving supplemental Zn. We hypothesized intestinal changes would be correlated with thermal shifts experienced by supplemented pigs. Gilts (n=96; initially 71.7 kg) were housed (1 of 5 pigs/pen) under thermoneutral (TN; 18.9–16.7°C) or cycling heat (HS) conditions simulating seasonal chronic heat (30-29°C/26-27°C for 12h:12h on days 24-63) with acute heat waves (32-33°C/29-30°C for 12h:12h on days 21-24, 42-45, and 63-64). Pens were randomly allotted to one of 12 temperature and diet treatment combinations across 8 weight blocks. Treatments were arranged as a 2×2×2 factorial (+ 4 treatments) with main effects of environment (HS vs. TN), supplemented Zn (50 vs. 130 mg kg^{-1} available Zn), and Zn source (inorganic from ZnO vs. organic/inorganic blend); the 4 additional diets represented 2 additional levels of available Zn (90 and 110 mg kg^{-1}) under both levels of environment. Core body temperatures (T_{core}) on days 42-45 were recorded continuously via indwelling vaginal thermometers and infrared thermal imaging was used to measure skin temperatures at 12-hour intervals. From a 64-gilt subset of the 2×2×2 treatments, jejunum and ileum samples were collected on day 65 for analysis of villus height, crypt depth, and jejunal gene expression of heat shock proteins (27, 70, 90), occludin, and mucin (MUC2). Pearson correlation coefficients were generated using SAS 9.4. The heat stress model induced thermoregulatory changes and increases ($P < 0.05$) in T_{core}. Day 42-45 ambient-temperature was negatively correlated with expression of HSP-27 (r=-0.42, P=0.047), HSP-90 (r=-0.49, P=0.014), and

occludin (r=-0.69, *P*<0.001) in HS pigs. In organic Zn supplemented pigs, ambient-temperature was positively correlated with expression of HSP-27 (r=0.42, *P*=0.034) and MUC2 (r=0.45, *P*=0.017) and negatively correlated with villus height in jejunum (r=-0.42, *P*=0.027) and ileum (r=-0.38, *P*=0.048). Thermal Circulation Index (measure of heat dissipation) of HS pigs was negatively correlated with their ileum villus height (r=-0.51, P=0.015) and positively correlated with HSP-70 expression (r=0.46, *P*=0.041). No relationship was found between T_{core} and most variables. In conclusion, thermal correlations with gut integrity characteristics existed for organic Zn supplemented and HS pigs. Degree of heat dissipation by heat stressed animals appeared to be associated with classic HS damage and intestinal responses which may be useful indicators of heat stress in the grow-finish pig.

Keywords: heat stress, pig, thermoregulation, gut.

3.2 Introduction

Heat stress (HS) jeopardizes animal health and welfare, increases mortality and morbidity, and decreases animal productive efficiency (see reviews by St-Pierre et al., 2003; Lacetera, 2019). Localized responses to heat cause intestinal damage which are of especial concern in the grow-finish pig because the majority of growth and feed consumption occurs during this production phase (Pearce et al., 2013; 2015b). Nutritional approaches to ameliorating HS reductions in growth encompass all nutrient classes including minerals (Rosero et al., 2012; reviewed by Mayorga et al., 2019). Zinc supplementation has proven effective in stimulating feed intake of pigs in the stressful post-weaning period and has been shown to improve intestinal integrity under short term exposure to heat (Feldpausch et al., 2018; Pearce et al., 2015b).

Because HS exacts a large toll on productive efficiency, there is value in identifying genetic, management, and/or nutritional interventions which improve thermotolerance particularly as different genetic lines can respond dissimilarly to HS (Nienaber et al., 1997). Genetic selection of higher yielding, faster growing animals is associated with greater basal metabolic heat production (Brown-Brandl et al., 2004) thereby leading to greater thermal stress susceptibility. Combined with anticipation of global temperature rise, HS risk is expanding to more temperate climates (Stott and Kettleborough, 2002; Hansen et al., 2006).

Ongoing research is advancing the understanding of genetic underpinnings of HS tolerance in swine (Fragomeni et al., 2016; Cross et al., 2018; Gourdine et al., 2017; reviewed by Misztal, 2017; Rauw et al., 2017; Kim et al., 2018). Identification of non-genetic factors which improve thermotolerance requires linking biomarkers to the physiological responses to heat which in turn correspond to productivity measurements (Bernabucci et al., 2010). Recent work by Dou and colleagues (2017) has worked to predict thermotolerance based on plasma biomarkers which are linked to production and physiological traits measured under heat stress (Dou et al., 2017) while work by Seibert and colleagues (2018) has sought to link changes in productive performance under heat stress with physiological parameters used as thermoregulatory markers.

With the hypothesis that intestinal changes are correlated with thermal shifts experienced by pigs, the study objective was to identify environmental and antemortem physiological measurements which may be candidates for future predictive modeling of chronic intestinal HS damage in finishing pigs receiving different zinc (**Zn**) supplementation strategies.

3.3 Materials and Methods

3.3.1 Study Design

The activities of this experiment were approved by the Purdue Animal Care and Use Committee (Protocol No. 1112000447). Crossbred commercial pigs (originally 72 kg body weight on day 0) from 2 different grow-finish pig groups 14 days apart were housed in 8 rooms of the Purdue University Swine Environmental Research Building in West Lafayette, IN (see Mills, 2018). Each room was equipped with a single propane heater, automated ventilation system, pit manure storage, artificial lighting, and contained 12 group pens over fully slatted concrete flooring. Each pen provided 0.84 m^2 per head and contained a single-hole stainless steel dry self-feeder and an adjustable wall mounted nipple drinker to provide pigs with *ad libitum* access to feed and water.

A representative animal from each pen (gilt closest to the study day 18 pen mean weight) was selected for thermal monitoring. Gilts (n = 96; initially 71.7 ± 4.7 kg) were housed (1 of 5 pigs per pen; total n = 480 pigs) for 65 days under thermoneutral (**TN**; 18.9–16.7°C) (Myer and Bucklin, 2001) or cycling diurnal heat stressing (**HS**) conditions simulating seasonal chronic heat. Target temperatures were 30-29 °C/26-27 °C for 12h:12h on days 24-63 with acute heat waves of 32-33 °C/29-30 °C for 12h:12h on days 21-24, days 42-45, and days 63-65. A brief heat acclimation period (28°C/24 °C for 12h:12h) on days 18-21 preceded the day 21-24 first acute heat event for the HS pigs. Throughout the study humidity was monitored but not controlled; daily average humidity recorded in the rooms during the acute heat periods ranged from 46 – 61% for the HS rooms and 41 – 60% for the TN rooms while during the chronic heat periods daily average humidity ranged from 45 – 59% for the HS rooms and 36 – 63% for the TN rooms. The upper temperatures selected for the acute heat stress events mimicked average historical summer daytime temperatures for swine-dense geographic locations in the United States (Ames, IA; Le Mars, IA; Raleigh, NC) and were slightly greater than average historical summer daytime high temperatures

in Worthington, MN. Diurnal temperature changes were designed to simulate normal daily temperature fluxes with warming and cooling periods occurring gradually over a several hour time period.

Pens were randomly allotted across 8 weight blocks to 1 of 12 temperature and diet treatments structured as a 2 (environment) × 2 (Zn source) × 2 (Zn level) factorial plus 4 additional treatments: 2 (additional Zn level) × 2 (environment). For the 2×2×2 factorial, the main effect of environment consisted of either TN or HS, the main effect of Zn level supplemented to the pig diet was 50 or 130 mg kg^{-1} available Zn, and the main effect of Zn source added to the pig diet consisted of either 100% inorganic from ZnO or 100% Zn from organic source (Availa®Zn; Zinpro Corp, Eden Prairie, MN) at 50 mg kg^{-1} level or 62% Zn from Availa®Zn and 38% from ZnO at the 130 mg kg^{-1} level. The 4 additional treatments consisted of 2 levels of added Zn (90 or 110 mg kg^{-1}) from an organic blend source (50 mg kg^{-1} added Zn from ZnO plus 40 or 60 mg kg^{-1} Zn from Availa®Zn) replicated within each environment (TN or HS). Diet tables and nutritional analysis are reported in Mills (2018).

3.3.2 Data Collection

Individual pig bodyweights were recorded periodically throughout the study (see Mills, 2018). Daily temperature extremes (high and low over 24-hour period; see Fig. 3.1) in each room were recorded via an AcuRite thermometer (Model 00305SBDI; Chaney Instrument Co., Lake Geneva, WI) and ambient temperature (**$T_{ambient}$**) and relative humidity were also recorded in each room every 5 minutes by an EasyLog Data Logger (Lascar Electronics, Erie, PA).

Real time body temperature responses were logged at 10-minute intervals before, during, and after each of the first two heat events during the trial. Indwelling vaginal thermometers were prepared using temperature logging devices (Maxim Integrated™ ibutton®, San Jose, CA) and

progesterone extracted EAZI-BREED™ CIDR® sheep inserts (Pfizer Inc, New York, NY). The tails of the inserts were trimmed and progesterone was extracted by soaking CIDR® inserts in 100% ethanol for a total of 12 hours and changing the ethanol bath every 3 hours. Surgical suture was epoxied to the circumference of ibuttons®, threaded through holes drilled in the CIDR®, and secured to the insert (Fig. 3.2). The prepared thermometers were inserted vaginally into one representative gilt of each pen for all 6 dietary treatments in 8 blocks (*n* = 96 gilts) for determination of core body temperature (**T_{core}**) responses to heat stress. Prior to insertion, the CIDR® with attached ibutton® was loaded into a CIDR® applicator, immersed in chlorhexidine solution (Nolvasan®, Pfizer Inc, New York, NY), and covered in obstetrical lube. Gilts were restrained by snaring and the vulva cleaned thrice with ethanol-soaked gauze followed by povidone-iodine soaked gauze. The loaded, sanitized applicator was fully inserted into the vulva up to the finger grips, then the finger grips were squeezed and the applicator gently retracted, depositing the thermometer in the anterior portion of the vagina. Thermometers were inserted on the morning of day 17 and remained inserted for the acclimation heating period (day 18 – 21; **pre-HSE1**) and through the duration of the first heat event (day 21 – 24; **HSE1**) until removal on day 26 (day 24 – 26; **post-HSE1**). Thermometers were again inserted the afternoon of day 41 prior to the start of the second heat event (**pre-HSE2**) and remained inserted for the duration of the heat event (day 42 – 45; **HSE2**) until removal on day 48 (**post-HSE2**).

On the days which T_{core} was measured, infrared thermal images of the same animal's trunk (Fig. 3.3) and posterior face of the ear (Fig. 3.4) were captured at approximately 07:00 and 19:00 h to measure average skin temperature (**T_{skin}**) over these areas using a FLIR® T440 Thermal Imaging Camera (Wilsonville, OR) with an emission factor setting of 0.90 - 1.00.

3.3.3 Image and Data Analysis

At each AM and PM timepoint, the average skin surface temperature of the trunk (**$T_{trunk\ skin}$**) and of the posterior face of the ear (**$T_{ear\ skin}$**) of each animal was calculated from the infrared images using FLIR® Tools software (version 5.11.16357.2007; 2016). For each of the gilts in the 2×2×2 factorial henceforward referred to as the "subset" (n = 64), the $T_{ambient}$ recorded in the room at the 5-minute interval closest to the T_{skin} time of recording and the T_{core} recorded for the gilt at the 10-minute interval closest to the T_{skin} time of recording were identified. From these measurements, a Thermal Circulation Index (**TCI**) was calculated to obtain a measure of heat dissipation by the animal for the regulation of core body temperature at each AM and PM timepoint (Kingma et al., 2014):

$$[T_{trunk\ skin} - T_{ambient}] \div [T_{core} - T_{trunk\ skin}]$$

3.3.4 Intestinal Stress Response Analysis

From the 64-gilt subset, jejunum and ileum samples were collected over three slaughter days from each gilt on study day 64 ± 1.0. Histological analysis of villus height and crypt depth, and analysis of the relative gene expression of jejunal mucosa heat shock protein (**HSP**) 27, HSP 70, HSP 90, tight junction protein occludin, and mucin-2 genes were performed as described in Mills (2018). Primer sequences are shown in Table 1. Briefly, two 15-cm sections of the ileum were removed 10 cm cranial to the ileocecal junction and two 15-cm jejunal sections were removed 163-193 cm cranial to the ileocecal junction. Sections were rinsed with phosphate buffered saline. Histology samples were fixed in 10% neutral buffered formalin, subsampled, embedded in wax, sectioned, and stained with hematoxylin and eosin, imaged, and measured (Purdue University College of Veterinary Medicine Histology Laboratory). Mucosal scrapings taken from the luminal surface of the rinsed jejunum section were snap frozen in TRIzol® reagent (Invitrogen™

ThermoFisher Scientific; Waltham, MA, USA). Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was performed according to the methods outlined in Mills (2018). Isolated mRNA purity as assessed by the 260/280 nm spectrophotometric absorbance ratio was >1.9 for all samples. The RNA was reverse transcribed then amplified using SYBR® Green dye-based PCR (iQ™ SYBR® green supermix, Bio-Rad, Hercules, CA) followed by a melt curve analysis to validate primer specificity. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a reference gene to which expression of the genes of interest were normalized, then relative fold-change expression of genes of interest was determined relative to that of a pooled composite sample representing the control treatment (thermoneutral, 50 mg kg^{-1} inorganic Zn) using the ΔΔCT calculation (Mills, 2018).

3.3.5 Statistical Analyses

The representative pig from each pen served as the experimental unit upon which measurements were made. For each timepoint (AM or PM) within each period (i.e. pre-HSE1, HSE1, post-HSE1, etc), thermal measurements for each individual animal were averaged. The main effects of environment, Zn source, and Zn level, as well as their interactions, were tested using predetermined non-orthogonal contrast statements. The effects of treatment were also analyzed as a one-way ANOVA in a randomized complete block design. The MIXED procedure of SAS (v9.4, SAS Institute Inc., Cary, NC) was used to model treatment as a fixed effect and initial weight block as a random effect.

For the 64-gilt subset, Pearson correlation coefficients between markers of intestinal integrity and thermal measurements during HSE2 were generated for different subsets of animals using SAS. Analysis was performed on log base 2 transformed gene expression values and using the $T_{ambient}$ and T_{core} recordings closest to time of T_{skin} measurement (time of infrared image capture)

for each individual pig. Correlation between bodyweight change during HSE2 with thermal measurements and with intestinal responses was similarly assessed. All results were considered statistically significant at $P \leq 0.05$; results with P-values > 0.05 and ≤ 0.10 were considered a tendency.

3.4 Results

3.4.1 Thermoregulation

At both the AM and PM time points, the ambient temperatures to which the animals were exposed in the HS rooms were greater ($P < 0.001$) than those by animals in the TN rooms during the pre-HSE1, HSE1, post-HSE1, pre-HSE2, HSE2, and post-HSE2 periods (see Tables 3.2 and 3.3). During each of these periods, the skin temperatures recorded on the trunk and the ear of the animals were greater ($P < 0.001$) for the HS animals than for TN animals in both the AM and PM (see Tables 3.4 – 3.7). Neither Zn level nor source affected skin temperature of the trunk or ear during HSE1 and HSE2 ($P > 0.05$) although at the AM timepoint of HSE1, there was an interactive effect of organic zinc level and environment on ear skin temperatures among pigs receiving organic source Zn.

Preceding and succeeding the acute heat stress events, several interactions were observed between zinc source, level, and environment on skin temperatures, but the effects were not sustained over subsequent periods. During the AM of the pre-HSE1 period, ear skin temperature was reduced (Zn Level Linear, $P = 0.039$) as Zn level linearly increased from 50 to 130 mg kg^{-1} across environments regardless of Zn source (see Tables 3.4 and 3.5). However, an interaction existed between Zn level and environment (Level Quadratic × Environment, $P = 0.029$) where under TN conditions, ear skin temperature increased then decreased as Zn level increased, while under HS conditions, ear temperature decreased then increased as Zn level increased regardless of

Zn source. Also at the AM timepoint of pre-HSE1, ear skin temperatures among just the pigs receiving organic source Zn increased then decreased as organic Zn level increased under TN conditions, while ear skin temperatures decreased then increased as organic Zn level increased under HS conditions (Org Quadratic × Environment, P = 0.023). A similar phenomenon was observed during HSE1 AM (Org Quadratic × Environment, P = 0.039). However, during pre-HSE1 PM among just the organic Zn source supplemented pigs, ear skin temperature decreased (Organic Linear, P = 0.049) as organic Zn level linearly increased across environments and Zn sources. During pre-HSE2 AM, ear skin temperatures were greater among organic Zn source fed pigs under TN conditions but were lower among organic Zn source fed pigs under HS conditions (Source × Environment, P = 0.032).

Less variability was observed among trunk skin temperatures (see Tables 3.6 and 3.7; Fig. 3.5). During pre-HSE1 AM and post-HSE1 PM, across environments at the 50 mg kg^{-1} level of Zn, organic source of Zn increased trunk skin temperature compared to inorganic source (50 InOrg vs. 50 Org, P = 0.047 and 0.007, respectively). Also, during post-HSE1 PM, increased (Organic Linear, P = 0.006) trunk skin temperature was observed as organic Zn level linearly increased among organic sources across environments. Post-HSE2 AM, across thermal environments at the 130 mg kg^{-1} level of Zn, organic source of Zn increased (130 InOrg vs. 130 Org, P = 0.034) trunk skin temperature compared to inorganic source.

There was a main effect of environment on T_{core} (Tables 3.8 and 3.9). The AM T_{core} was reduced (P = 0.018) in animals exposed to the initial heat during pre-HSE1. During HSE1, the T_{core} of animals was elevated (P < 0.001) by 0.45 °C by HS conditions at the PM timepoint. From the pre-HSE2 AM timepoint through the post-HSE2 PM timepoint, T_{core} of HS animals was on

average 0.22 – 0.45 °C greater ($P < 0.05$) than TN animals, and the magnitude of the difference was larger at the PM timepoint compared to at the AM timepoint.

Across Zn levels in both TN and HS environments, inorganic Zn source reduced T_{core} at post-HSE1 AM ($P = 0.044$) and PM ($P = 0.026$) timepoints, and also tended to reduce T_{core} at pre-HSE1 AM ($P = 0.068$) and HSE1 AM ($P = 0.083$) (Tables 3.8 and 3.9). The effect of Zn source on T_{core} of the representative animals occurred independently of a consistent impact of Zn source on the average daily feed intake (ADFI) for the pen during the HSE1 period; an interaction ($P < 0.001$) existed where inorganic Zn decreased ADFI of TN pigs but increased ADFI of HS pigs (Mills, 2018). Source of Zn did not appear to affect T_{core} during pre-HSE2, HSE2, or post-HSE2 periods, nor was T_{core} impacted ($P > 0.05$) by Zn level except at pre-HSE2 AM, T_{core} increased linearly (Organic Linear, $P = 0.022$) as level of organic Zn source increased regardless of environment.

Throughout the initial acute HS exposure during pre-HSE1 and during HSE1, timepoints with increased ($P < 0.10$) TCI between the HS and TN gilts of the 64-gilt subset corresponded to maintenance of T_{core} among all the HS animals while the increases in T_{core} were observed at timepoints when the TCI was not increased ($P > 0.10$; Tables 3.10 and 3.12). On days 42 - 48 during and following HSE2, the T_{core} of HS animals remained greater ($P < 0.05$) than that of the TN animals when TCI of the HS animals was not increased ($P > 0.10$). No main effect of Zn source was observed on the TCI measurements of the 64-gilt subset at any timepoint. Higher Zn level increased TCI measurements of the 64-gilt subset at the HSE1 PM (1.76 at 50 mg kg^{-1} vs. 2.08 at 130 mg kg^{-1}; $P = 0.041$) and post-HSE1 PM (1.64 at 50 mg kg^{-1} vs. 1.998 at 130 mg kg^{-1}; $P = 0.018$) timepoints but did not appear to affect TCI at any other timepoints.

For all the pigs in both environments, the body weight change measured from the beginning to the end of HSE2 was not correlated ($P > 0.05$) to ambient temperature, TCI, or intestinal measurements but was negatively correlated with T_{core} values measured at HSE2 AM and PM, and at post-HSE2 AM and PM (r = -0.29 to -0.23; $P \leq 0.027$). This correlation was also observed among the TN animals during the thermoneutral temperatures experienced during HSE2, as body weight change was negatively correlated with T_{core} measured at HSE2 AM and PM, and at post-HSE2 AM and PM (r = -0.45 to -0.33; $P \leq 0.024$; Fig. 3.6). However, for the HS animals experiencing acute heat during HSE2, body weight change had no relationship ($P > 0.05$) with T_{core} (Fig. 3.7). Body weight change during HSE2 was not correlated ($P > 0.05$) with intestinal measurements, TCI, or the ambient temperature during HSE2 for the HS pigs nor for the TN pigs.

3.4.2 Intestinal Responses Associated with Thermal Measures

For the 64-gilt subset during HSE2, the elevated T_{core} of animals had no relationship with most variables. For the HS pigs of the subset, jejunal villus height negatively correlated with PM $T_{trunk\ skin}$ (r = -0.38, $P = 0.036$) and jejunal expression of HSP-27 (r = -0.42, $P = 0.047$; Fig. 3.8), HSP-90 (r = -0.49, $P = 0.014$; Fig. 3.9), and occludin (r = -0.69, $P < 0.001$; Fig. 3.10) negatively correlated with PM $T_{ambient}$ during HSE2.

For organic Zn supplemented pigs of the subset during HSE2, PM $T_{ambient}$ correlated positively with expression of HSP-27 (r = 0.42, $P = 0.034$; Fig. 3.11) and MUC2 (r = 0.45, $P = 0.017$; Fig. 3.12) and negatively with villus height (jejunum, r = -0.43, $P = 0.025$; ileum, r = -0.38, $P = 0.050$; Fig. 3.13); villus height also negatively correlated with PM $T_{ear\ skin}$ (ileum, r = -0.37, $P = 0.040$) and PM T_{core} (jejunum, r = -0.45, $P = 0.012$).

In HS pigs of the subset, ileum villus height (r = -0.51, P = 0.015) negatively correlated with AM TCI (Fig. 3.14) and jejunal HSP-70 expression (r = 0.46, P = 0.041) positively correlated with PM TCI (Fig. 3.15) as well as with PM $T_{trunk\ skin}$ (r = 0.42, P = 0.023).

3.5 Discussion

Thermoregulation is the concerted effort of multiple body systems to balance heat production with heat loss to maintain body temperature at homeostatic equilibrium (Blatteis, 1998). Thermoregulation in pigs is accomplished through behavioral, physiological, and metabolic changes in response to thermal or cold stressors. In mammals, the physiologic response to thermal environment is modulated by the anterior hypothalamus while responses to absence of heat are governed by the posterior hypothalamus, through the sympathetic nervous system (Boulant, 1998; Morrison, 2016).

Surviving hyperthermia requires reducing endogenous heat production and increasing heat dissipation. Behavioral activities of the pig such as feeding generates body heat through the thermal metabolic increment of digestion and through musculoskeletal thermogenesis. Furthermore, these digestive and musculoskeletal processes present energetic and circulatory demands which compete with those needed for heat dissipation processes (Cabanac, 1998). Consequently, HS pigs reduce physical activity (Kerr et al., 2003), increase time spent lying (Huynh et al., 2005a), change feeding behavioral patterns (Quiniou et al., 2000) and reduce feed consumption (Nyachoti et al., 2004; Huynh et al., 2005b; da Fonseca de Oliveira et al., 2019). In addition to reductions in overall feed intake under HS, the larger amount of metabolic heat generated from dietary protein utilization relative to that of dietary fat and starch substrates together have considerable implication for lean growth of pigs under HS (Patience et al., 2015).

