2011

The effects of heat stress and nutritional status on metabolism and intestinal integrity in growing pigs

Sarah Pearce
Iowa State University

The effects of heat stress and nutritional status on metabolism and intestinal integrity in growing pigs

by

Sarah Christine Pearce

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Nutritional Sciences (Animal Nutrition)

Program of Study Committee:
Lance H. Baumgard, Major Professor
Nicholas K. Gabler
John F. Patience

Iowa State University

Ames, Iowa

2011

DEDICATION

I want to dedicate this to my mom and my grandparents. Thank you mom for always encouraging me and helping me to find my way in life, you are my hero. Thank you to my grandparents (Grammy and Big Al) for instilling the importance of education and showing a city girl from Phoenix how to be a rancher. I hope I can continue to make you all proud.

TABLE OF CONTENTS

LIST OF TABLES v
LIST OF FIGURES vii
LIST OF ABBREVIATIONS x
ABSTRACT xii

CHAPTER 1: LITERATURE REVIEW

Climate Change 1
Human Concerns 2
Present Standard Operating Procedures 4
Animal Agriculture 6
Pigs as a Biological Model 8
Heat Stress Effects on Physiology 11
    Heat-Related Illness 11
    Heat Dissipation 12
    Feed Intake 13
    Blood Flow 14
Gastrointestinal Tract Function 15
    Intestinal Macronutrient Transport 15
    Tight Junctions 17
    Intestinal Permeability Measurement 18
Metabolism 19
    Carbohydrates 19
    Lipid Metabolism 20
    Protein Metabolism 21
    Insulin 22
    AMPK-Activated Kinase 23
    Na^+/K^+ ATPase 23
Heat Stress Effects on Gastrointestinal Tract 24
    Measures of Intestinal Permeability 24
    Tight Junction Proteins 25
    Circulating Endotoxin 26
Heat Stress Effects on Metabolism 27
    Insulin 27
    Glucose Metabolism 29
    Lipid Metabolism 29
    Protein Metabolism 30
    AMPK-Activated Kinase 31
    Na^+/K^+ ATPase 32
Heat Stress Effects on Cytokines 34

CHAPTER 2: THE EFFECTS OF HEAT STRESS AND NUTRITIONAL STATUS ON METABOLISM IN GROWING PIGS

Abstract 35
Introduction 36
Materials and Methods 39
Design, Animals and Treatments 39
Lab Analyses 43
Statistical Analysis 49
Results 50
Discussion 53
Conclusions 61

CHAPTER 3: THE EFFECTS OF HEAT STRESS ON INTESTINAL INTEGRITY OF GROWING PIGS

Abstract 75
Introduction 76
Materials and Methods 78
Design, Animals and Treatments 78
Lab Analyses 81
Statistical Analysis 85
Results 86
Discussion 87
Conclusions 93

CHAPTER 4: INTEGRATIVE SUMMARY 102

LITERATURE CITED 105

APPENDIX 125

LIST OF TABLES

Table 1.1: Conditions with increased risk of heat-related illness 4

Table 1.2: Normal rectal temperature of various species 11

Table 1.3: Symptoms of heat-related illnesses from mild (1) to severe (5) 12

Table 1.4: Effects of heat stress on pig feed intake in varying environmental conditions 15

Table 1.5: Effects of heat stress on intestinal morphology measures in various species 25

Table 1.6: Effects of heat stress on intestinal permeability measures in various species 27

Table 1.7: Effect of heat stress on plasma metabolites in various species 33

Table 2.1: Diet composition and chemical analysis 41

Table 2.2: Western Blot antibody information 48

Table 2.3: Effects of heat stress and plane of nutrition on body temperature parameters in growing pigs 63

Table 2.4: Effects of heat stress on production parameters in growing pigs 66

Table 2.5: Effects of heat stress on plasma energetic variables in growing pigs 68

Table 2.6: Effects of heat stress on energetic variables of the Longissimus Dorsi (LD) and liver 70

Table 2.7 Effects of heat stress on adipose tissue lipogenic enzyme activity in growing pigs 73

Table 3.1: Effects of heat stress on measures of intestinal permeability in growing pigs 95

Table 3.2: Effects of heat stress on measures of intestinal nutrient transport in growing pigs 97

Table 3.3: Effects of heat stress on measures of intestinal morphology in growing pigs 99

Table A-1.1: Effects of heat stress and plane of nutrition on production parameters in growing pigs 128

Table A-1.2: Effects of heat stress and plane of nutrition on plasma energetic variables in growing pigs 128

Table A-1.3: Effects of heat stress and plane of nutrition on energetic variables of the Longissimus Dorsi (LD) and liver in growing pigs 129

Table A-1.4: Effects of heat stress and plane of nutrition on adipose tissue lipogenic enzyme activity in growing pigs 129

Table A-1.5: Effects of heat stress on plasma variables in growing pigs 138

Table A-1.6: Effects of heat stress and plane of nutrition on plasma variables in growing pigs 139

Table A-1.7: Effects of heat stress and plane of nutrition on intestinal permeability measures in growing pigs 152

Table A-1.8: Effects of heat stress and plane of nutrition on intestinal nutrient transport in growing pigs 152

Table A-1.9: Effects of heat stress and plane of nutrition on intestinal morphology measures in growing pigs 153

LIST OF FIGURES

Figure 1.1: Insulin effect on whole body metabolism in a healthy animal 22

Figure 2.1: Effects of heat stress and plane of nutrition on temporal pattern of rectal temperature and respiration rates in growing pigs 64

Figure 2.2: Effects of heat stress and plane of nutrition on temporal pattern of daily feed intake in growing pigs 65

Figure 2.3: Effects of heat stress and plane of nutrition on body weight change in growing pigs 67

Figure 2.4: Effects of heat stress and plane of nutrition on plasma energetic variables in growing pigs 69

Figure 2.5: Effects of heat stress and plane of nutrition on energetic variables of the Longissimus Dorsi (LD) and liver 71

Figure 2.6: Effects of heat stress and plane of nutrition on Longissimus Dorsi (LD) HSP-70 expression in growing pigs 72

Figure 2.7: Effects of heat stress and plane of nutrition on adipose tissue lipogenic enzyme activity in growing pigs 74

Figure 3.1: Effects of heat stress and plane of nutrition on jejunum Na^+/K^+ ATPase activity in growing pigs 94

Figure 3.2: Effects of heat stress and plane of nutrition on intestinal permeability variables in growing pigs 96

Figure 3.3: Effects of heat stress and plane of nutrition on intestinal nutrient transport in growing pigs 98

Figure 3.4: Effects of heat stress and plane of nutrition on intestinal morphology measures in growing pigs 100

Figure 3.5: Effects of heat stress and plane of nutrition on morphological alterations in growing pigs 101

Figure A-1.1: Effects of heat stress and plane of nutrition on temporal change in rectal temperature and respiration rates at all time points 125

Figure A-1.2: Effect of heat stress on temporal change in skin temperature at all time points 126

Figure A-1.3: Effects of heat stress and temporal pattern on average daily gain in growing pigs 127

Figure A-1.4: Effects of environment and plane of nutrition on plasma energetic variables.....130

Figure A-1.5: Effects of environment and plane of nutrition on plasma cholesterol in growing pigs.....131

Figure A-1.6: Effects