In the present study, the body weight gain during HSE2 is likely driven by feed intake under TN conditions, and modulated under HS conditions by increased water intake and urinary excretion along with reduced feed intake (Mills, 2018). As can be seen from the data, under TN conditions positive changes in body weight gain correspond to lower T_{core}. This may be because under higher T_{core}, nutrients are prioritized to other systems besides growth such as thermoregulation and to immune function; in addition, feed intake to support growth might be decreased to minimize additional metabolic heat production as would occur under experimentally induced HS (Patience et al., 2015). However, under our HS conditions, positive or negative body weight changes were not related to the T_{core} which were elevated due to the intense heat exposure.

Animals dissipate core body heat by transferring the heat into the surrounding environment via three fundamental types of heat transfer by which heat is gained or lost: conduction, convection, radiation (Morimoto, 1998; Werner, 1998). Transductional heat release occurs through behavioral modifications such as postural changes i.e. lying laterally on a cool concrete slat floor (Huynh et al., 2005a). Activity such as increasing water consumption theoretically increases musculoskeletal heat production, but the quantity of heat produced may be offset by the quantity of heat transfer from the body tissues into the cooler water which is then dissipated into the environment through urinary excretion (Song et al., 2011; Mills, 2018). Heat loss through convection occurs as ventilated air circulates around the pig and/or through its lungs (Morimoto, 1998).

Under heat stress, increased respiration rate (Pearce et al., 2013) facilitates both convective and evaporative cooling. Evaporative cooling is also a mechanism through which an animal can lose heat, such as through panting or water spray on the body surface. Pigs lack functional eccrine glands and lack the ability to sweat (Summerfield et al., 2015). Rather, pigs employ evaporative cooling through increased respiration rate. Vasodilation of peripheral blood vessels affords the

opportunity for heat loss via all 4 types of heat transfer and is an important mechanism by which pigs respond to the external temperature of their environment. When blood circulation is diverged to the cutaneous limbs, internal body heat is brought to the surface of the animal and can be dissipated thus lowering core body temperature and increasing surface temperature (Kingma et al., 2014).

The degree of heat transfer between a pig and its environment can be impacted by several factors, including body weight, ambient temperature, air speed, relative humidity, and surrounding surface temperatures (Werner, 1998; Cortus et al., 2017). The room temperatures used in the current modeling did not incorporate an adjustment for humidity, thus allowing comparison of results to field conditions where humidity is not a controllable factor or commonly recorded. Thus, the effective temperatures experienced by the heat stressed pigs of the present study were greater than the ambient temperature values. Subcutaneous adiposity is insulative and decreases heat transfer opportunity, and thus purportedly increases the risk factor for heat stress (Brown-Brandl et al., 2006). However, it is also opined that cutaneous blood flow axial to the subcutaneous fat layer is not impeded by the depth of the layer (Nielsen and Kaciuba-Uscilko, 1998). The TCI of the HS pigs in the present study was negatively correlated with day 64 10^{th} rib location backfat thickness (HSE1 AM, r = -0.51, P = 0.042), last rib backfat thickness (post-HSE2 PM, r = -0.43, P = 0.048), and last lumbar backfat thickness (HSE1 AM, r = -0.57, P = 0.020; HSE2 AM, r = -0.51, P = 0.016; HSE2 PM, r = -0.48, P = 0.023; post-HSE2 PM, r = -0.45, P = 0.034) thus supporting the former supposition.

The thermoneutral zone is the effective ambient temperature range over which temperature regulation i.e. maintenance of body temperature, occurs via vasomotor control and by conductive, convective, or radiative means without expending energy; above or below the bounds of this zone,

metabolic heat production or evaporative heat loss is employed (NRC, 1981; Kingma et al., 2012; Brownstein et al., 2017). Historically, the thermoneutral zone and optimum temperature range for achieving productive efficiency for growing pigs has had an upper critical temperature not exceeding 19-21 °C (Holmes and Close, 1977; Myer and Bucklin, 2001; see Nyachoti et al., 2004). The ambient temperatures recorded in the TN environment of the present study were at the upper end of this range. Because feed intake level, metabolic body size, and lean gain potential are the large determinants of endogenous heat production (NRC, 1981; Brown-Brandl et al., 2004), the thermoneutral zone may be lower and narrower for modern genetic lines which yield leaner pigs at heavier market weights (Brown-Brandl et al., 2013). Based on the relative humidity and ambient temperatures recorded in the HS rooms of the present study, the HS pigs experienced thermal indices considered "alert" and "danger" levels but were not exposed to "emergency" conditions in order to avoid extreme morbidity and potential mortality (Hahn et al., 2009).

Heat stress occurs when energy expenditure and/or evaporative heat loss i.e. increased respiration rate, is employed in an effort to maintain the balance between endogenous heat production and exogenous heat loss to avoid net thermal gain above the upper critical temperature (NRC, 1981; Kingma et al., 2012; Brownstein et al., 2017). As noted by Brown-Brandl et al. (2013), respiration rate is a key indicator of HS in pigs. Core body temperature is a function of all thermoregulatory processes (Blatteis, 1998) such that an increase in body temperature is a reflection of a failure to maintain equilibrium. During the cyclic HS pattern of the present study, the maintenance of core body temperature during the chronic periods of heat exposure indicate thermoregulatory responses can be adequate to maintain temperature equilibrium; however, during acute and repeated heat exposure thermoregulatory responses were no longer adequate to maintain temperature equilibrium. Despite this thermal gain, the core temperature parameter is still tightly

controlled within a narrow range and consequently, it may be difficult to detect significant correlations between core temperature and potential biomarkers of HS.

Using thermal imaging, Brown-Brandl et al. (2013) estimated that grower pigs increase surface blood flow at temperatures around 17.3 - 20.8 °C. At temperatures between 35.7 °C and 40.5 °C, surface blood flow (and potential dry heat loss) appeared to be maximized. Based on caretaker observations of the pigs in the present study, increased respiration rates (not recorded) indicative of evaporative cooling and heat stress occurred in pigs during the HSE1 and HSE2 periods. The ambient temperatures recorded in the HS rooms during these periods did not exceed 32 °C which is below the proposed temperature at which dry heat loss is maximized, which may indicate that heat stress occurs before blood flow and surface temperatures are maximized. However, the temperature at which blood flow maximization occurs could be lower for finishing pigs. As pig body weight increases, not only is more metabolic heat generated but the surface area to body mass decreases, making it more difficult to dissipate heat.

Because of greater vascularization, the $T_{ear\ skin}$ is more reflective of changes in cutaneous blood flow for the conservation or dissipation of deep body heat. As depicted in Fig. 3.5, $T_{ear\ skin}$ remained consistently elevated above $T_{trunk\ skin}$ for HS animals, while for TN animals the $T_{ear\ skin}$ was often less than $T_{trunk\ skin}$ especially in the cooler ambient temperatures of the AM timepoint. Since the T_{core} of HS animals was elevated despite the apparent increase in cutaneous blood flow to the ear and trunk, it should be concluded that the combined thermoregulatory responses were insufficient in maintaining T_{core} and preventing HS.

Under normal physiological conditions, the TCI appeared to have a diurnal pattern greater at PM and lower in AM which corresponds to more heat conservation at cooler AM timepoints and more heat dissipation at hotter PM timepoints (Table 3.12). Compared to 50 mg kg^{-1} Zn level,

the 130 mg kg^{-1} Zn level has been shown to improve pig body weight gain (Mills, 2018) and may explain the increased TCI observed for the 130 mg kg^{-1} Zn treatment independent of thermal environment and Zn source. As would be expected, increases in TCI corresponded to maintenance of T_{core} in the heat challenged animals of this study, while failure to increase heat loss corresponded to elevated T_{core}. Thus, TCI might have advantages over T_{core} as a predictor of gut level changes because of TCI's biological connection to the adaptive responses occurring within the dynamic system.

The T_{core} of the HS animals returned to baseline following the initial exposure to the acute heat of HSE1 but remained elevated even at the AM timepoints following HSE2. It is unclear if or when the T_{core} of the HS animals converged to baseline after post-HSE2 since T_{core} was only monitored for 48 hours after HSE2. There is a range of environmental temperatures at which changes in pig responses are elicited (Huynh et al., 2005b). Thus, various combinations of temperature, humidity, and time can be expected to elicit different responses in the pig. Further research would be useful to understand this progression and the consequences of extended durations of heat exposure versus repeated short-term exposure in addition to the magnitude of the heating and cooling periods. This would hopefully allow more targeted approaches to heat mitigation strategies.

Acute HS induces homeostatic responses within minutes or hours while chronic HS induces acclimatory responses over the course of days or weeks; this physiological acclimation to heat manifests itself in both short-term changes i.e. initiation of cellular signaling to preserve cellular homeostasis, and long-term changes i.e. altered endocrine milieu and reprogrammed gene expression and cellular response (Bernabucci et al., 2010; Collier et al., 2019). It is conceivable that through adaptation to prolonged cyclic heat, the pigs of the present study homeorhetically

adopted a new baseline body temperature. Indeed, *in utero* HS has been shown to increase the temperature setpoint in pigs (Johnson et al., 2015) while postnatal acclimation includes suppression of thyroid hormone and reduced metabolic rate (Horowitz, 1998). However, body temperatures of ruminants have been observed to return to baseline temperatures during periods of chronic heat exposure (Bernabucci et al., 1999; Hahn, 1999; Bernabucci et al., 2009). Interestingly, rectal temperatures of pigs under HS conditions have been observed to have a strong positive correlation with their rectal temperature under preceding TN conditions (Seibert et al., 2018), implying pre-HS factors influence susceptibility to HS on an individual basis.

Deleterious effects of heat stress can be observed on gut health attributes including absorptive capacity and reparative proliferation signified by villi length (height) and crypt depths, as well as gut integrity indicated by impaired tight junction barrier function and permeability (Pluske et al., 2018). In swine, acute heat exposure (≤ 24 hours) suppresses feed intake, damages intestinal integrity, reduces villous height and crypt depth, and elicits gut level responses such as upregulated mRNA expression and abundance of heat shock proteins, abundance of mucin, and tight junction protein prevalence (Pearce et al., 2013; 2015a; 2015b).

Heat Shock Protein 27 induces antioxidative functions (Arrigo, 2001; Arrigo et al., 2005; Lanneau et al., 2007; Batulan et al., 2016) and might have a role in cellular glucose uptake (Yuan et al., 2016). The positive correlations between ambient temperature with intestinal HSP27 gene expression and with mucin-2 gene expression in organic Zn supplemented gilts may be indicative of organic Zn facilitating an active response to sustained HS; this response was not apparent among the inorganic Zn supplemented pigs. However, among just the HS animals, the transcriptional responses of HSP27, HSP90, and occludin were surprisingly negatively correlated with the ambient temperature experienced by the animals 3 weeks prior. Because this subset of animals

would have experienced HS conditions for another 3 weeks until mRNA levels were assessed, it is possible that adaptive response signaling was exhausted under greater temperatures. However, speculation is difficult without more conclusive evidence of the how the gut adapts to hot temperatures and reduced feed intake over an extended time period. Moreover, the activity of HSP90 is ATP dependent and serves to promote protein synthesis, functions which might be downregulated under HS and reduced feed intake/energy levels (Hafen et al., 2019).

The functions of HSP70 include transporting partially-folded proteins into the mitochondria and blocking cathepsin release (Lanneau et al., 2007; see reviews by Voos, 2013, and by Craig, 2018). Among HS pigs, increased gene expression of HSP70 was positively correlated to the TCI of the animals prior to 3 additional weeks of HS conditions. Thus it would appear that for HS animals, the valued TCI activity is related to increased expression of intestinal HSP70 and may represent the multiple thermoregulatory responses occurring within the animal.

Reductions in jejunal villi height, crypt depths, and microvilli height have also been reported under a 3-day duration of HS (Yu et al., 2010). Yet there is scant data indicating how chronic exposure to cyclic heat impacts the intestinal morphology of grow-finish pigs. Among the HS animals, the negative correlation between villus height and TCI highlights the undesirable consequences which heat dissipation has on intestinal absorptive capacity still 3 weeks later. Alternatively, the negative correlations between ileal and villus heights with the HSE2 ambient temperature experienced by the organic Zn supplemented gilts could be caused by the continued elevation in HS ambient temperatures relative to the TN environmental conditions during the 3 weeks prior to gut morphology measurements being taken. Regardless, the negative impact of HS on finishing pig gut integrity is apparent.

In the present study, the negative impacts of HS on gut integrity were still evident within the animals receiving organic Zn supplementation. Accordingly, Liao et al. (2018) reported Zn from both inorganic and organic sources supplemented to poultry failed to ameliorate increased rectal temperature in heat stressed birds. However, Zn has demonstrated efficacy in maintaining feed intake of pigs in the stressful post-weaning period (Feldpausch et al., 2018). Moreover, dietary Zn is believed to have local beneficial action on the intestine and has been shown to improve tight junction complex perfusion in colitis-challenged rats (Sturniolo et al., 2002). Under thermal stress conditions, organic Zn supplementation has been observed to improve intestinal integrity and provide protection against blood endotoxin elevation in pigs during acute heat exposure (Pearce et al., 2015b). Furthermore, dietary Zn appears to reduce the HS induced expression of heat shock protein mRNA transcripts in tissues of other species which may indicate a protective effect of Zn against heat stress (Sheikh et al., 2017; Rajkumar et al., 2018). However, any beneficial action of Zn in the present study was obscured by the severity and/or duration of the HS.

Blood cortisol levels are commonly used to validate the stress response associated with acute heat exposure but have not been established as a reliable indicator of chronic HS in the pig (Pearce et al., 2013). Other non-invasive means of establishing the thermal stress response under chronic exposure might include salivary cortisol measurement which can be transient under HS (Song et al., 2011) or exploring the use of fecal glucocorticoids (Schatz and Palme, 2001; Keay et al., 2006). In addition, thermal monitoring might prove to be another useful means to measure the effects of HS. The present model demonstrated how repeated exposure to both chronic and acute heat challenge induced a thermoregulatory response which was inadequate to preserve core body temperature throughout the heat exposure period.

Perfusion of devices with capability for electronic data collection and transmission through the internet into daily functions, dubbed the Internet of Things, is a new frontier for scientific application in the swine industry. A review by Sorensen and Pederson (2015) describes how measuring skin temperatures using infrared technology is gaining practice across species and for applications from welfare to meat quality. Indeed, preliminary reports indicate successful implementation of thermal monitoring to detect changes in body temperature associated with social stressors and ambient temperature changes (Predicala, 2019). Identifying associations between thermal measurements and biomarkers thus holds tremendous potential for future refinement and application.

Trends in modern agriculture include a growing focus on applying modern technological tools and data acquisition techniques to improve agricultural output, a concept referred to as "smart farming" (Wolfert et al., 2017). Simultaneously, the swine industry continues to refine its knowledge of the interaction between genetics, environment, health, husbandry, and nutrition. Combined with this knowledge, identification of practical metrics and tools through which to apply big data will allow the industry to optimize production on smaller individual units, such as on a barn, pen, or even a litter basis.

3.6 Summary

Significant thermal correlations with gut integrity characteristics existed for HS pigs and existed in the presence of organic Zn supplementation. Gut level HS responses correlated with ambient temperature, skin temperature, and heat dissipation but very few correlated with core body temperature possibly due to tight regulation of this physiological parameter. Changes in body weight gain during a short period of acute HS did not correspond to changes in ambient and core body temperatures. Further research is needed to understand how both prolonged thermal

challenge as the grow-finish pig increases in body weight and recurrence of acute HS bouts impact the thermoregulatory ability of the grow-finish pig to maintain normal core body temperatures. Degree of heat dissipation by heat stressed animals appeared to be associated with classic HS damage and response mechanism. The correlation between ambient temperature and the transcriptional response under long-term thermal stress warrants further investigation. Additional research is needed to investigate whether strengths of the correlations between temperature measurements and gut integrity markers change with time post heat exposure and between chronic and acute heat stressors. Environmental and antemortem physiological measurements which may prove useful candidates for future predictive modeling of chronic HS damage in pigs include skin temperatures, Thermal Circulation Index, intestinal villus height, and heat shock protein expression.

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Table 3.1. Primer sequences for reverse transcription-qPCR (Mills, 2018).

Gene	Sense (5'→ 3') – Forward	Antisense (5'→ 3') - Reverse	References
HSP 27	AGGAGCGGCAGGATGAG	GGACAGGGAGGAGGAGAC	Kamanga-Sollo et al., 2011
HSP 70	GCCCTGAATCCGCAGAATA	TCCCCACGGTAGGAAACG	Pilon et al., 2003
HSP 90	CGCTGAGAAAGTGACCGTTATC	ACCTTTGTTCCACGACCCATAG	Kamanga-Sollo et al., 2011
Occludin	TCGTCCAACGGGAAAGTGAA	ATCAGTGGAAGTTCCTGAACCA	Pearce et al., 2015b
Mucin 2	CTGTGTGGGGCCTGACAA	AGTGCTTGCAGTCGAACTCA	Pearce et al., 2015b
GAPDH	GAAGGTCGGAGTGAACGGAT	CATGGGTAGAATCATACTGGAACA	Li et al., 2005

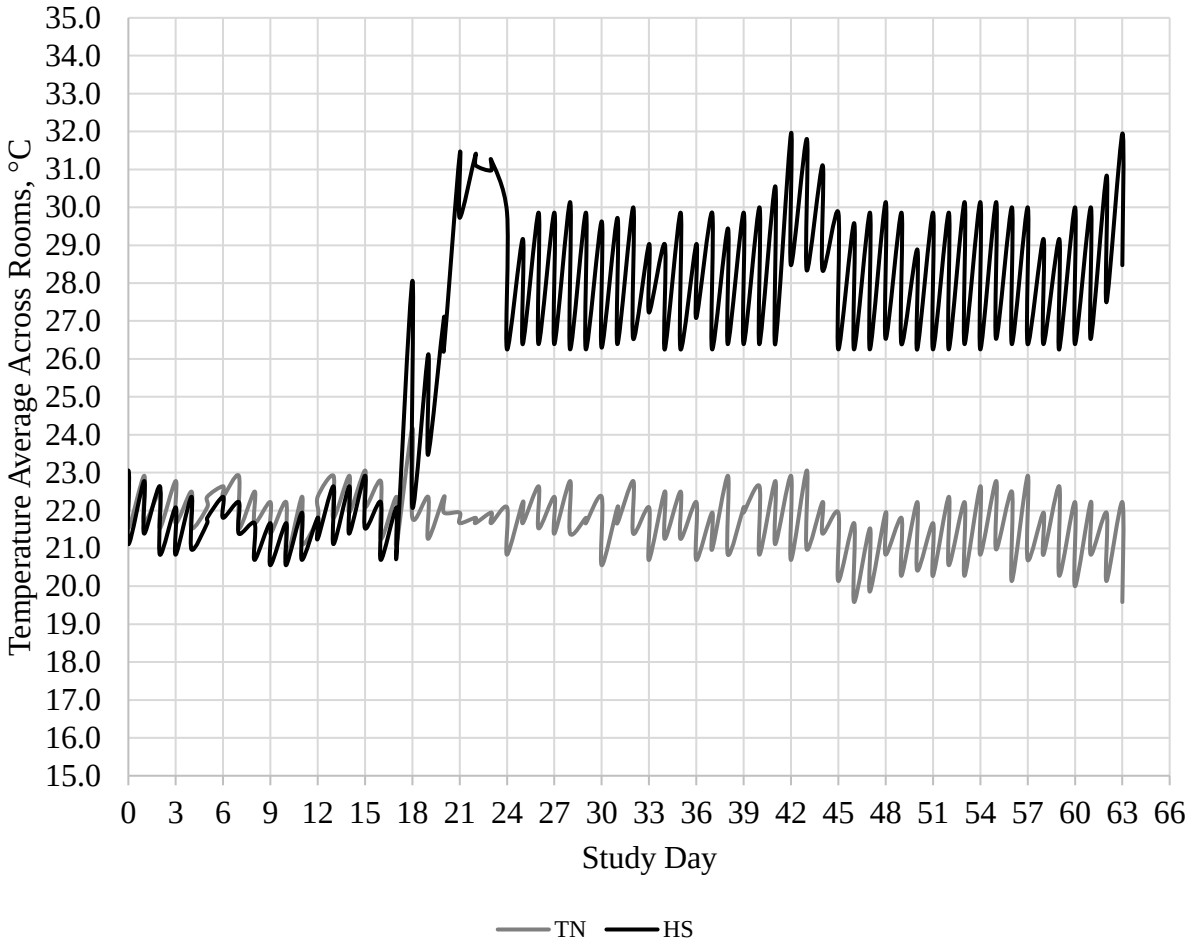

Figure 3.1. Environmental temperature extremes (24-hour high and 24-hour low) recorded in environmental rooms throughout the study from day 0 to day 63 in the thermoneutral (TN) rooms (n = 4) and heat stress (HS) rooms (n = 4). Temperatures were logged for the preceding 24-hour period up until pig marketing began on day 64±1.

Figure 3.2. T_{core} recording device.

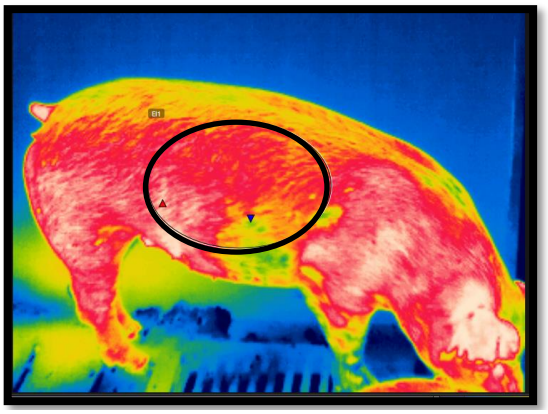

Figure 3.3. Thermal image of trunk area.

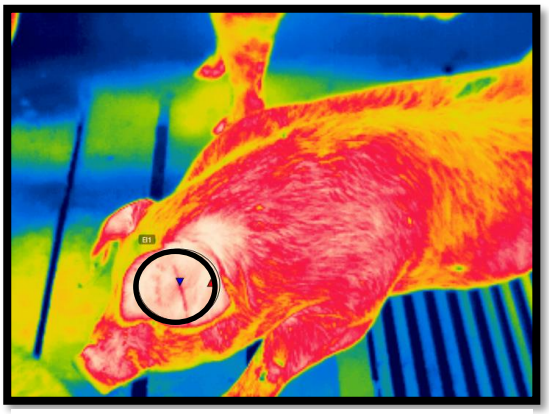

Figure 3.4. Thermal image of ear area.

Table 3.2. Environmental thermal data at AM and PM timepoints experienced by pigs (n = 96) fed varying zinc level & source under environmental heat stress (interactive LSMEANS).

Temperature:	Thermoneutral							Heat Stress							
Diet:[1]	1	2	3	4	5	6		1	2	3	4	5	6		SEM
n=	8	8	8	8	8	8		8	8	8	8	8	8		
Ambient,[2] °C															
Pre-HSE1 AM [a]	21.53	21.64	21.58	21.60	21.65	21.64		24.13	24.21	24.16	24.08	24.21	24.15		0.181
Pre-HSE1 PM [a]	22.03	21.98	21.99	22.00	22.13	22.03		27.13	27.13	27.13	27.11	27.10	27.08		0.171
HSE1 AM [a]	21.65	21.73	21.70	21.68	21.70	21.73		29.83	29.87	29.90	29.87	29.87	30.05		0.200
HSE1 PM [a]	21.78	21.70	21.75	21.65	21.75	21.70		31.97	32.00	31.97	32.00	32.00	32.00		0.183
Post HSE1 AM [a]	21.40	21.40	21.44	21.40	21.40	21.44		26.49	26.53	26.53	26.56	26.59	26.56		0.339
Post HSE1 PM [a]	21.88	21.81	21.83	21.84	21.83	21.88		29.34	29.44	29.31	29.29	29.24	29.34		0.220
Pre-HSE2 AM [a]	21.95	21.89	22.01	21.96	21.89	21.95		27.09	27.00	27.09	27.00	27.17	27.00		0.164
HSE2 AM [a]	20.89	21.00	20.88	20.83	20.88	20.89		28.74	28.77	28.77	28.77	28.74	28.77		0.324
HSE2 PM [a]	21.25	21.38	21.30	21.28	21.38	21.28		31.46	31.39	31.46	31.42	31.42	31.42		0.437
Post HSE2 AM [a]	20.64	20.67	20.71	20.57	20.64	20.67		26.58	26.55	26.55	26.58	26.62	26.58		0.199
Post HSE2 PM [a]	21.13	21.12	21.13	21.12	21.12	21.13		29.57	29.57	29.57	29.57	29.57	29.57		0.165

[1]Diet 1: 50 mg kg^{-1} ZnO; Diet 2: 130 mg kg^{-1} ZnO; Diet 3: 50 mg kg^{-1} organic Zn from Availa®Zn; Diet 4) 50 mg kg^{-1} ZnO + 40 mg kg^{-1} organic Zn; Diet 5: 50 mg kg^{-1} ZnO + 60 mg kg^{-1} organic Zn; and Diet 6: 50 mg kg^{-1} ZnO + 80 mg kg^{-1} organic Zn.

[2]Ambient temperatures recorded at timepoints during the acclimation heating period (day 18 – 21; pre-HSE1), first acute heat event (day 21 – 24; HSE1), until vaginal thermometer removal on day 26 (day 24 – 26; post-HSE1), after vaginal thermometer insertion the afternoon of day 41 until the start of the second acute heat event (day 41 – 42; pre-HSE2), second acute heat event (day 42 – 45; HSE2), and until vaginal thermometer removal on day 48 (day 45 – 48 post-HSE2).

[a] For each timepoint, probability of overall treatment effect was found to be statistically significant ($P < 0.001$).

Table 3.3. Statistical analysis of ambient temperature experienced at AM and PM timepoints by individual pigs (n = 96) fed varying zinc level & source under environmental heat stress (*P*-values).

Thermal measurement:	Ambient Temperature										
Event period:[1]	Pre-HSE1 AM	Pre-HSE1 PM	HSE1 AM	HSE1 PM	Post-HSE1 AM	Post-HSE1 PM	Pre-HSE2 AM	HSE2 AM	HSE2 PM	Post-HSE2 AM	Post-HSE2 PM
Contrasts											
Environment[2]	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Zn Source[3]	0.903	0.919	0.717	0.937	0.820	0.634	0.726	0.809	0.996	0.968	1.000
Zn Level[4] Linear	0.277	0.948	0.643	0.976	0.896	0.945	0.546	0.856	0.951	0.948	0.961
Zn Level[4] Quadratic	0.827	0.773	0.756	0.895	0.942	0.637	0.934	0.790	1.000	0.815	0.951
Source x Environment	0.679	0.649	0.800	0.874	0.918	0.655	0.931	0.765	0.969	0.903	1.000
Level Linear x Environment	0.277	0.948	0.643	0.976	0.896	0.945	0.546	0.856	0.951	0.948	0.961
Level Quadratic x Environment	0.651	0.749	0.891	0.796	0.848	0.725	0.864	0.811	0.983	0.622	0.951
Organic[5] Linear	0.367	0.953	0.449	0.982	0.825	0.954	0.845	0.938	0.970	0.851	1.000
Organic[5] Quadratic	0.736	0.759	0.777	0.932	0.929	0.620	0.927	0.911	0.917	0.888	0.938
50 InOrg vs. 50 Org	0.677	0.852	0.756	0.942	0.886	0.806	0.808	0.965	0.936	0.921	1.000
130 InOrg vs. 130 Org	0.766	1.000	0.625	1.000	0.886	0.912	0.808	0.812	0.914	0.921	0.956
Org Linear x Environment	0.792	0.652	0.736	0.836	0.938	0.972	0.992	0.991	0.889	0.900	1.000
Org Quadratic x Environment	0.800	0.826	0.777	0.823	0.844	0.896	0.714	0.911	0.857	0.779	0.938

[1]Temperatures recorded at timepoints during the acclimation heating period (day 18 – 21; pre-HSE1), first acute heat event (day 21 – 24; HSE1), until vaginal thermometer removal on day 26 (day 24 – 26; post-HSE1), after vaginal thermometer insertion the afternoon of day 41 until the start of the second acute heat event (day 41 – 42; pre-HSE2), second acute heat event (day 42 – 45; HSE2), and until vaginal thermometer removal on day 48 (day 45 – 48 post-HSE2).

[2]Environment = Heat stress (HS) vs Thermoneutral (TN).

[3]Source = Inorganic vs Organic; diets 1, 2 vs 3, 4, 5, 6.

[4]Contrast "Level" (Level) = Total level available Zn regardless of source of either 50 (2 diets), 90, 110, 130 mg kg^{-1} (2 diets); diets 1, 3 vs 4 vs 5 vs 2, 6.

[5]Contrast "Organic" (Org) = 50 ZnO basal with added organic zinc of (0), 40, 60, or 80 mg kg^{-1}; diets 1 vs 4 vs 5 vs 6.

Table 3.4. Ear skin thermal data at AM and PM timepoints of pigs (n = 96) fed varying zinc level & source under environmental heat stress (interactive LSMEANS).

Temperature:	Thermoneutral							Heat Stress							
Diet:[1]	1	2	3	4	5	6		1	2	3	4	5	6		SEM
n=	8	8	8	8	8	8		8	8	8	8	8	8		
Ear skin,[2] °C															
Pre-HSE1 AM [a]	33.25	31.61	33.43	33.63	33.83	30.98		35.13	34.16	35.26	34.38	34.33	35.14		0.732
Pre-HSE1 PM [a]	34.51	34.18	34.19	34.93	33.06	33.20		36.83	36.95	36.84	37.03	36.43	36.75		0.547
HSE1 AM [a]	31.76	32.13	32.88	33.13	32.44	31.46		36.99	36.78	36.83	36.14	37.04	37.44		0.626
HSE1 PM [a]	34.13	33.64	33.50	34.40	31.63	34.13		38.09	38.14	37.84	37.85	37.99	38.00		0.525
Post HSE1 AM [a]	31.84	31.06	31.41	33.08	29.75	32.81		36.03	34.14	35.81	34.59	34.46	35.19		0.990
Post HSE1 PM [a]	33.43	33.91	33.76	33.48	32.01	33.93		36.83	36.90	37.08	36.64	36.83	36.60		0.623
Pre-HSE2 AM [a]	33.74	32.44	34.26	34.70	34.33	33.69		35.95	35.93	35.19	35.49	35.51	35.95		0.615
HSE2 AM [a]	32.85	30.30	30.95	30.66	31.94	31.88		36.48	36.11	35.96	35.83	36.33	36.11		0.710
HSE2 PM [a]	33.03	32.59	33.14	34.14	32.95	33.29		37.48	37.25	37.04	37.43	37.34	36.56		0.530
Post HSE2 AM [a]	31.66	29.13	28.94	31.74	30.59	31.03		34.94	34.13	34.95	33.95	34.56	34.95		0.828
Post HSE2 PM [a]	32.73	32.56	32.90	33.26	30.80	31.96		36.54	36.70	36.28	36.84	36.66	36.63		0.574

[1]Diet 1: 50 mg kg^{-1} ZnO; Diet 2: 130 mg kg^{-1} ZnO; Diet 3: 50 mg kg^{-1} organic Zn from Availa®Zn; Diet 4) 50 mg kg^{-1} ZnO + 40 mg kg^{-1} organic Zn; Diet 5: 50 mg kg^{-1} ZnO + 60 mg kg^{-1} organic Zn; and Diet 6: 50 mg kg^{-1} ZnO + 80 mg kg^{-1} organic Zn.

[2]Ear skin temperatures recorded at timepoints during the acclimation heating period (day 18 – 21; pre-HSE1), first acute heat event (day 21 – 24; HSE1), until vaginal thermometer removal on day 26 (day 24 – 26; post-HSE1), after vaginal thermometer insertion the afternoon of day 41 until the start of the second acute heat event (day 41 – 42; pre-HSE2), second acute heat event (day 42 – 45; HSE2), and until vaginal thermometer removal on day 48 (day 45 – 48 post-HSE2).

[a] For each timepoint, probability of overall treatment effect was found to be statistically significant ($P < 0.001$).

Table 3.5. Statistical analysis of ear skin temperature at AM and PM timepoints of pigs (n = 96) fed varying zinc level & source under environmental heat stress (*P*-values).

Thermal measurement:	Ear Skin Temperature										
Event period:[1]	Pre-HSE1 AM	Pre-HSE1 PM	HSE1 AM	HSE1 PM	Post-HSE1 AM	Post-HSE1 PM	Pre-HSE2 AM	HSE2 AM	HSE2 PM	Post-HSE2 AM	Post-HSE2 PM
Contrasts											
Environment[2]	<.001	0.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Zn Source[3]	0.460	0.331	0.492	0.236	0.825	0.531	0.296	0.599	0.641	0.805	0.514
Zn Level[4] Linear	0.039	0.111	0.833	0.304	0.133	0.598	0.598	0.615	0.454	0.612	0.216
Zn Level[4] Quadratic	0.272	0.630	0.699	0.383	0.676	0.219	0.266	0.578	0.107	0.549	0.801
Source x Environment	0.655	0.564	0.456	0.622	0.730	0.681	0.032	0.983	0.192	0.917	0.551
Level Linear x Environment	0.039	0.111	0.833	0.304	0.133	0.598	0.598	0.615	0.454	0.612	0.216
Level Quadratic x Environment	0.029	0.594	0.060	0.668	0.488	0.380	0.062	0.848	0.550	0.102	0.690
Organic[5] Linear	0.154	0.049	0.862	0.315	0.595	0.904	0.893	0.618	0.333	0.645	0.174
Organic[5] Quadratic	0.423	0.920	0.525	0.057	0.121	0.276	0.673	0.203	0.314	0.458	0.850
50 InOrg vs. 50 Org	0.831	0.767	0.433	0.337	0.724	0.619	0.840	0.092	0.757	0.104	0.935
130 InOrg vs. 130 Org	0.818	0.266	1.000	0.700	0.124	0.808	0.279	0.268	0.991	0.102	0.531
Org Linear x Environment	0.156	0.138	0.317	0.358	0.695	0.888	0.882	0.813	0.466	0.479	0.158
Org Quadratic x Environment	0.023	0.788	0.039	0.127	0.895	0.257	0.134	0.401	0.973	0.665	0.528

[1]Temperatures recorded at timepoints during the acclimation heating period (day 18 – 21; pre-HSE1), first acute heat event (day 21 – 24; HSE1), until vaginal thermometer removal on day 26 (day 24 – 26; post-HSE1), after vaginal thermometer insertion the afternoon of day 41 until the start of the second acute heat event (day 41 – 42; pre-HSE2), second acute heat event (day 42 – 45; HSE2), and until vaginal thermometer removal on day 48 (day 45 – 48 post-HSE2).

[2]Environment = Heat stress (HS) vs Thermoneutral (TN).

[3]Source = Inorganic vs Organic; diets 1, 2 vs 3, 4, 5, 6.

[4]Contrast "Level" (Level) = Total level available Zn regardless of source of either 50 (2 diets), 90, 110, 130 mg kg^{-1} (2 diets); diets 1, 3 vs 4 vs 5 vs 2, 6.

[5]Contrast "Organic" (Org) = 50 ZnO basal with added organic zinc of (0), 40, 60, or 80 mg kg^{-1}; diets 1 vs 4 vs 5 vs 6.

Table 3.6. Trunk skin thermal data at AM and PM timepoints of pigs (n = 96) fed varying zinc level & source under environmental heat stress (interactive LSMEANS).

Temperature:	Thermoneutral							Heat Stress							
Diet:[1]	1	2	3	4	5	6		1	2	3	4	5	6		SEM
n=	8	8	8	8	8	8		8	8	8	8	8	8		
Trunk skin,[2] °C															
Pre-HSE1 AM [a]	32.54	33.04	33.70	32.95	33.10	33.03		34.00	33.66	34.10	33.79	33.98	34.63		0.330
Pre-HSE1 PM [a]	33.05	33.64	33.74	33.64	33.10	33.40		35.40	35.55	35.54	35.49	35.54	35.79		0.304
HSE1 AM [a]	32.76	32.95	33.20	32.99	32.76	33.16		36.28	35.55	35.90	35.79	36.13	36.65		0.357
HSE1 PM [a]	33.03	33.25	33.75	33.23	32.90	33.30		37.15	37.31	36.70	36.65	37.06	37.25		0.324
Post HSE1 AM [a]	32.14	32.94	33.09	32.50	32.11	33.26		34.84	34.30	34.95	34.20	34.64	34.61		0.378
Post HSE1 PM [a]	32.29	33.41	33.56	33.18	33.03	33.63		35.99	35.98	36.16	35.73	35.83	36.23		0.287
Pre-HSE2 AM [a]	32.71	32.84	33.68	33.34	33.44	33.23		35.06	34.91	34.46	34.89	35.33	34.88		0.389
HSE2 AM [a]	32.59	32.39	32.45	32.13	32.44	31.64		35.46	34.96	35.38	35.10	35.41	35.59		0.312
HSE2 PM [a]	32.65	32.74	33.04	33.14	32.80	33.08		36.49	36.56	36.20	35.99	36.15	36.15		0.345
Post HSE2 AM [a]	31.66	31.80	31.78	32.25	32.29	32.45		34.18	33.73	34.23	33.99	34.35	34.51		0.349
Post HSE2 PM [a]	32.55	32.94	32.64	32.49	32.09	32.63		35.71	35.51	35.38	35.46	35.70	35.61		0.358

[1]Diet 1: 50 mg kg^{-1} ZnO; Diet 2: 130 mg kg^{-1} ZnO; Diet 3: 50 mg kg^{-1} organic Zn from Availa®Zn; Diet 4) 50 mg kg^{-1} ZnO + 40 mg kg^{-1} organic Zn; Diet 5: 50 mg kg^{-1} ZnO + 60 mg kg^{-1} organic Zn; and Diet 6: 50 mg kg^{-1} ZnO + 80 mg kg^{-1} organic Zn.

[2]Trunk skin temperatures recorded at timepoints during the acclimation heating period (day 18 – 21; pre-HSE1), first acute heat event (day 21 – 24; HSE1), until vaginal thermometer removal on day 26 (day 24 – 26; post-HSE1), after vaginal thermometer insertion the afternoon of day 41 until the start of the second acute heat event (day 41 – 42; pre-HSE2), second acute heat event (day 42 – 45; HSE2), and until vaginal thermometer removal on day 48 (day 45 – 48 post-HSE2).

[a] For each timepoint, probability of overall treatment effect was found to be statistically significant ($P < 0.001$).

Table 3.7. Statistical analysis of trunk skin temperature at AM and PM timepoints of pigs (n = 96) fed varying zinc level & source under environmental heat stress (*P*-values).

Thermal measurement:	Trunk Skin Temperature										
Event period:[1]	Pre-HSE1 AM	Pre-HSE1 PM	HSE1 AM	HSE1 PM	Post-HSE1 AM	Post-HSE1 PM	Pre-HSE2 AM	HSE2 AM	HSE2 PM	Post-HSE2 AM	Post-HSE2 PM
Contrasts											
Environment[2]	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Zn Source[3]	0.073	0.506	0.364	0.657	0.572	0.123	0.231	0.654	0.833	0.061	0.409
Zn Level[4] Linear	0.960	0.708	0.930	0.760	0.788	0.218	0.669	0.277	0.975	0.317	0.893
Zn Level[4] Quadratic	0.422	0.809	0.474	0.143	0.060	0.156	0.323	0.749	0.693	0.539	0.424
Source x Environment	0.764	0.972	0.940	0.190	0.678	0.127	0.103	0.203	0.075	0.732	0.630
Level Linear x Environment	0.960	0.708	0.930	0.760	0.788	0.218	0.669	0.277	0.975	0.317	0.893
Level Quadratic x Environment	0.641	0.844	0.848	0.707	0.588	0.581	0.869	0.727	0.278	0.412	0.458
Organic[5] Linear	0.067	0.351	0.254	0.513	0.201	0.006	0.518	0.342	0.966	0.078	0.916
Organic[5] Quadratic	0.673	0.879	0.214	0.285	0.146	0.614	0.288	0.818	0.756	0.937	0.448
50 InOrg vs. 50 Org	0.047	0.160	0.926	0.639	0.119	0.007	0.624	0.714	0.879	0.808	0.724
130 InOrg vs. 130 Org	0.133	1.000	0.054	0.983	0.347	0.379	0.636	0.839	0.909	0.034	0.764
Org Linear x Environment	0.821	0.704	0.819	0.909	0.134	0.069	0.451	0.099	0.389	0.629	0.960
Org Quadratic x Environment	0.132	0.585	0.380	0.556	0.855	0.203	0.590	0.315	0.442	0.414	0.663

[1]Temperatures recorded at timepoints during the acclimation heating period (day 18 – 21; pre-HSE1), first acute heat event (day 21 – 24; HSE1), until vaginal thermometer removal on day 26 (day 24 – 26; post-HSE1), after vaginal thermometer insertion the afternoon of day 41 until the start of the second acute heat event (day 41 – 42; pre-HSE2), second acute heat event (day 42 – 45; HSE2), and until vaginal thermometer removal on day 48 (day 45 – 48 post-HSE2).

[2]Environment = Heat stress (HS) vs Thermoneutral (TN).

[3]Source = Inorganic vs Organic; diets 1, 2 vs 3, 4, 5, 6.

[4]Contrast "Level" (Level) = Total level available Zn regardless of source of either 50 (2 diets), 90, 110, 130 mg kg^{-1} (2 diets); diets 1, 3 vs 4 vs 5 vs 2, 6.

[5]Contrast "Organic" (Org) = 50 ZnO basal with added organic zinc of (0), 40, 60, or 80 mg kg^{-1}; diets 1 vs 4 vs 5 vs 6.

Table 3.8. Internal core body thermal data at AM and PM timepoints of pigs (n = 96) fed varying zinc level & source under environmental heat stress (interactive LSMEANS means).

Temperature:	Thermoneutral							Heat Stress							
Diet:[1]	1	2	3	4	5	6		1	2	3	4	5	6		SEM
n=	8	8	8	8	8	8		8	8	8	8	8	8		
Internal core,[2] °C															
Pre-HSE1 AM [a]	39.53	39.57	39.95	39.72	39.74	39.73		39.54	39.40	39.34	39.59	39.63	39.60		0.185
Pre-HSE1 PM [b]	39.46	39.71	39.92	39.70	39.80	39.83		39.86	39.75	39.46	39.90	39.68	39.83		0.197
HSE1 AM [b]	39.39	39.57	39.87	39.58	39.56	39.83		39.72	39.57	39.54	39.69	39.83	39.88		0.207
HSE1 PM [a]	39.47	39.64	39.80	39.64	39.68	39.63		40.12	40.17	39.74	40.23	39.98	40.33		0.200
Post HSE1 AM [b]	39.44	39.48	40.10	39.58	39.66	39.55		39.48	39.18	39.48	39.59	39.53	39.40		0.275
Post HSE1 PM [b]	39.41	39.53	40.08	39.56	39.90	39.75		39.70	39.48	39.66	39.88	39.68	39.74		0.288
Pre-HSE2 AM [c]	39.28	39.24	39.45	39.36	39.35	39.70		39.58	39.74	39.43	39.60	39.70	39.68		0.134
HSE2 AM [d]	39.28	39.27	39.38	39.48	39.48	39.51		39.61	39.69	39.67	39.63	39.58	39.79		0.150
HSE2 PM [a]	39.31	39.36	39.58	39.41	39.56	39.54		39.91	39.89	39.95	39.80	39.93	39.99		0.136
Post HSE2 AM [b]	39.33	39.20	39.35	39.34	39.51	39.34		39.51	39.51	39.54	39.50	39.63	39.46		0.148
Post HSE2 PM [d]	39.48	39.29	39.53	39.45	39.58	39.44		39.79	39.73	39.78	39.78	39.88	39.69		0.151

[1]Diet 1: 50 mg kg^{-1} ZnO; Diet 2: 130 mg kg^{-1} ZnO; Diet 3: 50 mg kg^{-1} organic Zn from Availa®Zn; Diet 4) 50 mg kg^{-1} ZnO + 40 mg kg^{-1} organic Zn; Diet 5: 50 mg kg^{-1} ZnO + 60 mg kg^{-1} organic Zn; and Diet 6: 50 mg kg^{-1} ZnO + 80 mg kg^{-1} organic Zn.

[2]Internal core body temperatures recorded at timepoints during the acclimation heating period (day 18 – 21; pre-HSE1), first acute heat event (day 21 – 24; HSE1), until vaginal thermometer removal on day 26 (day 24 – 26; post-HSE1), after vaginal thermometer insertion the afternoon of day 41 until the start of the second acute heat event (day 41 – 42; pre-HSE2), second acute heat event (day 42 – 45; HSE2), and until vaginal thermometer removal on day 48 (day 45 – 48 post-HSE2).

[a] For each timepoint, probability of overall treatment effect was found to be statistically significant ($P < 0.001$).

[b] For each timepoint, probability of overall treatment effect was found to be statistically nonsignificant ($P > 0.100$).

[c] For each timepoint, probability of overall treatment effect was found to be statistically significant ($P < 0.050$).

[d] For each timepoint, probability of overall treatment effect was found to be statistically significant ($P < 0.100$).

Table 3.9. Statistical analysis of internal core temperature at AM and PM timepoints of pigs (n = 96) fed varying zinc level & source under environmental heat stress (*P*-values).

Thermal measurement:	Internal Core Temperature										
Event period:[1]	Pre-HSE1 AM	Pre-HSE1 PM	HSE1 AM	HSE1 PM	Post-HSE1 AM	Post-HSE1 PM	Pre-HSE2 AM	HSE2 AM	HSE2 PM	Post-HSE2 AM	Post-HSE2 PM
Contrasts											
Environment[2]	0.018	0.910	0.425	<.001	0.063	0.870	0.001	0.001	<.001	0.013	<.001
Zn Source[3]	0.068	0.427	0.083	0.751	0.044	0.026	0.288	0.199	0.172	0.352	0.390
Zn Level[4] Linear	0.887	0.359	0.408	0.226	0.146	0.732	0.063	0.423	0.703	0.962	0.558
Zn Level[4] Quadratic	0.342	0.645	0.796	0.823	0.401	0.489	0.704	0.893	0.615	0.389	0.398
Source x Environment	0.308	0.076	0.444	0.246	0.657	0.351	0.066	0.273	0.248	0.505	0.568
Level Linear x Environment	0.887	0.359	0.408	0.226	0.146	0.732	0.063	0.423	0.703	0.962	0.558
Level Quadratic x Environment	0.611	0.611	0.448	0.962	0.323	0.424	0.577	0.213	0.471	0.696	0.986
Organic[5] Linear	0.359	0.351	0.064	0.361	0.934	0.332	0.022	0.134	0.119	0.809	0.820
Organic[5] Quadratic	0.493	0.828	0.700	0.929	0.347	0.448	0.506	0.915	0.881	0.332	0.436
50 InOrg vs. 50 Org	0.417	0.837	0.324	0.861	0.056	0.081	0.913	0.542	0.225	0.826	0.870
130 InOrg vs. 130 Org	0.216	0.513	0.079	0.616	0.477	0.259	0.090	0.182	0.246	0.722	0.667
Org Linear x Environment	0.653	0.124	0.503	0.892	0.573	0.275	0.229	0.763	0.526	0.759	0.797
Org Quadratic x Environment	0.750	0.436	0.979	0.273	0.983	0.728	0.329	0.304	0.371	0.914	0.865

[1]Temperatures recorded at timepoints during the acclimation heating period (day 18 – 21; pre-HSE1), first acute heat event (day 21 – 24; HSE1), until vaginal thermometer removal on day 26 (day 24 – 26; post-HSE1), after vaginal thermometer insertion the afternoon of day 41 until the start of the second acute heat event (day 41 – 42; pre-HSE2), second acute heat event (day 42 – 45; HSE2), and until vaginal thermometer removal on day 48 (day 45 – 48 post-HSE2).

[2]Environment = Heat stress (HS) vs Thermoneutral (TN).

[3]Source = Inorganic vs Organic; diets 1, 2 vs 3, 4, 5, 6.

[4]Contrast "Level" (Level) = Total level available Zn regardless of source of either 50 (2 diets), 90, 110, 130 mg kg^{-1} (2 diets); diets 1, 3 vs 4 vs 5 vs 2, 6.

[5]Contrast "Organic" (Org) = 50 ZnO basal with added organic zinc of (0), 40, 60, or 80 mg kg^{-1}; diets 1 vs 4 vs 5 vs 6.

Table 3.10. Main effect of environment on ambient and animal thermal data at AM and PM timepoints (LSMEANS).

Environment:	Thermoneutral	Heat Stress		SEM		Main Effect, *P*-value
n=	48	48				
Ambient,[1] °C						
Pre-HSE1 AM	21.60	24.16		0.153		<0.001
Pre-HSE1 PM	22.02	27.11		0.145		<0.001
HSE1 AM	21.70	29.90		0.081		<0.001
HSE1 PM	21.72	31.99		0.075		<0.001
Post HSE1 AM	21.41	26.54		0.240		<0.001
Post HSE1 PM	21.84	29.33		0.146		<0.001
Pre-HSE2 AM	21.94	27.06		0.107		<0.001
HSE2 AM	20.89	28.76		0.229		<0.001
HSE2 PM	21.31	31.43		0.316		<0.001
Post HSE2 AM	20.65	26.58		0.128		<0.001
Post HSE2 PM	21.13	29.57		0.090		<0.001
Trunk skin,[1] °C						
Pre-HSE1 AM	33.06	34.03		0.164		<0.001
Pre-HSE1 PM	33.43	35.55		0.149		<0.001
HSE1 AM	32.97	36.05		0.184		<0.001
HSE1 PM	33.24	37.02		0.185		<0.001
Post HSE1 AM	32.67	34.59		0.220		<0.001
Post HSE1 PM	33.18	35.98		0.160		<0.001
Pre-HSE2 AM	33.20	34.92		0.197		<0.001
HSE2 AM	32.27	35.32		0.140		<0.001
HSE2 PM	32.91	36.26		0.174		<0.001
Post HSE2 AM	32.04	34.16		0.171		<0.001
Post HSE2 PM	32.55	35.56		0.156		<0.001
Ear skin,[1] °C						
Pre-HSE1 AM	32.79	34.73		0.305		<0.001
Pre-HSE1 PM	34.01	36.80		0.265		0.001
HSE1 AM	32.30	36.87		0.299		<0.001
HSE1 PM	33.57	37.98		0.323		<0.001
Post HSE1 AM	31.66	35.04		0.553		<0.001
Post HSE1 PM	33.42	36.81		0.317		<0.001
Pre-HSE2 AM	33.86	35.67		0.306		<0.001
HSE2 AM	31.43	36.14		0.297		<0.001
HSE2 PM	33.19	37.18		0.229		<0.001
Post HSE2 AM	30.51	34.58		0.346		<0.001
Post HSE2 PM	32.37	36.61		0.300		<0.001
Internal core,[1] °C						
Pre-HSE1 AM	39.71	39.52		0.058		0.018
Pre-HSE1 PM	39.74	39.75		0.060		0.910
HSE1 AM	39.63	39.70		0.065		0.425
HSE1 PM	39.64	40.09		0.063		<0.001
Post HSE1 AM	39.64	39.44		0.077		0.063
Post HSE1 PM	39.71	39.69		0.081		0.870
Pre-HSE2 AM	39.40	39.62		0.056		0.001
HSE2 AM	39.40	39.66		0.067		0.001
HSE2 PM	39.46	39.91		0.049		<0.001
Post HSE2 AM	39.34	39.53		0.065		0.013
Post HSE2 PM	39.46	39.77		0.056		<0.001

[1]Temperatures recorded at timepoints during the acclimation heating period (day 18 – 21; pre-HSE1), first acute heat event (day 21 – 24; HSE1), until vaginal thermometer removal on day 26 (day 24 – 26; post-HSE1), after vaginal thermometer insertion the afternoon of day 41 until the start of the second acute heat event (day 41 – 42; pre-HSE2), second acute heat event (day 42 – 45; HSE2), and until vaginal thermometer removal on day 48 (day 45 – 48 post-HSE2).

Table 3.11. Effect of feeding varying zinc level and source across thermal environments on ambient and animal thermal data at AM and PM timepoints (LSMEANS).

	Diet: [1]	1	2	3	4	5	6		SEM
	n=	16	16	16	16	16	16		
Ambient, [2] °C	Pre-HSE1 AM	22.83	22.93	22.87	22.84	22.93	22.89		0.165
	Pre-HSE1 PM	24.58	24.55	24.56	24.56	24.61	24.55		0.156
	HSE1 AM	25.74	25.80	25.80	25.77	25.78	25.89		0.132
	HSE1 PM	26.87	26.85	26.86	26.83	26.88	26.85		0.121
	Post HSE1 AM	23.95	23.96	23.98	23.98	24.00	24.00		0.273
	Post HSE1 PM	25.61	25.63	25.57	25.57	25.53	25.61		0.171
	Pre-HSE2 AM	24.52	24.45	24.55	24.48	24.53	24.48		0.127
	HSE2 AM	24.81	24.89	24.82	24.80	24.81	24.83		0.261
	HSE2 PM	26.35	26.38	26.38	26.35	26.40	26.35		0.355
	Post HSE2 AM	23.61	23.61	23.63	23.58	23.63	23.63		0.157
	Post HSE2 PM	25.35	25.34	25.35	25.34	25.34	25.35		0.124
Trunk skin, [2] °C	Pre-HSE1 AM	33.27	33.35	33.90	33.37	33.54	33.83		0.244
	Pre-HSE1 PM	34.23	34.59	34.64	34.56	34.32	34.59		0.224
	HSE1 AM	34.52	34.25	34.55	34.39	34.44	34.91		0.267
	HSE1 PM	35.09	35.28	35.23	34.94	34.98	35.28		0.250
	Post HSE1 AM	33.49	33.62	34.02	33.35	33.38	33.94		0.294
	Post HSE1 PM	34.14	34.69	34.86	34.45	34.43	34.93		0.220
	Pre-HSE2 AM	33.89	33.88	34.07	34.11	34.38	34.05		0.290
	HSE2 AM	34.03	33.68	33.91	33.61	33.93	33.61		0.226
	HSE2 PM	34.57	34.65	34.62	34.56	34.48	34.61		0.256
	Post HSE2 AM	32.92	32.76	33.00	33.12	33.32	33.48		0.258
	Post HSE2 PM	34.13	34.23	34.01	33.98	33.89	34.12		0.257
Ear skin, [2] °C	Pre-HSE1 AM	34.19	32.89	34.34	34.00	34.08	33.06		0.520
	Pre-HSE1 PM	35.67	35.56	35.51	35.98	34.74	34.98		0.402
	HSE1 AM	34.38	34.45	34.85	34.63	34.74	34.45		0.459
	HSE1 PM	36.11	35.89	35.67	36.13	34.81	36.06		0.415
	Post HSE1 AM	33.93	32.60	33.61	33.83	32.11	34.00		0.759
	Post HSE1 PM	35.13	35.41	35.42	35.06	34.42	35.26		0.464
	Pre-HSE2 AM	34.84	34.18	34.73	35.09	34.92	34.82		0.455
	HSE2 AM	34.66	33.21	33.46	33.24	34.13	33.99		0.505
	HSE2 PM	35.25	34.92	35.09	35.78	35.14	34.93		0.379
	Post HSE2 AM	33.30	31.63	31.94	32.84	32.58	32.99		0.588
	Post HSE2 PM	34.63	34.63	34.59	35.05	33.73	34.29		0.431
Internal core, [2] °C	Pre-HSE1 AM	39.53	39.49	39.65	39.65	39.68	39.67		0.114
	Pre-HSE1 PM	39.66	39.73	39.69	39.80	39.74	39.83		0.121
	HSE1 AM	39.55	39.57	39.70	39.63	39.69	39.86		0.127
	HSE1 PM	39.80	39.90	39.77	39.93	39.83	39.98		0.122
	Post HSE1 AM	39.46	39.33	39.79	39.58	39.59	39.48		0.163
	Post HSE1 PM	39.56	39.51	39.87	39.72	39.79	39.75		0.129
	Pre-HSE2 AM	39.43	39.49	39.44	39.48	39.53	39.69		0.091
	HSE2 AM	39.44	39.48	39.52	39.55	39.53	39.65		0.104
	HSE2 PM	39.61	39.62	39.76	39.61	39.74	39.76		0.090
	Post HSE2 AM	39.42	39.36	39.45	39.42	39.57	39.40		0.102
	Post HSE2 PM	39.63	39.51	39.65	39.61	39.73	39.56		0.100

[1]Diet 1: 50 mg kg^{-1} ZnO; Diet 2: 130 mg kg^{-1} ZnO; Diet 3: 50 mg kg^{-1} organic Zn from Availa®Zn; Diet 4) 50 mg kg^{-1} ZnO + 40 mg kg^{-1} organic Zn; Diet 5: 50 mg kg^{-1} ZnO + 60 mg kg^{-1} organic Zn; and Diet 6: 50 mg kg^{-1} ZnO + 80 mg kg^{-1} organic Zn.

[2]Temperatures recorded at timepoints during the acclimation heating period (day 18 – 21; pre-HSE1), first acute heat event (day 21 – 24; HSE1), until vaginal thermometer removal on day 26 (day 24 – 26; post-HSE1), after vaginal thermometer insertion the afternoon of day 41 until the start of the second acute heat event (day 41 – 42; pre-HSE2), second acute heat event (day 42 – 45; HSE2), and until vaginal thermometer removal on day 48 (day 45 – 48 post-HSE2).

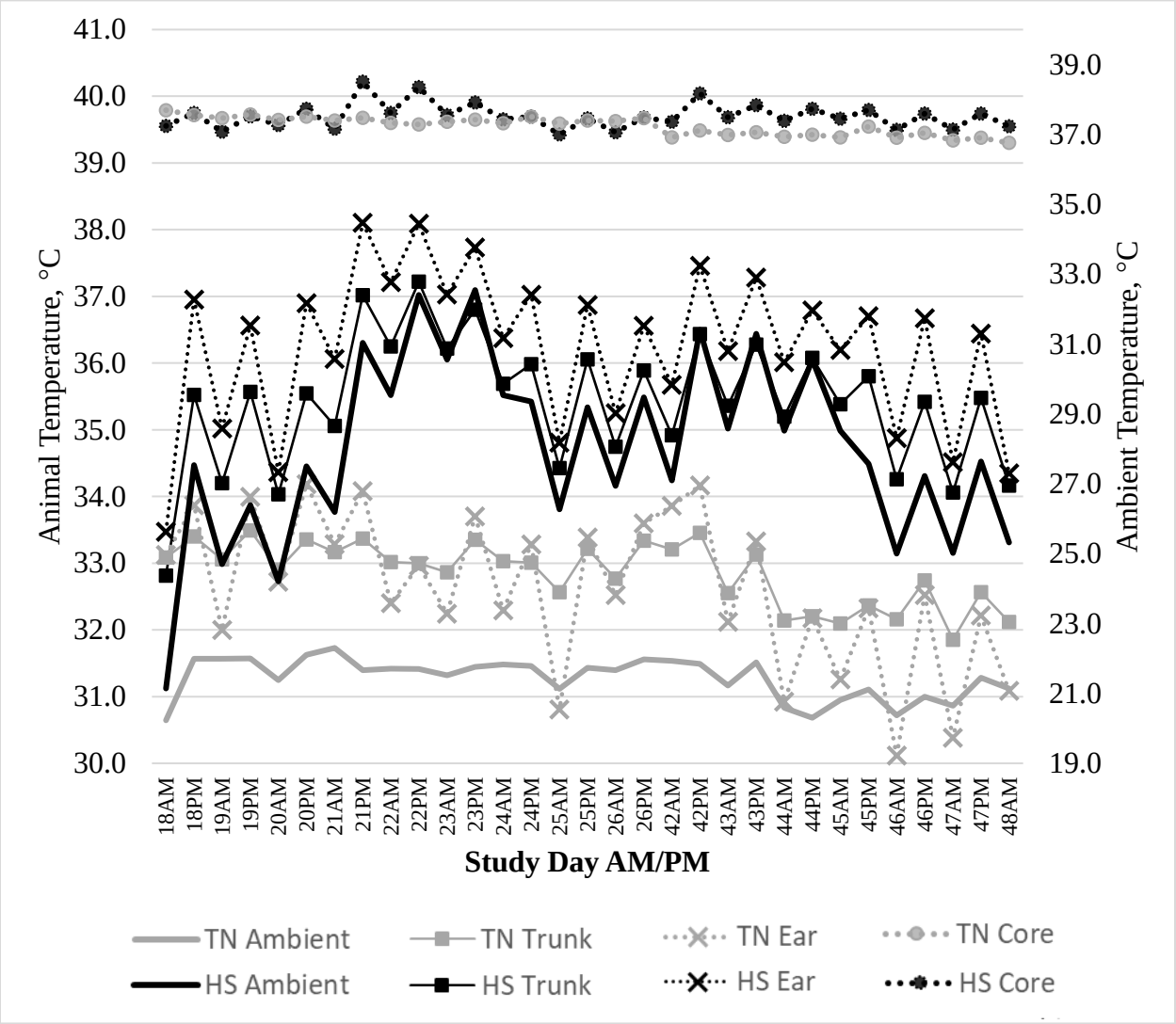

Figure 3.5. Thermal data at AM and PM timepoints shows ambient temperature is greater under heat stress environment [n = 4 rooms heat stress (HS), 4 rooms thermoneutral (TN)] and causes elevation of core body temperature despite ear skin temperatures being consistently greater than trunk skin temperatures among pigs supplemented with varying zinc level & source (n = 96 gilts).

Table 3.12. Thermal Circulation Index (TCI) of 64-gilt subset housed under thermoneutral (TN) or chronic, cyclic heat stressing (HS) conditions.

Environment:	TN	HS		SEM		HS vs. TN, *P* =
n=	*32*	*32*				
TCI[1]						
Pre-HSE1 AM[2]	1.78	2.07		0.102		0.031
Pre-HSE1 PM[3]	1.83	2.22		0.104		0.011
HSE1 AM[3]	1.73	2.11		0.164		0.091
HSE1 PM[3]	1.94	1.90		0.158		0.823
Post HSE1 AM[2]	1.64	2.02		0.121		0.036
Post HSE1 PM[2]	1.82	1.82		0.101		0.984
Pre-HSE2 AM[3]	1.89	1.66		0.120		0.117
HSE2 AM[3]	1.70	1.77		0.108		0.613
HSE2 PM[3]	1.86	1.67		0.115		0.120
Post HSE2 AM[3]	1.48	1.67		0.116		0.147
Post HSE2 PM[3]	1.76	1.62		0.280		0.411

[1]Thermal circulation index: $[T_{trunk\ skin} - T_{ambient}] \div [T_{core} - T_{trunk\ skin}]$ (Kingma et al., 2014).
[2]Overall treatment effect, $0.10 \geq P \geq 0.05$.
[3]Overall treatment effect, $P \geq 0.10$.

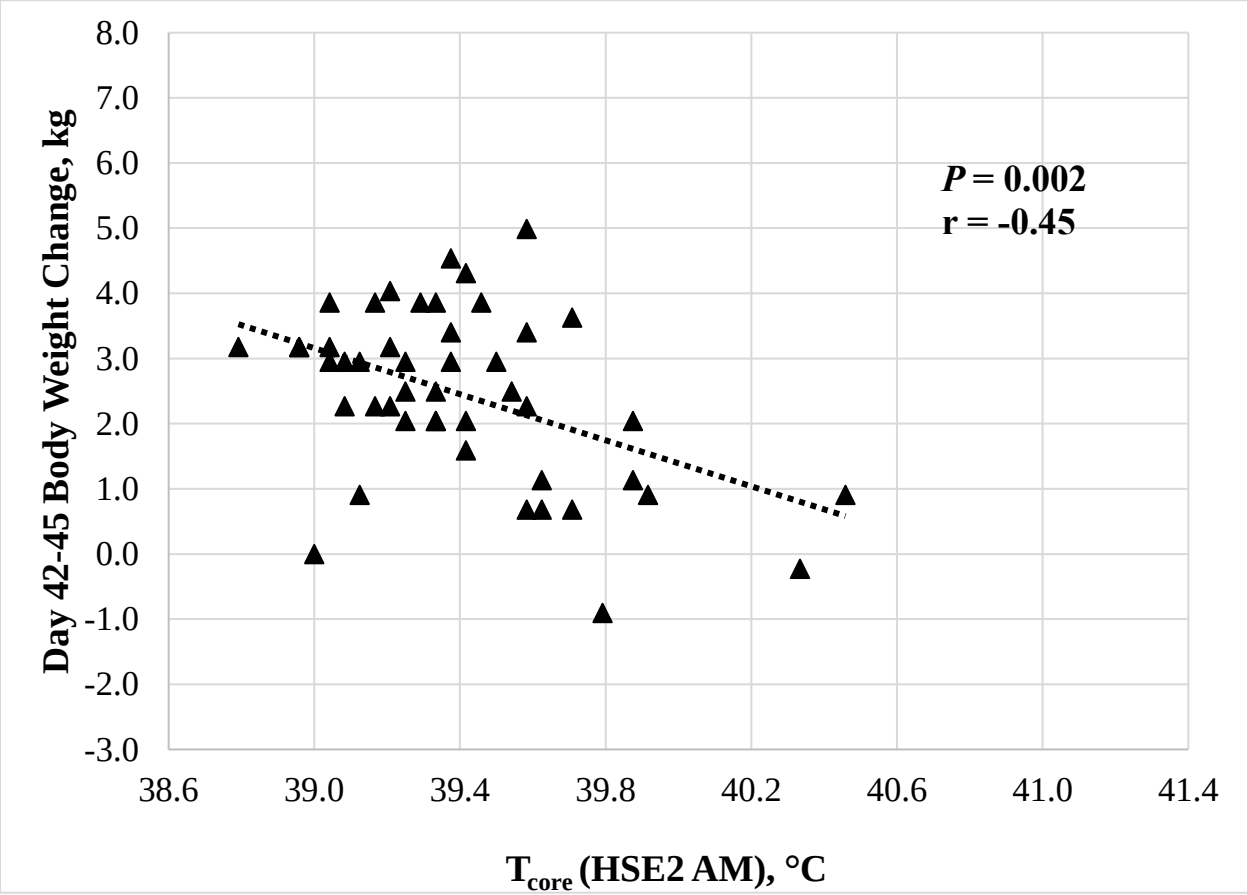

Figure 3.6. Correlation between body weight change under thermoneutral conditions (TN) and core body temperature (T_{core}) on days 42 – 45 (HSE2) for TN gilts (n = 48).

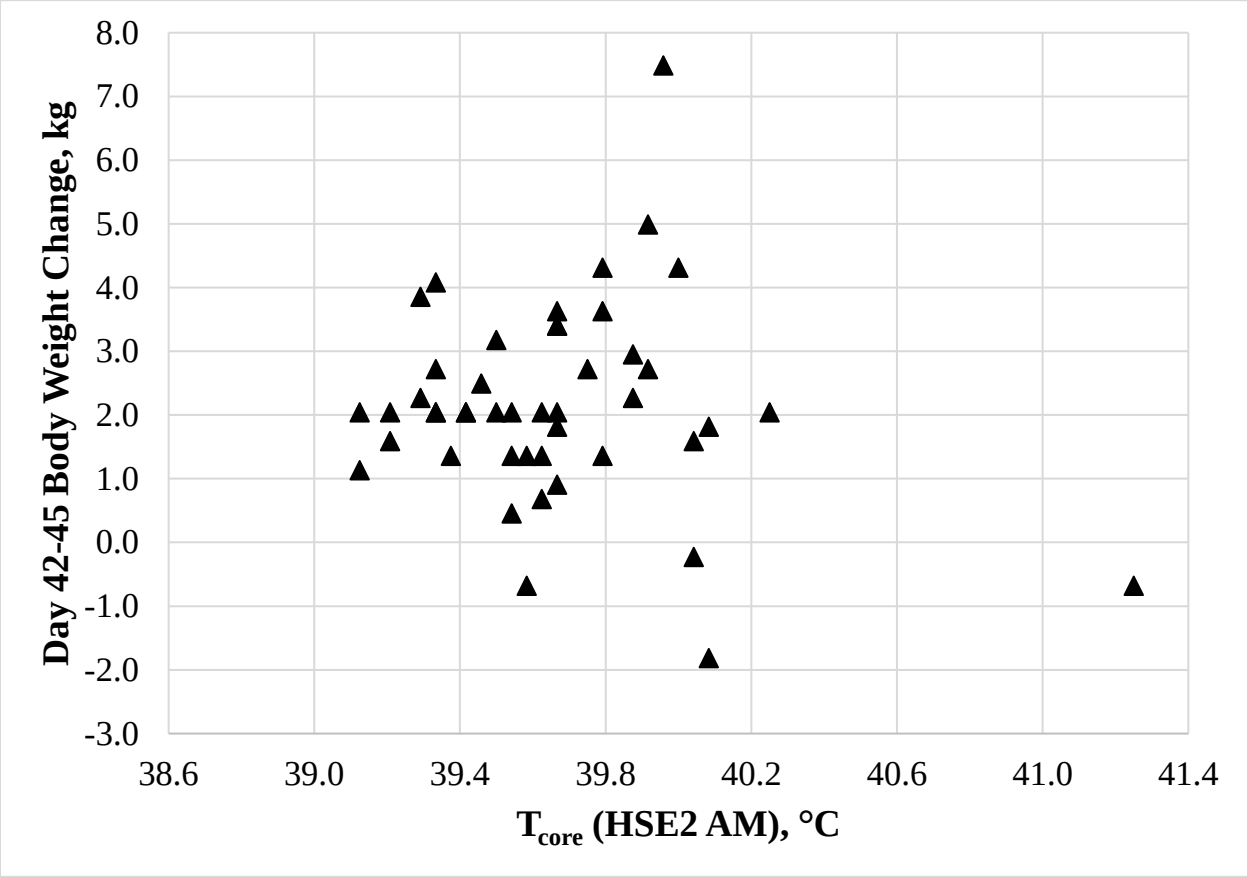

Figure 3.7. No correlation existed between body weight change under heat stress (HS) conditions and core body temperature (T_{core}) on days 42 - 45 (HSE2) for HS gilts (n = 48).

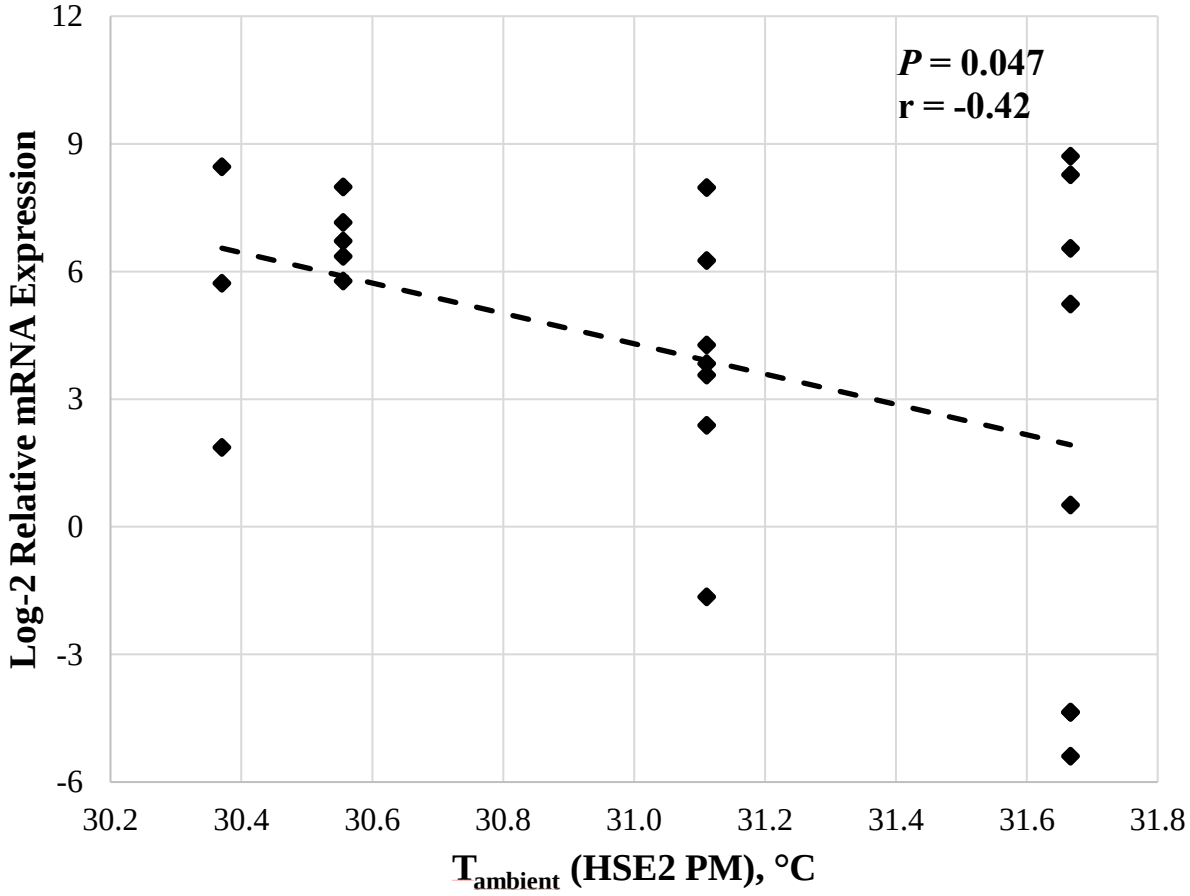

Figure 3.8. Correlation between jejunal HSP27 gene expression and ambient temperature recorded at the PM timepoint on days 42 – 45 (HSE2) for heat stressed gilts of the subset.

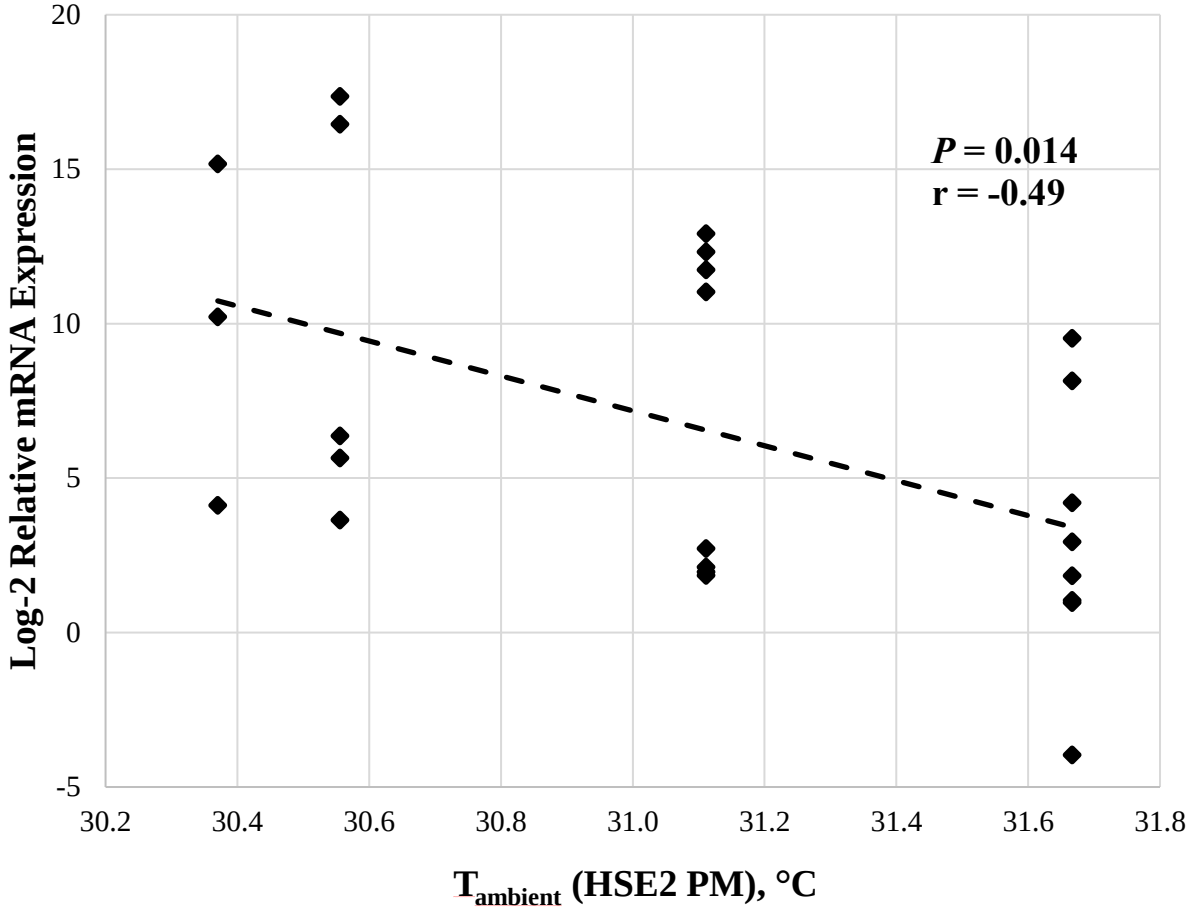

Figure 3.9. Correlation between jejunal HSP90 gene expression and ambient temperature recorded at the PM timepoint on days 42 – 45 (HSE2) for heat stressed gilts of the subset.

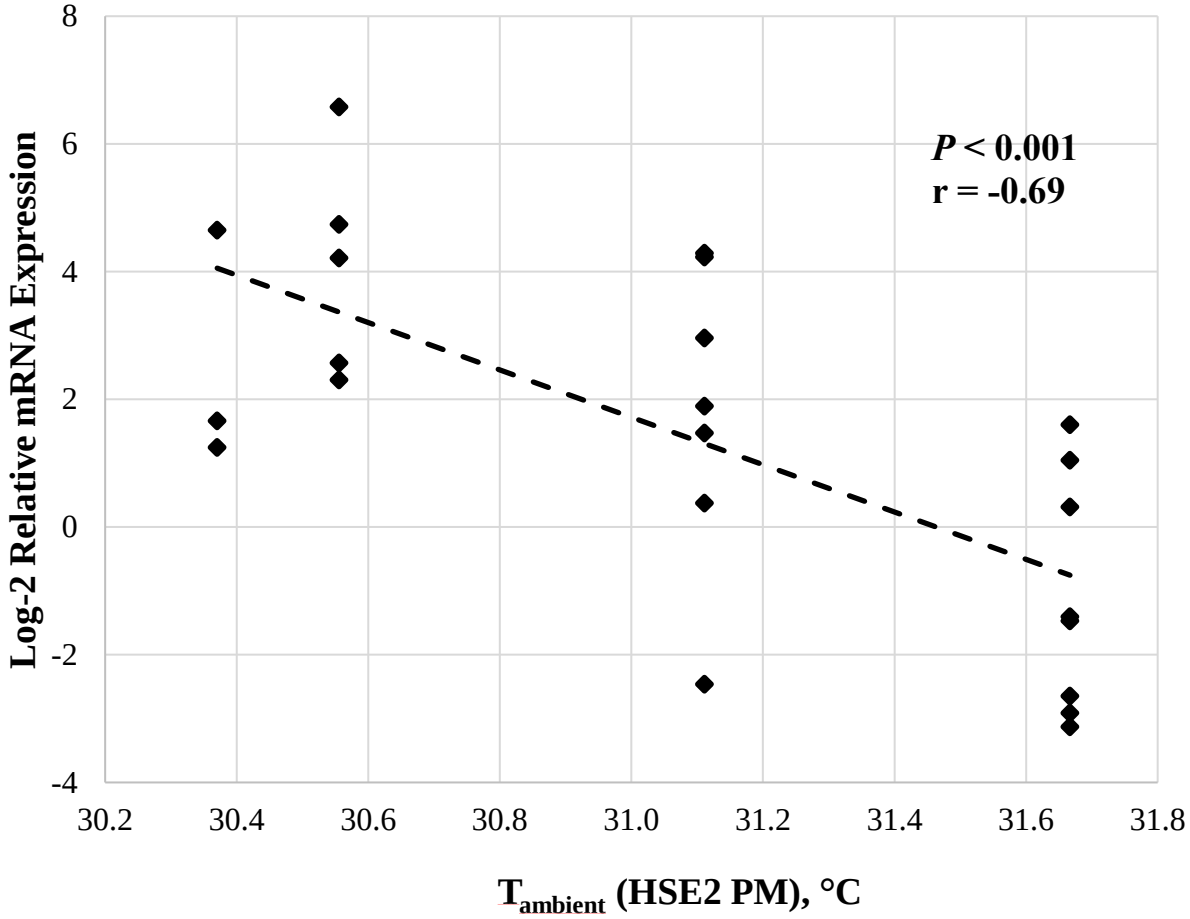

Figure 3.10. Correlation between jejunal occludin gene expression and ambient temperature recorded at the PM timepoint on days 42 – 45 (HSE2) for heat stressed gilts of the subset.

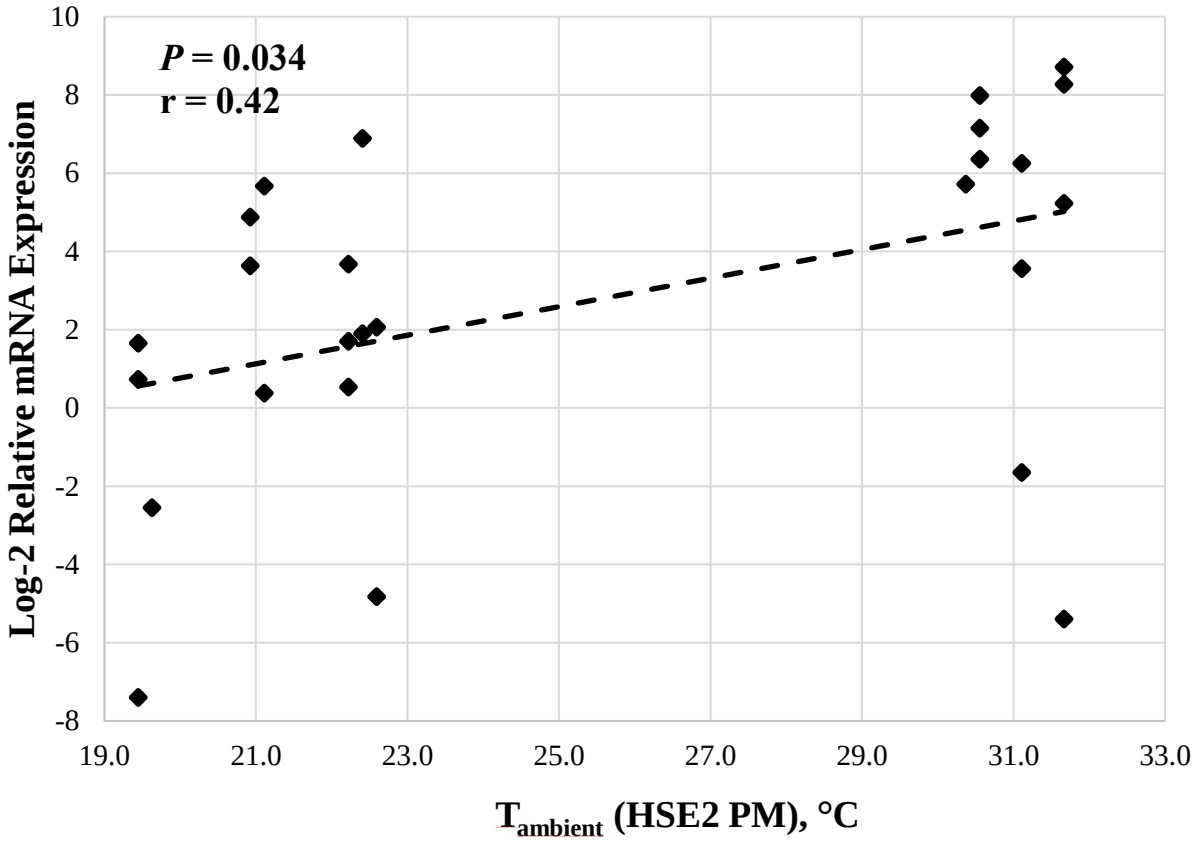

Figure 3.11. Correlation between jejunal HSP27 gene expression and ambient temperature recorded at the PM timepoint on days 42 – 45 (HSE2) for gilts of the subset supplemented with an organic Zn source.

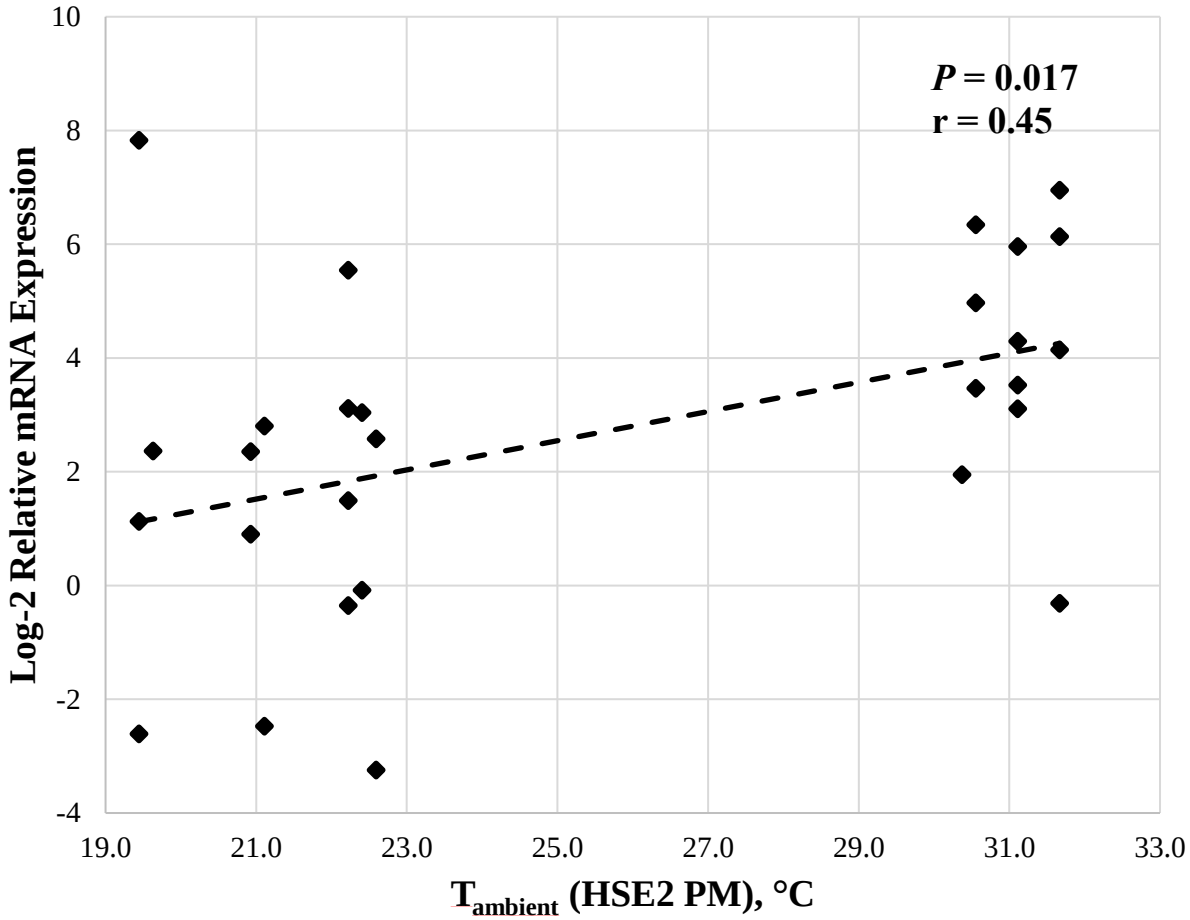

Figure 3.12. Correlation between jejunal mucin-2 gene expression and ambient temperature recorded at the PM timepoint on days 42 – 45 (HSE2) for gilts of the subset supplemented with an organic Zn source.

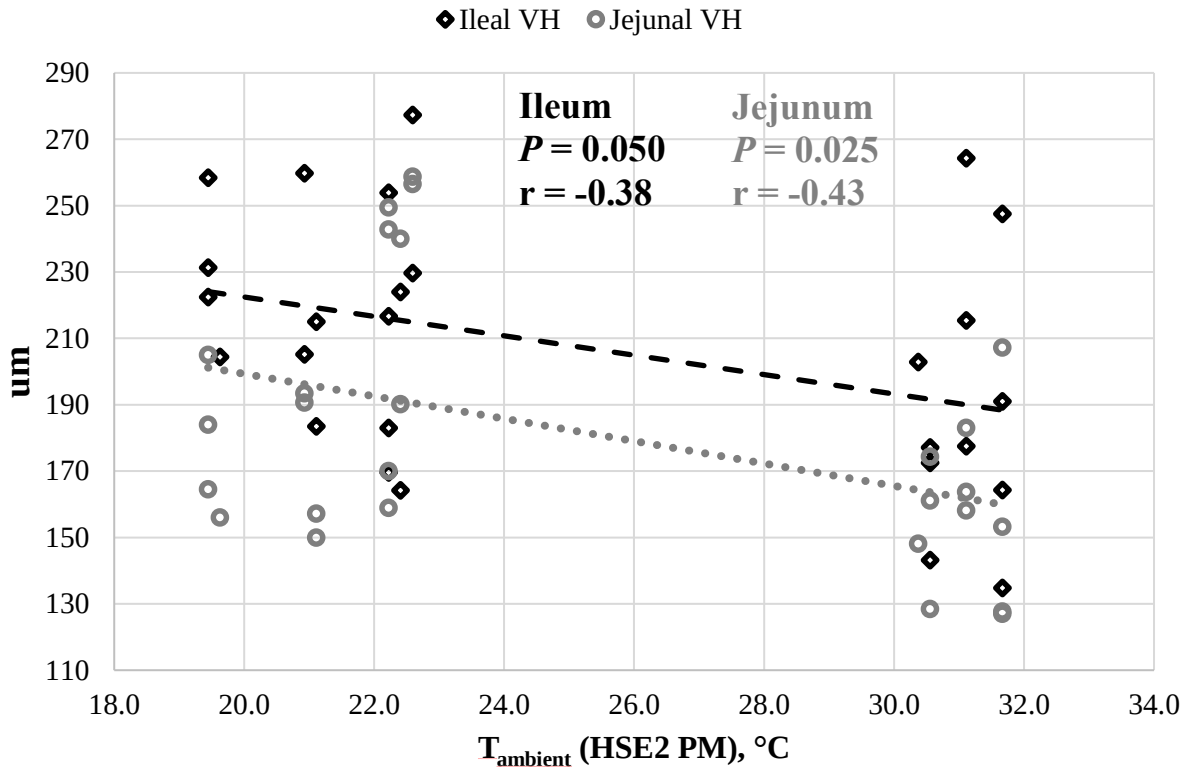

Figure 3.13. Correlation between villus height and ambient temperature recorded at the PM timepoint on days 42 – 45 (HSE2) for gilts of the subset supplemented with an organic Zn source.

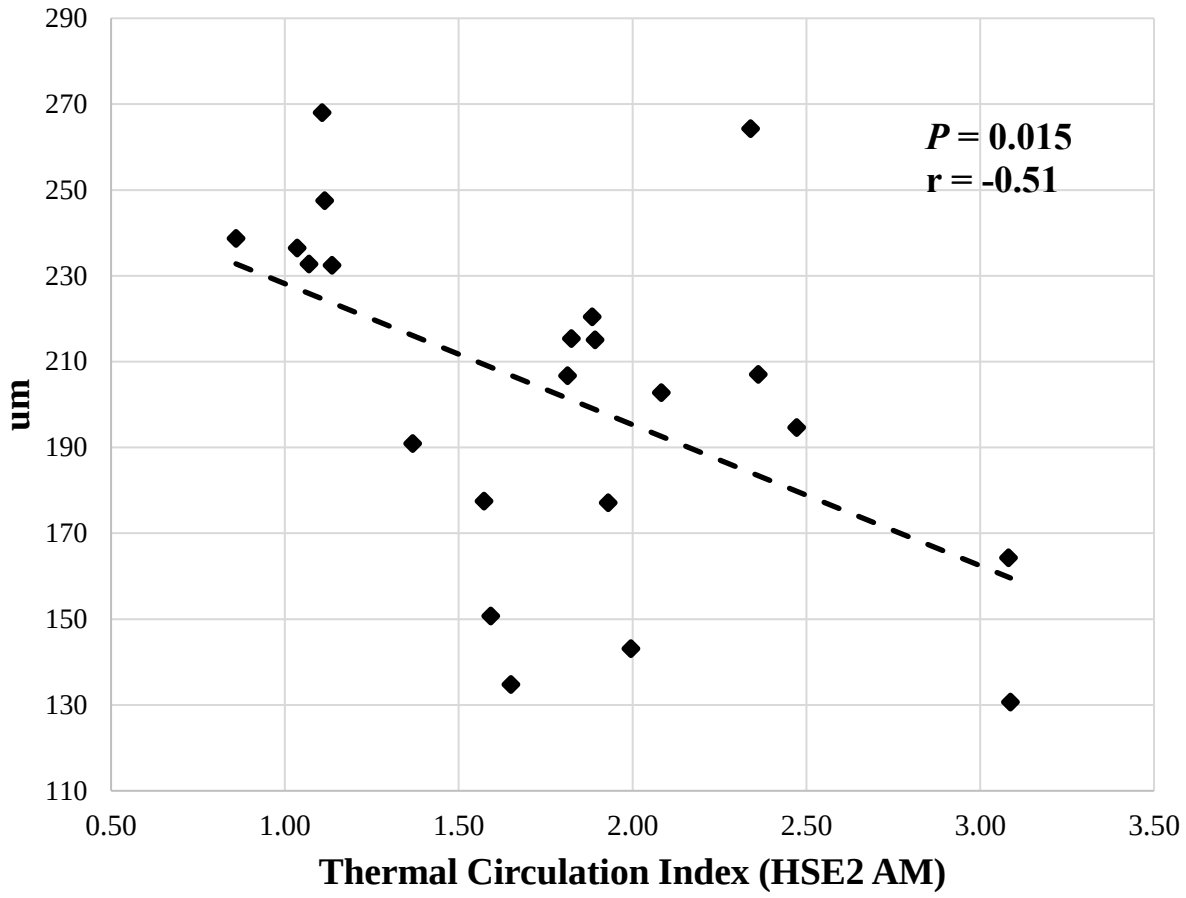

Figure 3.14. Correlation between ileal villus height and Thermal Circulation Index measured at AM timepoint on days 42 – 45 (HSE2) for heat stressed gilts of the subset.

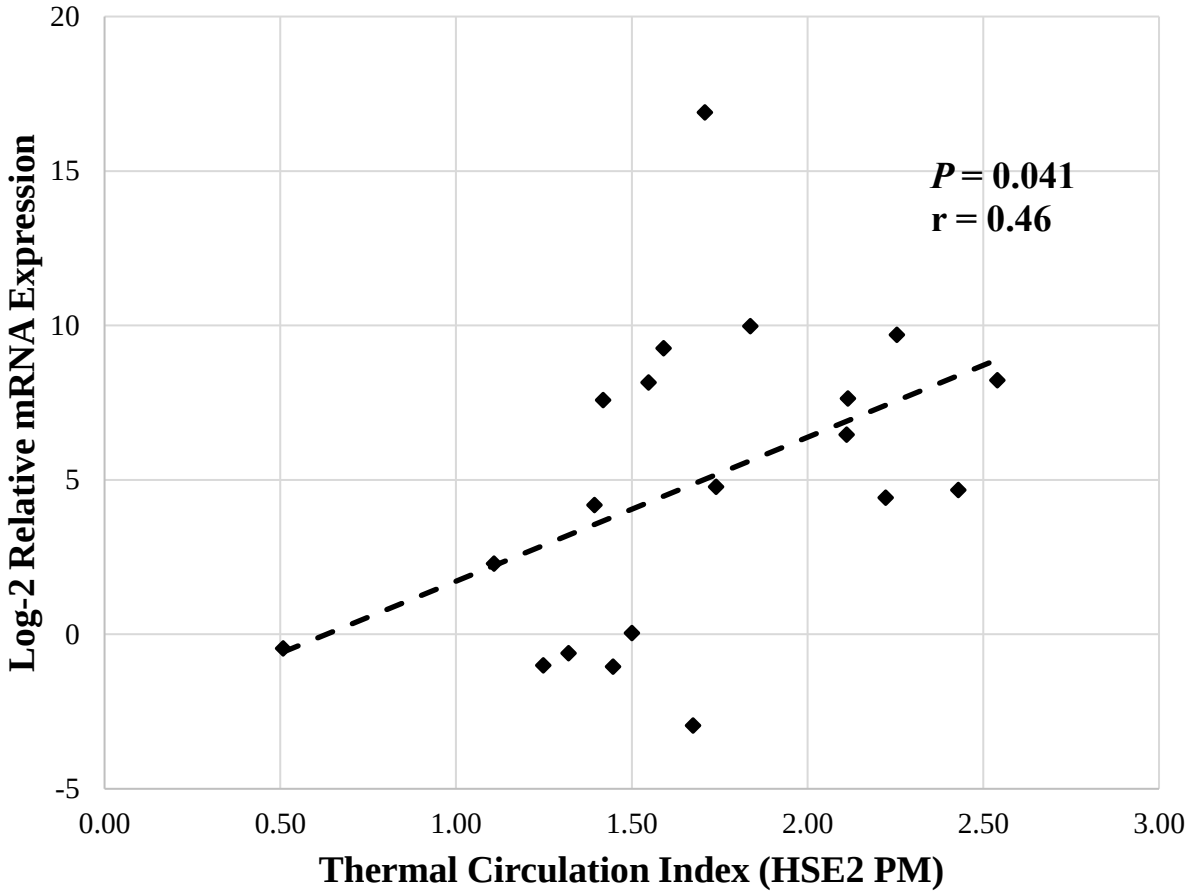

Figure 3.15. Correlation between jejunal HSP70 gene expression and Thermal Circulation Index measured at the PM timepoint on days 42 – 45 (HSE2) for heat stressed gilts of the subset.

CHAPTER 4. AGRICULTURAL STUDENT PERCEPTIONS OF CAREER SUCCESS FACTORS: RANKING ATTRIBUTES OF COLLEGIATE EXPERIENCES

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4.1 Abstract

There is an ever-imminent need for career-ready graduates from collegiate agriculture programs. Generational shifts in attitudes and background experiences of agricultural students challenge educators to maintain curricular programming which establishes a successful career trajectory for students. Students studying animal science and/or agricultural economics were surveyed to understand their perception of how collegiate curricular, co-curricular, and extracurricular experiences (coursework, club participation, relevant work, international experience, advising/mentoring, college life, and professional networking) contribute to their anticipated career success. A best-worst scaling experiment was used to force respondents to make tradeoffs between the collegiate experience attributes in a manner designed to be free of scale biases. Responses were related back to additional demographical and experience/perception characteristics of respondents through various approaches. Based on their responses, students solely in a pre-veterinary Animal Science curriculum represented a particularly interesting category of students regarding their beliefs and reported experiences. Students indicated relevant work experience was overwhelmingly the most critical of the 7 factors they were asked to evaluate. Further research should investigate possible disconnects between student perceptions and reality in higher education.

Keywords: agricultural student; career success; work experience; pre-veterinary; financial stress

4.2 Introduction

There is a projected shortage of U.S. college graduates with bachelor's degrees to fill job openings within the related industries of agriculture, food, environment and natural resources (Goecker et al., 2015). Projected growth in domestic meat production and exportation indicates a critical need for college graduates with a career interest in one of the largest sectors of U.S. agriculture, the meat and poultry industry, according to the North American Meat Institute (2017). Certainly, one of the goals of agricultural higher education is effective workforce preparation and as such, has been deemed a research priority by several institutions (T. G. Roberts et al., 2016).

Designers of an effective collegiate curriculum must consider the knowledge gaps existing between first year students and expected competencies of program graduates. Background experiences of agricultural students have changed over time as students now predominantly hail from urban upbringings and a low percentage have enrolled in high school agriculture courses (Swan and De Lay, 2014). The student body is predominantly female, and among animal sciences, there is prevalent interest in companion animals; the most common career objective is veterinary medicine, and a shrinking number of students have career interests in food animal production (Lyvers Peffer, 2011; Swan and De Lay, 2014).

Educational opportunities and priorities are also changing, thereby requiring the use of co-curricular experiences to enhance student learning. High impact learning initiatives are supported at the university, college, and departmental levels, but when it comes to the perceived value of these experiences, differences in perception among agricultural educators may be biased by the number of years of teaching experience (Murphrey et al., 2016). Since perceptions vary among individuals, understanding student perception of the relative value of common college experience

attributes is critical to meeting student expectations and/or countering misbeliefs among educators and employers.

The undergraduate curriculum is increasingly tasked with the development of noncognitive competencies of students. The highest ranking competencies of graduates valued by employers are noncognitive, even to the exclusion of major-relevant skills and technical knowledge for some employers (Finch et al., 2013; Gillespie and Bampasidou, 2018; National Association of Colleges and Employers [NACE], 2017). Consequently, few employers consider college transcripts helpful predictors of job candidate success (Hart Research Associates, 2015) and although employers traditionally rely upon grade point average (GPA) as a screening factor and/or potential job qualification (Henrich, 2016; NACE, 2017), employers may be losing confidence in the importance of GPA (Pearce, 2017). Regardless of whether students are acutely aware of these shifting employer attitudes, a myriad of experiences extraneous to coursework vie for time in the student's schedule.

Study abroad outcomes are generally hailed as beneficial for agricultural students and can make candidates more attractive to potential employers (Harder et al., 2015; White, 2016). However, incongruity exists among faculty perceptions of the curricular importance and prioritization of study abroad for preparation of globally competent graduates (Rampold et al., 2018). This divergence of opinion might be partially attributable to the variability in duration of study abroad experiences and student outcomes (Gaia, 2015). Yet regardless of the perceived value, student financial concerns might present a barrier to pursuing international experience since collegiate funding status appears to influence student intentions to study abroad (Salisbury et al., 2011). Consequently, considerable effort has been focused on documenting study abroad impact and expanding study abroad opportunities for students while less effort devoted to understanding

how agricultural students perceive their career success being relatively benefited by international experience.

Employers in animal science and related industries ranked internships, relevant work experience, and general employment during college among the most important experiences potential employees should have (Robinson and Mulvaney, 2018). Internships can serve as an avenue for employment offers by the sponsoring organization (Lyvers Peffer, 2012). Unsurprisingly then, work experience and internships are linked to career success factors such as expedited post-graduation job placement, employment in a role consistent with one's college major, and larger starting salary (Blau et al., 2014, 2017). However, students may or may not perceive these benefits if they are engaging in part-time work during college for financial reasons, a circumstance which students believe encumbers their involvement in collegiate activities and academic achievement (Furr and Elling, 2000).

Student engagement in extracurricular activities is also impactful to career preparation. Employers value extracurricular activity involvement at a similar level to high GPA and regard leadership experience more highly than either; over 70% of employers evaluate college graduates for leadership skills (NACE, 2017). However, students have demonstrated overvaluing group or club activities and internship participation while undervaluing farm work experiences compared to post-graduates now employed in agribusiness (Radhakrishna and Bruening, 1994).

The need for formal academic advising arose as collegiate programs became more discipline specific and student diversity increased (Himes & Schulenberg, 2016). Presently, advising has greater potential to impact students since agriculture programs are recruiting more diverse students with less familiarity with agriculture (Setterbo et al., 2017). However, perceptions of academic advising can differ by student gender, particularly regarding career and internship

opportunities (Suvedi et al., 2015). Fouad et al. (2006) found that although students voice a need for more information on occupations as well as on stages of the career development process, students appear to seek career advice from professional advisors only as much as they do from other sources such as parents, family members, friends, and peers, and slightly more frequently than from professors. Despite being aware of campus resources, students choose to underutilize professional counseling services.

College living environment has considerable implication for learning and career outcomes. Integration of coursework and college life through living/learning communities or Freshmen Interest Groups has been shown to improve the graduation rate among agricultural students (Purdie et al., 2007). Students who do not live on campus are less engaged especially in university-based career and professional development opportunities (Blau et al., 2014; Kuh et al., 2001). Conversely, campus life provides opportunities for stronger community and mentoring connections such as those derived from participation in Greek letter organizations (Hale, 2015).

Networking and mentoring relationship opportunities have been identified as valuable curriculum components by both students and agriculture industry stakeholders (Curtis and Mahon, 2010; Trexler et al., 2006). Networking opportunities such as career fairs give students crucial exposure to tangible career opportunities and allow them to establish lines of communication with potential employers (Payne and Sumter, 2005). Another engagement opportunity is Student Professional Organization involvement, which has been shown to be correlated with securing employment consistent with one's major upon graduation (Blau et al., 2017). Despite the majority of professional jobs being obtained through networking and networking's impact on career advancement in terms of salary and promotion, students often do not fully appreciate the value of networking (de Janasz and Forret, 2008).

4.3 Theoretical Framework

Commonly used techniques to ascertain rankings or preferences, including Likert scale questions and forced ranking of attributes, are relatively simple and flexible across various subject matters. However, Likert scale questions allow respondents to rate all attributes or factors equally, without tradeoffs. Forced ranking questions elicit tradeoffs but can be complex and difficult for respondents. Best-worst scaling (BWS) has advantages over rating methods, because it forces respondents to make tradeoffs between attributes and is free of scale biases (Lusk and Briggeman, 2009; Wolf and Tonsor, 2013). Best-worse scaling is rooted in random utility theory in that respondents make choices within the model to maximize their utility (Scarpa et al., 2012). The presentation of every possible combination of attributes to respondents would require many choice sets (questions) and would likely result in survey fatigue, which is known to negatively impact responses (Galesic and Bosnjak, 2009). Therefore, a partial factorial designed using the SAS %MkrtBSize macro with a block design efficiency criterion of 100 was employed (SAS, 2018). The particular experimental design chosen included showing three attributes to respondents per choice set from which to choose their most-important/least-important attribute. Each attribute appeared seven times, and seven choice sets were required (SAS, 2018). Employing data from a designed BWS experiment to prioritize attributes involves identification of the most important and least important attributes from the BWS experiment to determine each attribute's location along the continuum. For attribute *j*, the location on the scale of most important to least important attribute is represented by λ_j. How important a respondent views a particular attribute, for respondent *i* is:

$$I_{ij} = \lambda_j + \varepsilon_{ij} \quad (1)$$

where ε_{ij} represents a random error term. For respondent i, the probability that he/she chooses attribute j as most important and attribute k as least important is the probability that the difference between I_{ij} and I_{ik} is greater than all potential differences available from the choices presented to each respondent. Assuming the error term is independently and identically distributed type I extreme value, the probability of choosing any most important-least important combination takes the multinomial logit form (Lusk and Briggeman, 2009) of:

$$Prob(j = best \cap k = worst) = \frac{e^{\lambda_j - \lambda_k}}{\sum_{l=1}^{J}\sum_{m=1}^{J} e^{\lambda_l - \lambda_m - J}}. \quad (2)$$

The parameter λ_j, estimated using maximum-likelihood estimation, represents how important attribute j is relative to the attribute that is least important. An attribute must be normalized to zero in order to prevent multicollinearity, which is caused by the dummy variable trap (Lusk and Briggeman, 2009).

4.4 Purpose and Objectives

The purpose of this study was to gain insight on the perceptions undergraduate agricultural students have of various collegiate factors with respect to their relative contributions to one's personal career success. Understanding students' views of how various college experience attributes contribute to career success will provide educators, administrators, and employers awareness of attitudes which may be contradictory to or corroborate stakeholder value beliefs. Consequently, the study employed known methodology, rooted in theory surrounding prioritization and relative ranking, to attributes contributing to students' own successes. Specific objectives were to:

1) determine the undergraduate agricultural student's ranking of the relative importance of seven different attributes of the college experience to his/her intended career success utilizing BWS;

2) investigate how student experiences are related to various demographical characteristics with special attention to college funding, career interest, and motivation for career path; and

3) establish how the relative perception of collegiate factors contributing to career success varies among agricultural students according to personal characteristics and experiences.

4.5 Methods

4.5.1 Survey Instrument and Data Collection

A survey was administered in the spring semester of 2018 to undergraduate students within the two largest departments based on undergraduate enrollment numbers within the Purdue University College of Agriculture: Agricultural Economics (AGEC) and Animal Sciences (ANSC). The 51-question survey was made available to students from February 20, 2018 to March 9, 2018, in both electronic (Qualtrics®; Provo, Utah) and nine-page paper booklet formats available through the respective departments' advising offices. Survey responses from 487 students were obtained via the electronic (n=462) and paper booklet (n=25) formats. All responses were voluntary and collected anonymously. Students were not offered extra credit of any kind to participate, although course instructors in both departments did allow announcements by researchers that the data collection effort was taking place. Data collection procedures were approved by the Purdue Institutional Review Board (IRB) Human Research Protection Program (Protocol No. 1801020154).

The survey instrument began with a BWS experimental design to elicit rankings of key student success factors. This task was accomplished by presenting the student with a series of choice sets (questions) where they were tasked with choosing the most (best) and least (worst) important attribute included in that choice set (Figure 4.1). Students were presented with seven unique choice sets; each was configured as a combination of three of the seven attributes using

SAS (v9.4, SAS Institute Inc., Cary, NC) to optimize positional frequencies of each of the attributes across the seven choice set questions.

In this particular experimental design, students were asked to select what they believed was the most important and least important attribute contributing towards their future career success. Attributes were briefly defined for students and included: coursework, club participation, relevant work experience, international experience, advising/mentoring, college life, and professional networking. After completing the BWS experiment, information on student gender, age, race, current and youth living environment, engagement in animal/agricultural activities, academics, career aspirations, and collegiate experiences was sought. Respondents were also asked to characterize 27 factors according to the level of influence each had on their decision to pursue their intended career, self-report their financial stress and funding means for college, and indicate their academic performance level.

4.5.2 Data Analysis

Summary statistics, including means and frequencies of responses, for both the whole sample as well as subsamples of interest (i.e. by department or by key identified demographics of interest) were developed. Cross-tabulations of student responses were evaluated by chi-square analysis (z-test, unadjusted; 95% confidence interval) using IBM Statistical Package for the Social Sciences (SPSS 24) software.

The student's choices of the most important and least important attributes from the BWS experiment were used to determine each attribute's location along the continuum from most to least important attribute. The BWS experiment was modeled in NLOGIT 6.0 using two distinct methods for incorporating student heterogeneity of preferences, a random parameters logit (RPL) and a latent class model (LCM). The RPL model allows for continuous heterogeneity amongst

individuals and the individual-specific parameter estimates were used to calculate individual-specific preference shares. Parameter estimates are not intuitive to interpret, thus shares of preferences were calculated following Wolf and Tonsor (2013) and must sum to one across the seven attributes:

$$share_j = \frac{e^{\lambda_j}}{\sum_{k=1}^{J} e^{\lambda_k}} \quad (3)$$

The Krinsky-Robb method was used to determine confidence intervals for the result of the RPL preference shares (Krinsky and Robb, 1986). Overlapping confidence intervals method was used to determine if the sizes of the preference shares were statistically different from one-another within the RPL model results; it is acknowledged that comparing 95% confidence intervals and examining overlap is more conservative than the standard method of significance testing (Schenker & Gentleman, 2001).

Within the LCM, people have homogenous preferences within a class, and heterogeneous preferences across classes (Boxall and Adamowicz, 2002). The latent class model classifies individuals into a class (*S*), based on their attitudes and preferences. Parameters for each class are simultaneously estimated, and individual respondents are assigned to an unobserved latent class (Swait, 1994). Given the respondent belongs to a specific latent class, denoted as *s*, the conditional probability of choices is:

$$(Prob(j = best \cap k = worst)|s) = \frac{e^{\lambda_{js} - \lambda_{ks}}}{\sum_{l=1}^{J} \sum_{m=1}^{J} e^{\lambda_{ls} - \lambda_{ms} - J}} \quad (4)$$

where the λ_{js} and λ_{ks} parameters are class specific (Ouma et al., 2007). The probability of membership in the unobservable classes has the multinomial logit form:

$$Prob(s) = \frac{e^{(\theta_s Z)}}{\sum_{s=1}^{S} e^{\theta_s Z k}} \quad (5)$$

where Z is a set of hypothesized drivers of class membership, the s^{th} parameter vector is normalized to zero for model identification, and θ_s characterizes the impact the drivers have on class membership (Ouma et al., 2007). Preference shares for each latent class were also calculated using equation 3.

4.5.3 Linking Preferences with Respondent Demographics

Although the RPL provides information regarding respondent preferences and relative ranking of the attributes, it does not include the relationship between preferences and the respondent demographics. Therefore, Pearson correlations (Pearson, 1894; Pearson, 1895) were used to evaluate the relationship between RPL preference shares and respondent demographics.

The LCM estimates the probability of class membership for each individual respondent. Post-estimation, individual respondents were assigned to the class in which they had the highest probability of membership following Lai (2017). Respondents who did not have at least a 50% difference in probability between their first and second highest probability classes were not included in the demographic analysis. A total of n=316 respondents were included for the LCM demographic analysis.

4.6 Results and Discussion

4.6.1 Student Sample Demographics

Respondents (n=487) reported studying a major in the department of Agricultural Economics, a major in the department of Animal Sciences, or majors in both (n=96). The majority of respondents was female, between the ages of 18 and 21, and described themselves as White/Caucasian (Table 1). Fall 2017 undergraduate enrollment in the Purdue University College of Agriculture consisted of a female majority (59%) and respondent ethnicity mirrored percentages

of Asian (2.9%), Black/African American (1.8%), and Hispanic/Latino (4.0%) students enrolled in the college (Purdue University Data Digest, 2017).

Across class level, freshmen students comprised the largest percent of survey participants, while sophomores, juniors, and seniors participated at similar rates. Of those reporting majoring in ANSC, 45% selected pre-veterinary curriculum as their sole current area of study; this subset of ANSC students, hereby PREVET, predominantly consisted of female freshmen. Respondents studying in the department of AGEC were most often majoring in agribusiness (42.9%) or undecided (32.9%).

Rural backgrounds were reported by the majority of AGEC students and by the majority of ANSC students not exclusively in the pre-veterinary curriculum track (non-PREVET; Table 2). PREVET students reported an urban background environment at twice the rate of non-PREVET students. PREVET students were distinguished by having the most youth engagement with companion animals but consistently had the lowest involvement in agronomic production, meat animal husbandry, and animal product related activities relative to non-PREVET and AGEC students. The highest percentage of 1st generation college attendance was reported among PREVET students as well as the most international exposure (data not shown), possibly associated with this subset of students also having the most ethnic diversity.

Regarding coursework, over 84% of respondents self-reported typically obtaining A or B grades. Across major and academic year, B grades were reported more frequently than A grades with the sole exception of PREVET students. Non-PREVET students were more similar to AGEC students than PREVET students who reported the greatest frequencies of having earned high school advanced placement (AP) but the fewest community college credits. The majority of

students indicated they perceived departmental course content (82.3%), timing and scheduling (71.0%), and class size (68.2%) to be generally sufficient.

4.6.2 BWS Model Results for Student Perception of Collegiate Experience Attributes

The results of the RPL model showed that respondents ranked relevant work experience as the most important attribute when considering their anticipated career success (Table 3). There was a tie between coursework and professional networking for second in terms of size of preference share. Advising/mentorship ranked 3rd, and club participation ranked 4th with preference shares of less than 5% each. Statistically, there was a tie for last (5th) between international experience and college life.

Based on BIC and class size, a four class model was determined to be the most appropriate for the LCM. The first class had a large preference share for relevant work experience and coursework so it was named "Work and Study" (Table 4.3 and Figure 4.2). The second class had a large preference share for relevant work experience and professional networking so the class was named "Outside the Classroom." The third class was characterized by its large preference share for relevant work experience, coursework, and professional networking so it was named "Classic Collegiate" in consideration of the traditional collegiate marketing themes of scholarship, workforce preparation, student clubs, professional networking, and study abroad. Class 4 had fairly equal preference shares for all attributes, so it was named "Everything/Nothing" because it is indeterminable whether the respondents had high preference for all of the attributes or simply did not care for any of the attributes.

4.6.3 Relationships Between Respondent Career Aspirations, Demographics, and Experiences

When asked directly, 75.3% of students claimed professional networking was necessary for helping them obtain their desired jobs. The majority of AGEC students anticipated working in the agriculture/animal industry post-graduation but only about half as many ANSC students planned to do the same (Table 4.4). Rather, the majority of ANSC majors planned to continue their education post-baccalaureate by either attending veterinary school or graduate school. When asked to indicate career area(s) they were preparing for, over 50% of all ANSC students indicated they were targeting veterinary medicine; for AGEC students, the most common career area was agriculture business management. Meat industry sales/service was reported as an area of career interest by a small but similar share of AGEC and ANSC respondents and over 20% of all respondents expressed interest in a career involving live animal husbandry.

Across academic grade level, there was a reduction in the percentage of students planning to attend veterinary school post-graduation (Table 4.5). Correspondingly, the percent of freshmen intending to enter the agriculture/animal industry post-graduation was smaller than the percent of sophomores, juniors, and seniors who had the same intention. We surmise this phenomenon to be the consequence of veterinary career minded freshmen changing their career intention during their undergraduate education. Results also showed that the percentage of students with high career commitment who indicated a veterinary medicine career aspiration was greater than the percentage of those with low career commitment who aspired to a veterinary career. This may indicate a strong allegiance to the veterinary profession among PREVET curriculum students and corroborates with their self-reported lack of major change. Alternatively, since PREVET students were more often freshman, the lack of major change could simply be due to shorter time in college at the time of data collection.

As discussed by Kogan and colleagues, advisers are challenged to assist pre-veterinary students with a preparatory curriculum that gives students a successful dossier for the highly competitive veterinary school admission process (Kogan et al., 2011). Simultaneously, advisers must also help students be cognizant of the value in preparing themselves for an alternate career path. The percent of ANSC graduates actually involved in some aspect of the veterinary profession is far less than the percentage of interested students (Dodson and Benson, 2010) and many students experience a delay between undergraduate graduation and veterinary school (Kedrowicz et al., 2015). Alignment of student initiative with a curriculum which facilitates noncognitive outcomes without neglecting technical training could be augmented by further investigation of the impact of high career commitment on narrowness of approach when electing co-curricular activities.

Encouragingly, 71.5% of respondents indicated they considered animal product study a necessary component of their college education and 28% of them reported they had completed an ANSC meat science course at Purdue University. Analysis showed that students who indicated they had taken a meat science course and/or meat science laboratory were more interested in a meats career area of focus than those who had not. Moreover, the percent of freshmen who were highly influenced in their career choice by college courses was smaller than the percent of seniors also highly influenced by college courses, which is perhaps not surprising as freshmen respondents were only in their second semester (Table 4.5). Including a variety of applied courses in curriculum coursework may be a strategy to stimulate career interests and goals among students with limited background exposure to agriculture (Swan and De Lay, 2014). With broadened interests and identification of alternative opportunities, graduates might also be advantaged in navigating short term labor market entry barriers within a particular field such as veterinary medicine.

To investigate factors influencing the career choices of students enrolled in the agricultural curriculum, respondents were asked to categorize certain factors for having a "high," "low," or "non-applicable" level of influence on their career choice. Passion for career area was the factor most frequently considered highly influential among all students and AGEC students (Table 4.6), with desire to work with animals only slightly surpassing it as the top influence among ANSC students. Passion for career area and financial considerations have previously been reported as highly influential factors (Hegerfeld-Baker et al., 2015), yet respondents appeared to be relatively uninfluenced by monetary consideration as a relatively low number of respondents reported starting salary was a high influence. Notably, almost all respondents who said starting salary was a high influence also indicated that salary potential was a highly influential factor (Table 4.5). Participation in youth programs such as 4-H and FFA was not directly assessed in the present study; however, the majority of AGEG students and over 25% of ANSC PREVET considered 4-H involvement highly influential to their career aspiration (Table 4.6). This finding is notable because previous involvement in 4-H/FFA programming seems to be declining among collegiate agricultural students (Russell, 1993; Swan and De Lay, 2014) despite the success of 4-H programs in preparing participants for the workforce (Nash and Sant, 2005; Rusk et al., 2002). The percent of freshmen who were highly influenced in their career choice by volunteer work was greater than the percent of sophomores and juniors, which may be an artifact of the high interest in veterinary medicine among freshmen. Veterinary school admissions staff value animal exposure experiences, which the American Veterinary Medical Association (2018) suggests can be acquired through volunteer work at animal shelters or veterinary clinics.

A considerable majority (85.5%) of PREVET students considered animal handling and management a necessary component of their college education while 63.5% of AGEC students

thought it necessary. Concerning the delivery method of animal handling and management education, 82.5% of respondents indicated they thought hands-on experience the best format (over lecture-based discussion). Given the option of obtaining skills important for success through either computer simulation or via on-farm visits, almost all students (82.8%) chose on-farm experiences. Intensifying pressures on university resources have driven educators towards increasingly creative mechanisms to expose students to on-farm and out-of-classroom experiences. It is recognized that funding and staffing challenges have strained many Land Grant Universities in these endeavors (R. M. Roberts et al., 2009). Consequently, conserving the ability to provide students with on-farm animal experiences while developing industry partnerships to provide students with networking opportunities, exposure to various work and career options, and employer values in settings outside the classroom may be a desirable approach**.**

Relevant work experience was perceived by students as highly beneficial for future career success, and many students reported working a college job either related or unrelated to their major. As expected due to compounding experiences, the number of students having obtained work experience increased with academic grade level (Table 4.5). Although a large number of both AGEC and non-PREVET students reported relevant work experience, an internship, or both, a smaller number of PREVET students indicated the same (Table 4.7).

Over 71% of upperclassmen reported funding college through working a job. Although a majority of non-PREVET students reported working a job to fund their college experience, a considerably smaller percentage of PREVET students indicated so and only a quarter of PREVET students reported working while school was in session (Table 4.7). The PREVET students had the lowest percent of respondents indicating they were funding college via personal savings, working a job, or scholarship receipt, but the largest percentage of respondents funding college via familial

support. Perhaps this financial situation makes PREVET students less inclined to pursue (paid) internships and relevant work experience than their peers. This is a potentially concerning situation because PREVET students are prone to changing their career intention during their undergraduate years and because of the importance of gaining relevant work experience during this period (Blau et al., 2014, 2017; Robinson and Mulvaney, 2018).

Over 20% of the agricultural students responding to the survey reported participation in at least one collegiate study abroad experience; yet overall, international experience was not highly ranked in the BWS experiment. Further investigation suggested study abroad participation may be related to financial outlook as has been previously observed (Whatley, 2017). The percentage of students studying abroad at least once who indicated high financial stress (4 or 5 on 1 – 5 Likert scale) was smaller than the percent of highly stressed students never studying abroad (Table 4.8). Of students having studied abroad, there were greater percentages of students who were upperclassmen, who reported working a job in college, who were obtaining relevant work experience and/or internship, and a smaller percentage of PREVET students than among students who had never studied abroad. Regarding coursework, of students who had studied abroad there was a larger percent of A-grade students and a smaller percent of B-grade students than among students who had not studied abroad.

Over 55% of respondents indicated they were actively engaged in at least one collegiate club or organization and financial stress did not appear to be associated with involvement in extracurricular activities. Participation in more than one extracurricular activity was greater among freshmen and lower among upperclassmen compared to non-involvement within each grade level (Table 4.8).

However, while only 10.3% of freshmen reported leadership in a club or activity, 45.4% of seniors reported having provided leadership in a club or organization. Of the students involved in more than one extracurricular activity, there was a greater percentage of PREVET students and a smaller percentage of C-grade students than among those students not involved in any extracurriculars (Table 4.8). These observations suggest that although students do not consider extracurricular activities a high contributor to their career success, nevertheless the activities provide opportunities for the development of leadership skills which are highly valued by employers (NACE, 2017).

4.6.4 Linking Perceptions of Success Factors and Respondent Demographics

To inform curriculum development and advising endeavors, correlations were assessed to further establish how perceptions vary among agricultural students according to personal characteristics, values, and experiences. The size of preference share for relevant work experience was negatively correlated with all other shares of preference (Table 4.9). Also, the size of preference share for relevant work experience was positively correlated with a student being an upper classman and negatively correlated with a student living on campus and with a student planning on going to graduate school/veterinary school. Rather, living on campus and planning on going to graduate school/veterinary school were both positively correlated with the coursework share of preference. Corroborating with the cross-tabulation results, being an upper classman was negatively correlated with the share of preference for coursework.

The share of preference for professional networking was positively correlated with that for college life. Not surprisingly, the student believing class content offerings are sufficient, and the student believing the timing/scheduling options of departmental course offerings are sufficient were negatively correlated with the size of preference share for advising/mentorship (data not

shown). However, the student planning on going to graduate school or veterinary school was curiously negatively correlated with the advising/mentorship share of preference (Table 4.9). Furthermore, the student planning on going to graduate school or veterinary school was negatively correlated with the size of preference shares for college life and professional networking.

Club participation share of preference was positively correlated with that for advising/mentorship, international experience, and professional networking; the size of preference share for these latter two were positively correlated with each other (Table 4.9). The size of preference share for professional networking was also positively correlated with the student living on campus. Forty-six percent of respondents indicated they were living on campus in a dormitory/residence hall, yet college life did not rate among the highest preference shares. Of students reporting living in residence halls, an overwhelming percent were ANSC major (Table 4.8). The percentage of residence hall students who reported relevant work and/or internship experience was smaller than the percentages in the other housing arrangements. These observations may be due in part to the housing distribution of lower and upper classmen as percentage of students living in a residence hall consisted of a larger percent freshmen and smaller percent upperclassmen relative to the percentages inhabiting other housing types.

As the level of stress created by paying for college increased, the size of the preference share for international experience decreased ($r = -0.1320$, $p = 0.0079$), consistent with the cross-tabulation findings. Level of financial stress was not correlated to any preference shares other than that for international experience.

When evaluating the demographics of the four identified LCM classes, a high percentage of students who indicated their major was in AGEC were members of Class 2 “Outside the Classroom” (Table 4.10). Students who indicated they were female were more likely to be

members of Class 1 “Work and Study” and Class 2. Seniors were more likely to be members of Class 2 which had a very small preference share for coursework. Given the decreasing focus on coursework as grade level of respondents increased, this class membership is particularly revealing. Respondents who reported earning mostly A’s were more likely to be members of Class 1 and Class 2. While A students probabilistically belonging to Class 1 is expected, membership in Class 2 with the small preference share for coursework is less anticipated.

Students who completed a paid internship were more likely to be members of Class 1 as were students who attended a career fair within the last two years. Respondents with the intention of attending veterinary school or graduate school after graduation had a higher probability of being members of Class 1, which had the highest focus on coursework. If the respondent intends to work for the family’s agricultural business/farm after graduation they were more likely to be members of Class 4 “Everything/Nothing” while those with predominant home environment of rural on a farm/ranch were more likely to be members of Class 2.

4.6.5 Perception of Career Success Factors for Self Versus Others

In the final question of the survey, respondents were asked to choose which two attributes (of the seven BWS attributes) they thought contributed most to their peers’ career success. Their directly stated selection for peers was compared to their top two largest estimated preference shares for themselves, which had been discreetly obtained through BWS. As shown in Figure 4.3, relevant work experience and professional networking were more frequently ranked in the top two for self than considered in the top two for others; conversely, club participation, college life, international experience, and advising/mentoring were more frequently considered expedient for others than for self. On an individual respondent basis, the percentage of students who selected at least one of their own top two preference shares as one of the top two factors for others as well (%

match) was over 50% for relevant work experience, coursework, international experience and club participation. Interestingly, the factors that had a high percent match can all be characterized as having a rather objectively tangible, social nature, i.e. could be included on a résumé and serve as an interview point of discussion, while those factors which had the low percent match have rather subjective intangible, personalistic outcomes.

4.7 Conclusions and Recommendations

The surveyed student body is a sample of agricultural students from a large Land Grant agricultural institution. The demographics of these students are predominantly female and nearly one-half are focused on veterinary careers with limited agricultural backgrounds and experiences. Special attention should be given to students enrolled exclusively in the pre-veterinary curriculum due to their unique demographic, tendency to change career intention during their undergraduate years, and lessened proclivity to pursue internships and relevant work experiences. Educators and advisers need to be especially cognizant of novel ways to provide PREVET students the experiences needed for success in their eventual career regardless of whether that is the student's currently intended career. Since the career interest in veterinary medicine is highest among Freshmen and the largest proportion of Freshmen live on-campus, developing college life related activities and opportunities through approaches such as living-learning communities may be an especially valuable strategy.

When asked indirectly, students ranked obtaining relevant work experience more valuable to their career success than even coursework; international experience and college life were deemed least important. However, over 75% of students agreed professional networking was necessary and students were overwhelmingly motivated by passion for their career area. Further analysis of the student demographics illustrates the importance of maintaining extracurricular

activities and club opportunities for students to develop leadership skills, and the importance of continued efforts to make study abroad opportunities more affordable for students.

Educators should beware of the variation that exists among individuals as highlighted by the RPL and latent class modeling. Furthermore, student beliefs for themselves did not closely match their beliefs for peers when their opinion was directly solicited. The degree to which students' career preparation activities are influenced by the expectations their peers have for them warrants further study as this may provide career advisers insight to effectively convey guidance.

The emphasis which students placed on obtaining relevant work experience appears to align with the changing priorities of employers (NACE, 2017; Robinson and Mulvaney, 2018) and is a signal to educators to ensure all students have access to networking and relevant work opportunities. With over 70% of the upperclassmen in the present study already working to fund their college education, departments should support internship or on-campus work opportunities so students can pursue work that is relevant to their anticipated career. Additional research should be conducted to investigate how students are obtaining work experience and what barriers exist, and also address whether student perceptions are realistic and actually predictive of career success. Although, regardless of whether perceptions are predictive of future success, perceptions of students drive their behaviors and allocation of efforts, and thus are valuable to further understand.

Understanding students' perceptions and demographics benefits effective curriculum design. The current findings suggest that coursework can influence career interests so providing young students exposure to a variety of major-specific courses early may assist them in identifying their final career goal and efficiently focusing their career preparation efforts. The responses of the present study also very clearly demonstrate a reduction in the perceived value of coursework among upper classmen. Consequently, structuring the curriculum to facilitate more

internship/relevant work i.e. undergraduate research, and networking activities at this point in the undergraduate program may behoove all parties involved – students, educators, and employers.

More work is also needed to investigate how closely academic administration, industry employers, and student perceptions align or are disconnected. As curriculum designers collaborate with employers regarding competencies and success factors for program graduates, students' perceptions should not be overlooked. This study is a single example of what represents a possible disconnect between student perceptions and reality in higher education. Once recognized, potential gaps and opportunities to rectify or foster accurate student beliefs can inform allocation of scarce stakeholder resources.

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Figure 4.1. The survey began with a best-worst scaling (BWS) experimental design which presented respondents with a series of choice sets. Factor definitions and the seven BWS choice set questions are shown here.

Table 4.1. Demographic Information of Respondents

Demographic variable	**% of all respondents (*N*=487)**	**% of AGEC major (*N*=170)**	**% of ANSC major (*N*=413)**	**% of ANSC PREVET (*N*=186)**	**% of ANSC non-PREVET (*N*=227)**
Gender[a]	***n=462***	***n=163***	***n=395***	***n = 180***	***n = 215***
Female	78.4	62.6	85.1	86.1	84.2
Age[a]	***n=463***	***n=163***	***n=396***	***n=180***	***n=216***
18 - 19	48.2	39.3	51.8	69.4	37.0
20 - 21	39.5	44.2	36.6	25.6	45.8
22 - 23	11.2	14.1	10.6	4.4	15.7
24 +	1.1	2.5	1.0	0.6	1.4
Class[a]	***n=464***	***n=163***	***n=397***	***n=180***	***n=217***
Freshman	37.7	31.3	40.8	57.2	27.2
Sophomore	19.2	19.6	17.9	17.2	18.4
Junior	22.2	22.1	21.9	13.3	29.0
Senior	20.9	27.0	19.4	12.2	25.3
Race[a]	***n=464***	***n=163***	***n=397***	***n=180***	***n=217***
White/Caucasian	90.3	92.0	89.4	85.0	93.1
Asian	3.7	3.7	4.0	5.6	2.8
Black/African American	1.7	1.2	2.0	2.8	1.4
Hispanic/Latino	3.0	2.5	3.3	4.4	2.3
Other/prefer not to answer	1.3	0.6	1.3	2.2	0.5
Residency	***n=487***	***n=170***	***n=413***	***n=186***	***n=227***
In-state student	79.3	83.5	78.7	73.7	82.8
International student	1.6	2.9	1.2	1.6	0.9
Type	***n=487***	***n=170***	***n=413***	***n=186***	***n=227***
Full-time student	90.1	86.5	91.0	92.5	89.9
Changed major at least once	37.6	44.7	37.0	19.4	51.5

[a] Response rate among all students was ≥ 95%.

Table 4.2. Background and Educational Experiences of Respondents

Characteristic	% of all respondents (*N*=487)	% of AGEC major (*N*=170)	% of ANSC major (*N*=413)	% of ANSC PREVET (*N*=186)	% of ANSC non-PREVET (*N*=227)
Background environment[a]	***n=459***	***n=160***	***n=392***	***n=178***	***n=214***
Urban	7.8	8.8	8.2	11.2	5.6
Non-urban	92.2	91.2	91.8	88.8	94.4
Suburban	28.3	21.3	31.1	42.1	22.0
Rural	63.8	70.0	60.7	46.6	72.4
Rural, farm/ranch	38.6	48.8	35.7	23.0	46.3
Rural, non-farm/ranch	25.3	21.3	25.0	23.6	26.2
Youth ag involvement	***n=487***	***n=170***	***n=413***	***n=186***	***n=227***
Agronomic production	34.9	45.3	31.0	21.5	38.8
Animal husbandry					
Companion animals	58.9	45.3	64.2	72.6	57.3
Horses	25.9	22.4	28.8	28.5	29.1
Meat animal	30.6	37.6	28.6	18.8	36.6
Animal products					
Meat processing	13.3	15.9	12.6	7.0	17.2
Non-meat production	26.3	29.4	25.9	21.5	29.5
Husbandry &/or products	73.3	68.8	75.8	76.3	75.3
Education	***n=487***	***n=170***	***n=413***	***n=186***	***n=227***
High school ag courses					
Available	48.9	53.5	46.2	40.9	50.7
Enrolled	44.8	49.4	42.1	33.3	49.3
College 1st generation	23.0	20.6	25.2	26.9	23.8
Typical grades[a]	***n=463***	***n=163***	***n=396***	***n=180***	***n=216***
A's	33.3	32.5	32.1	41.7	24.1
B's	55.5	55.2	56.3	52.2	59.7
C's	10.6	12.3	10.9	6.1	14.8
D's	0.6	0	0.8	0	1.4
Non-university college credit	***n=487***	***n=170***	***n=413***	***n=186***	***n=227***
High school AP	67.1	66.5	68.3	71.0	66.1
Community college	38.8	38.8	39.0	32.8	44.1
Other	9.7	8.8	10.4	14.5	7.0

[a] Response rate among all students was ≥ 94%.

Table 4.3. Analysis of Students' Best-Worst Rankings of Attributes for Career Success. (*n*=405)

	Random Parameters Logit				**Latent Class Model – Coefficients [a]**				**Latent Class Model - Preference shares**			
	Coefficient	Standard deviation	Shares of Preference	Rank[b]	Class 1	Class 2	Class 3	Class 4	Class 1 "Work and Study"	Class 2 "Outside the classroom"	Class 3 "By the Book"	Class 4 "Everything/Nothing
Coursework	-0.279***	1.490***	14%	2	1.252***	-2.377***	0.2824	0.081	34%	3%	20%	20%
	0.098	0.109	[0.121, 0.175]		0.331	0.232	0.212	0.179				
Club participation	-1.967***	0.989***	3%	4	-2.40***	-2.644***	-1.615***	-0.429***	1%	2%	3%	12%
	0.096	0.103	[0.022, 0.032]		0.330	0.257	0.216	0.151				
Relevant work experience	1.090***	1.111***	57%	1	1.677***	0.480***	1.133***	0.145	52%	55%	47%	21%
	0.111	0.137	[0.514, 0.621]		0.236	0.183	0.200	0.151				
International experience	-2.588***	1.624***	1%	5	-3.164***	-3.660***	-0.372*	-0.915***	0%	1%	10%	7%
	0.129	0.128	[0.011, 0.019]		0.391	0.272	0.198	0.178				
Advising/mentorship	-1.496***	0.752***	4%	3	-1.163***	-2.089***	-1.603***	-0.788***	3%	4%	3%	8%
	0.084	0.114	[0.036, 0.051]		0.238	0.226	0.200	0.156				
College life	-2.891***	1.692***	1%	5	-2.886***	-3.796***	-2.707***	-0.296*	1%	1%	1%	14%
	0.133	0.137	[0.008, 0.014]		0.327	0.270	0.271	0.160				
Professional networking	-	-	19%	2	-	-	-	-	10%	34%	15%	18%
	-	-	[0.164, 0.220]		-	-	-	-				
Avg. class probability constant					-0.326	0.420	-1.458**		0.291	0.332	0.209	0.168
Student is pre-vet					0.597	-0.906**	0.876*					
Student is female					0.816*	0.792*	1.545**					

Note. ***indicates significance at 0.001 level, **significance at 0.05 level, *significance at 0.10 level.

[a] To prevent multicollinearity, the category "Professional Networking" was dropped from the model. All coefficients are in reference to this dropped variable.

[b] Ranks were determined by examining overlapping confidence intervals established using the Krinsky-Robb method (1986).

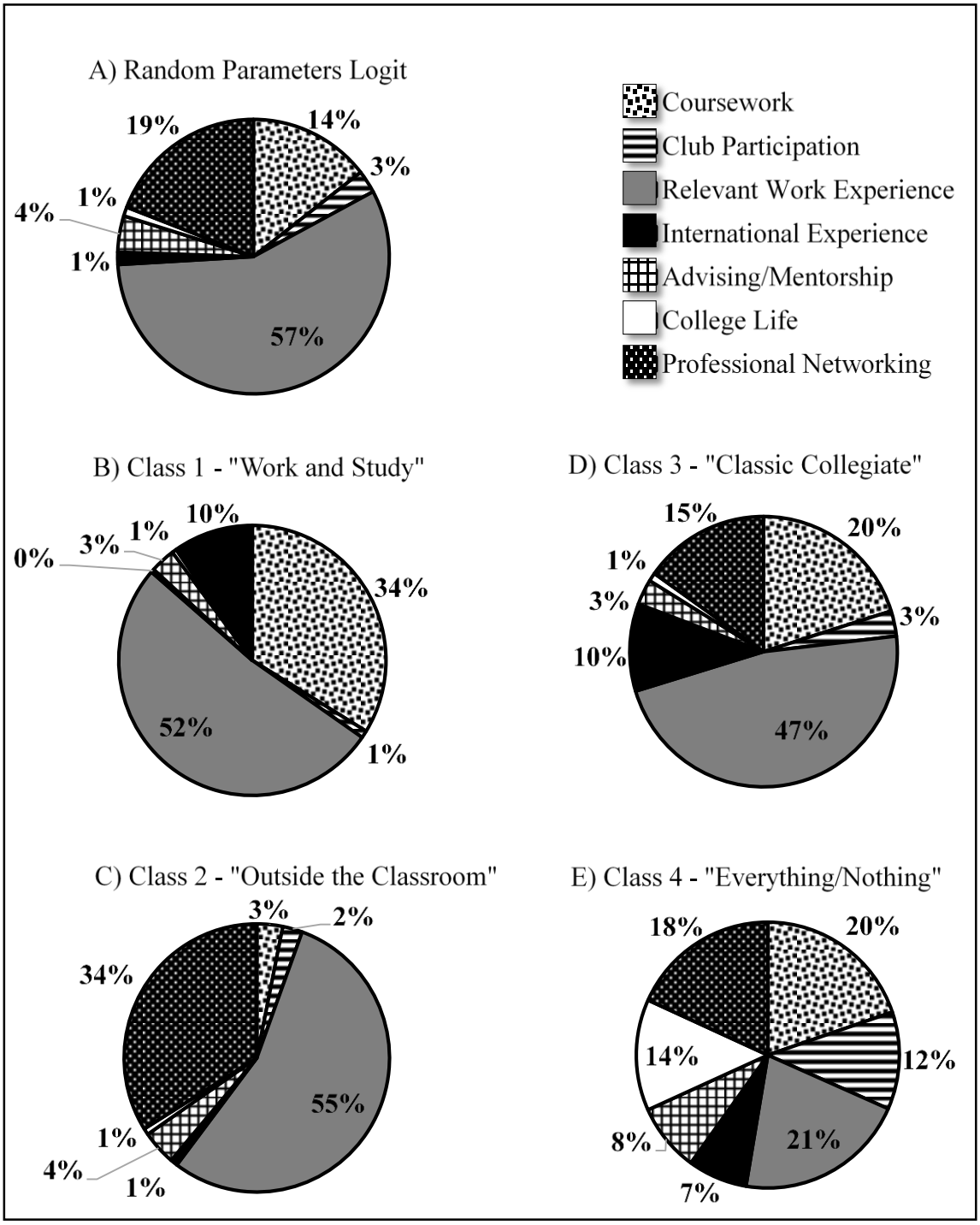

Figure 4.2. Preference shares of attributes students believe are the most important to their career success as determined by (A) Random Parameters Logit, n=405, and by (B – E) Latent Class Model, individual classes, n=405.

Table 4.4. Career Aspirations of Respondents (% of Respondents)

Characteristic	**% of all respondents** ***N=487***	**% of AGEC Major** ***N=170***	**% of ANSC Major** ***N=413***	**% of Fresh.** ***N=174***	**% of Soph.** ***N=89***	**% of Juniors** ***N=103***	**% of Seniors** ***N=97***
Post-grad plan (Response rate of all students = 95%)							
Work	*n=464*	*n=163*	*n=397*	*n=174*	*n=89*	*n=103*	*n=97*
Work in ag or animal industry	43.8	68.1	35.8	28.0	48.3	54.4	56.7
Family operation	5.4	12.9	2.5	2.9	7.9	7.8	5.2
Ag/animal sci. industry	38.4	55.2	33.2	25.1	40.4	46.6	51.5
Work in non-ag industry	3.4	3.7	3.3	2.3	2.2	6.8	3.1
Continue education	50.4	26.4	58.4	67.4	47.2	36.9	37.1
Veterinary school	39.4	19.0	46.1	53.7	41.6	28.2	23.7
Graduate school	11.0	7.4	12.3	13.7	5.6	8.7	13.4
Other	2.4	1.8	2.5	2.3	2.2	1.9	3.1
Career intention(s)							
Veterinary medicine	43.7	22.9	51.3	60.3	48.3	34.0	30.9
Nutritional research/sales/service	17.2	14.1	20.1	17.2	22.5	15.5	18.6
Genetics research/sales/service	13.8	15.3	14.8	15.5	11.2	12.6	17.5
Animal health research/sales	27.1	22.4	31.2	25.3	22.5	32.0	36.1
Agronomic research/sale/service	7.8	21.2	2.9	5.7	13.5	6.8	9.3
Crop farm production	8.0	20.0	2.7	6.3	12.4	5.8	11.3
Live animal husbandry	21.4	18.8	24.2	16.1	18.0	34.0	25.8
Farm business management	13.6	28.2	9.7	9.8	18.0	16.5	16.5
Ag business management	19.9	44.7	11.6	12.6	24.7	21.4	32.0
Dairy/egg industry sales/service	7.0	7.6	7.3	3.4	9.0	9.7	10.3
Meat industry sales/service	9.0	8.2	9.7	8.6	5.6	15.5	8.2
Academia	5.3	4.1	5.3	2.3	6.7	7.8	8.2
Other	11.1	11.2	10.2	7.5	11.2	16.5	14.4
Undecided	8.8	5.9	9.9	11.5	6.7	11.7	5.2

Table 4.5. Cross Tabulations of Respondent Career Salary Influence and Class to Career Goal, Other Factors of Influence, and Work (% of Respondents)

	Starting salary influence		Grade level			
	High	**Low**	**Fr.**	**Soph.**	**Jr.**	**Sr.**
Career choice	***n=415***		***n=463***			
Ag industry inc. family ag	42.8_{a}	44.8_{a}	28.2_{a}	48.3_{b}	54.4_{b}	56.7_{b}
Non-ag industry/other	6.2_{a}	4.4_{a}	4.6_{a}	4.5_{a}	8.7_{a}	6.2_{a}
Graduate school	9.7_{a}	11.9_{a}	13.8_{a}	5.6_{b}	8.7_{ab}	13.4_{ab}
Veterinary medicine	41.4_{a}	38.9_{a}	53.4_{a}	41.6_{ab}	28.2_{bc}	23.7_{c}
Continue education	***n=413***		***n=463***			
No	49.0_{a}	49.3_{a}	32.8_{a}	52.8_{b}	63.1_{b}	62.9_{b}
Yes	51.0_{a}	50.7_{a}	67.2_{a}	47.7_{b}	36.9_{b}	37.1_{b}
% of respondents reporting:[a]						
Career highly influenced by:	***n=359 - 411***		***n=385 - 435***			
College course(s)	82.0_{a}	72.9_{b}	69.2_{a}	73.2_{ab}	78.6_{ab}	85.2_{b}
Previous job	69.0_{a}	70.9_{a}	68.8_{a}	71.2_{a}	70.9_{a}	74.4_{a}
Volunteer work	73.9_{a}	63.9_{b}	77.4_{a}	59.3_{b}	60.9_{b}	65.9_{ab}
Starting salary	---	---	35.8_{a}	35.0_{a}	31.2_{a}	37.3_{a}
Salary potential	95.1_{a}	17.2_{b}	46.3_{a}	44.4_{a}	37.5_{a}	48.8_{a}
Parent(s)	79.3_{a}	67.7_{b}	80.1_{a}	71.1_{ab}	60.8_{b}	70.5_{ab}
Desire to work with animals	87.1_{a}	88.3_{a}	92.9_{a}	86.4_{ab}	84.8_{b}	84.9_{b}
Engagement in:	***n=415***		***n=463***			
Work during college	76.6_{a}	75.6_{a}	59.8_{a}	77.5_{c}	92.2_{b}	88.7_{b}
Work, major related	57.9_{a}	63.0_{a}	40.8_{a}	66.3_{b}	75.7_{bc}	79.4_{c}
Work, major unrelated	50.3_{a}	44.8_{a}	36.2_{a}	39.3_{a}	63.1_{b}	59.8_{b}
Related work &/or internship	63.4_{a}	67.8_{a}	43.7_{a}	73.0_{b}	80.6_{bc}	88.7_{c}
Internship, paid	24.1_{a}	20.0_{a}	2.9_{a}	20.2_{b}	31.1_{b}	47.4_{c}
Internship, unpaid	7.6_{a}	12.6_{a}	6.9_{a}	6.7_{a}	10.7_{a}	22.7_{b}
Internship, any	28.3_{a}	30.7_{a}	9.8_{a}	27.0_{b}	37.9_{b}	61.9_{c}
Career fair attendance	89.0_{a}	88.5_{a}	91.4_{a}	87.6_{a}	85.4_{a}	85.6_{a}

Note. For a specific variable, percentages within a row lacking a common subscript significantly differ from each other.

[a] "Reporting" means an affirmation was given; absence of affirmation does not necessarily mean no (responses to questions were not forced).

Table 4.6. Factors Influencing Career Aspirations of Respondents (% of Respondents)

Highly influential factor	**% of all respon-dents (*N*=487)**	**% of AGEC major (*N*=170)**	**% of ANSC major (*N*=413)**	**% of ANSC PREVET (*N*=186)**
High school courses	39.8	35.9	40.7	45.7
High school teacher	41.7	41.2	42.9	41.4
National Honors Society	16.2	18.8	16.9	18.3
Student Council	7.2	7.1	8.2	9.7
College visit	46.6	43.5	48.2	52.7
College courses	66.1	60.0	67.6	68.8
College professor	34.1	34.7	34.6	25.3
4-H involvement	45.6	55.9	43.1	26.3
FFA involvement	31.4	38.2	29.3	18.8
Previous job	56.1	57.6	56.7	54.3
Volunteer work	58.5	41.2	65.9	78.0
Previous or current employer	50.7	53.5	50.1	48.4
Friends	49.5	52.9	49.2	51.6
Parents	64.3	65.3	63.4	66.7
Relatives other than parents	48.3	53.5	47.5	46.2
Similarity to parent career	18.5	31.2	14.3	9.7
Starting salary	29.8	32.9	29.5	30.1
Salary potential	38.2	40.0	37.8	43.5
Graduation placement rate	35.7	40.0	33.2	33.9
Job security	49.1	50.6	47.9	52.7
Employment opp. & demand	58.3	60.0	57.9	60.2
Geographic location	46.0	52.9	39.4	44.6
Long-term professional goals	75.2	70.0	76.5	82.8
Competency & proficiency	63.2	58.8	63.9	71.0
Passion for career area	85.4	80.0	86.2	88.7
Desire to work with animals	78.9	60.6	87.9	89.2
Desire to work with plants	13.6	30.0	8.2	3.8

Note. Students were asked to rank each factor of influence as having a low influence, high influence, or as non-applicable to their career choice; for brevity, only the percentages of respondents ranking the factor as a high influence are shown here.

Table 4.7. Financial Security of Respondents (% of Respondents)

Characteristic	**% of all respon-dents (*N*=487)**	**% of AGEC major (*N*=170)**	**% of ANSC major (*N*=413)**	**% of ANSC PREVET (*N*=186)**	**% of ANSC non-PREVET (*N*=227)**
Financial stress					
Level of stress	3.1	2.9	3.2	3.2	3.3
[1 low to 5 hi]	(1.31 SD)	(1.30 SD)	(1.29 SD)	(1.30 SD)	(1.28 SD)
College funding sources					
Parental/family	59.3	55.9	60.0	64.5	56.4
Personal savings	38.6	42.9	36.8	34.4	38.8
Student loan	39.2	34.1	42.4	40.9	43.6
Working a job	61.6	61.8	61.5	54.8	67.0
Work between sessions	53.2	55.3	51.8	47.3	55.5
Work during sessions	33.9	36.5	34.1	25.3	41.4
Part-time	32.6	33.5	32.7	24.2	39.6
Full-time	1.4	2.9	1.7	1.1	2.2
Work during sessions + loan	17.9	20.0	18.4	12.9	22.9
Financial aid/grant	49.5	47.1	51.1	50.0	52.0
Scholarship	67.8	67.6	67.3	64.5	69.6
Scholarships					
Scholarship recipient	71.0	72.4	70.2	70.5	69.9
Need-based scholarship	45.8	47.1	46.2	44.6	47.6
Merit-based scholarship	59.3	62.9	57.1	55.9	58.1
Departmental opportunities to apply					
Sufficient	70.4	75.3	69.2	65.6	72.2
Insufficient	9.4	5.3	10.7	11.8	9.7
Work experience					
Internship	28.7	37.6	24.9	18.8	30.0
Paid	20.7	31.8	16.2	7.0	23.8
Unpaid	10.5	7.6	11.1	14.0	8.8
Work a college job	72.7	78.8	72.6	64.5	79.3
Related to major	58.5	67.6	57.4	50.0	63.4
Unrelated to major	45.4	47.6	45.8	39.8	50.7
Relevant work &/or internship	63.7	72.9	62.5	53.8	69.6
Attended a career fair	83.8	80.0	83.5	81.7	85.0
Attended fair & did internship	25.5	32.9	21.5	14.0	27.8

Table 4.8. Cross-Tabulations of Respondents' Collegiate Activities and Living Environment with Academic and Financial Factors (% of Respondents)

	No. active extracurriculars			Study abroad		Housing			
	0	1	>1	Never	At least 1x	Res. hall	Greek	Apartm't	Other
Gender	***n=461***			***n=451***		***n=457***			
Female	75.0_a	82.7_a	78.1_a	77.5_a	81.1_a	84.3_a	31.4_b	80.2_a	78.1_a
Male	25.0_a	17.3_a	21.9_a	22.5_a	18.9_a	15.7_a	68.6_b	19.8_a	21.9_a
Grade level	***n=463***			***n=453***		***n=459***			
Freshman	23.2_a	46.0_b	46.9_b	45.1_a	11.5_b	65.6_a	17.1_b	5.4_c	33.3_b
Sophomore	20.9_a	13.7_a	22.4_a	20.7_a	13.5_a	19.6_a	14.3_a	21.0_a	12.1_a
Junior	28.2_a	20.9_{ab}	16.3_b	19.6_a	31.3_b	9.8_a	37.1_b	35.9_b	21.2_{ab}
Senior	27.7_a	19.4_{ab}	14.3_b	14.6_a	43.8_b	4.9_a	31.4_b	37.7_b	33.3_b
ANSC major	***n=487***			***n=453***		***n=459***			
No	19.9_a	15.1_{ab}	8.8_b	13.2_a	19.8_a	6.3_a	45.7_c	17.4_b	24.2_{bc}
Yes	80.1_a	84.9_{ab}	91.2_b	86.8_a	80.2_a	93.8_a	54.3_c	82.6_b	75.8_{bc}
ANSC curriculum	***n=413***			***n=387***		***n=392***			
Non pre-vet	62.7_a	52.5_{ab}	47.8_b	51.6_a	66.2_b	38.1_a	68.4_b	73.2_b	80.0_b
Pre-vet	37.3_a	47.5_{ab}	52.2_b	48.4_a	33.8_b	61.9_a	31.6_b	26.8_b	20.0_b
Financial stress level [a]	***n=458***			***n=448***		***n=454***			
High	43.4_a	34.1_a	41.4_a	42.0_a	29.7_b	38.8_a	28.6_a	43.9_a	35.5_a
Intermediate	25.1_a	26.8_a	29.7_a	28.6_a	24.2_a	30.4_a	22.9_a	24.4_a	25.8_a
Low	31.4_a	39.1_a	29.0_a	29.4_a	46.2_b	30.8_a	48.6_b	31.7_{ab}	38.7_{ab}
Related work &/or internship	***n=487***			***n=453***		***n=459***			
No	37.3_a	36.0_a	35.4_a	38.7_a	13.5_b	48.2_a	20.0_b	19.2_b	15.2_b
Yes	62.7_a	64.0_a	64.6_a	61.3_a	86.5_b	51.8_a	80.0_b	80.8_b	84.8_b
Working while in college [b]	***n=487***			***n=453***					
No	29.4_a	25.9_a	25.9_a	27.5_a	10.4_b				
Yes	70.6_a	74.1_a	74.1_a	72.5_a	89.6_b				
Grades	***n=459***			***n=449***					
A	28.9_a	36.0_a	36.7_a	30.2_a	45.3_b				
B	57.2_a	51.1_a	58.5_a	58.5_a	46.3_b				
C	13.9_a	12.9_a	4.8_b	11.3_a	8.4_a				

Note. For a specific variable, percentages within a row lacking a common superscript significantly differ.

[a] Likert scale selections of 1 or 2 were classified as "low," 3 as "intermediate," and 4 or 5 as "high." [b] Students were asked if they had worked during their time in college. When asked specifically if they had worked during the semester to fund their college experience, no differences existed among No. active extracurriculars nor among study abroad participation for those indicating that they had.

Table 4.9. Pearson Correlations (p-values) of Preference Shares for Attributes Students Believe are Important for Their Anticipated Career Success and Student Demographics (*n*=405)

	Coursework preference share	Club Participation preference share	Relevant work experience preference share	International experience preference share	Advising/ mentorship preference share	College life preference share	Professional networking preference share
Share of preference							
Club participation	-0.1703***	1					
Relevant work experience	-0.7197****	-0.2667****	1				
International experience	-0.0707	0.0885	-0.2756****	1			
Advising/mentorship	-0.1814***	0.2162****	-0.2661****	0.0009	1		
College life	0.0435	0.0663	-0.3126****	0.0159	0.1144*	1	
Professional networking	-0.2517****	0.4039****	-0.3817****	0.1463**	0.5655****	0.1695***	1
Student is upper classman	-0.1045*	-0.0271	0.1438**	-0.0275	-0.0210	-0.0111	-0.0909
Student lives on campus	0.1454**	0.0102	-0.1753***	0.1067*	-0.0220	-0.0047	0.0561
Student is planning on grad or vet school	0.2337****	-0.0957	-0.1092*	0.0722	-0.1677***	-0.1134*	-0.1401*

Note. * indicates significance at 0.05 level, ** at 0.01 level, *** at 0.001 level, **** at 0.0001 level.

Table 4.10. Demographic Analysis of the Latent Class Model (LCM) Estimated Class Members (n=316)

	Class 1 (*n*=93)	Class 2 (*n*=103)	Class 3 (*n*=65)	Class 4 (*n*=55)
Department, n=377 (number of selections)				
Agricultural Economics	21%	45%	13%	21%
Animal Sciences	32%	28%	23%	16%
Gender, n=316				
Female	31%	31%	25%	13%
Male	23%	39%	5%	33%
Class, n=313				
Freshman	28%	29%	25%	18%
Sophomore	37%	31%	15%	18%
Junior	33%	30%	15%	22%
Senior	18%	45%	25%	11%
Grades, n=315				
A's	35%	29%	22%	14%
B's	27%	34%	21%	18%
C's	20%	40%	17%	23%
Ways respondent was funding their college education, n=1114 (number of selections)				
Parental or family member monetary support	27%	34%	23%	16%
Scholarship	30%	34%	19%	17%
Financial aid/grant(s)	31%	37%	19%	13%
Student loan(s)	31%	35%	20%	14%
Part time work while classes are in session	31%	36%	17%	17%
Full time work while classes are in session	17%	33%	17%	33%
Summer or between semester work/job	26%	36%	21%	17%
Personal savings	32%	34%	17%	17%
Ways respondent has worked during college, n=728 (number of selections)				
Worked in a field related to my major	29%	33%	22%	16%
Worked in an unrelated field to my major	30%	32%	19%	19%
Completed a paid internship	20%	46%	14%	20%
Completed an unpaid internship	32%	24%	22%	22%
Attended a career fair within the last 2 years	27%	35%	22%	16%
Primary intention following graduation, n=457 (number of selections)				
Work for family's agricultural business/farm	25%	25%	8%	42%
Work in the agriculture or ANSC industry	18%	56%	11%	15%
Work in a non-ag industry	25%	38%	13%	25%
Attend veterinary school	42%	13%	31%	15%
Attend graduate school	32%	25%	21%	21%
Other	22%	22%	28%	28%
Predominant home environment before the age of 18, n=312				
Rural, on a farm/ranch	24%	42%	15%	19%
Rural, NOT on a farm/ranch	31%	37%	18%	13%
Suburban	34%	19%	33%	14%
Urban	25%	25%	17%	33%

Note. Latent class modeling was used to estimate the probability of class membership for each individual respondent (Class 1 "Work and Study," Class 2 "Outside the Classroom," Class 3 "Classic Collegiate," or Class 4 "Everything/Nothing"). The table shows the percentage of respondents in each left hand sub-category who answered the best-worst question and whose probability of being in a class was at least 0.5 greater than the probability of being in their next highest class.

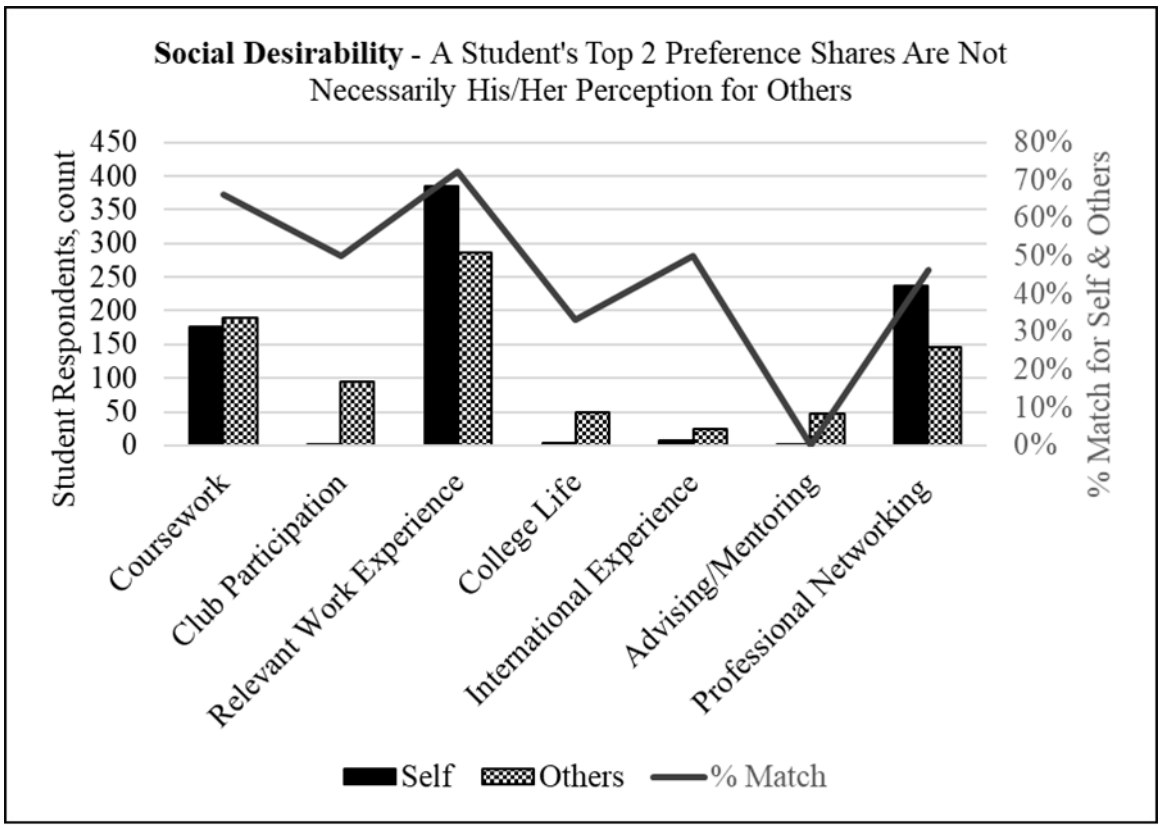

Figure 4.3. Students were asked which attributes they thought contributed most to their peers' career success and responses were compared on an individual basis to the attributes a student indicated as most important for self (% Match, percent of students who selected at least 1 of self's top 2 for others as well).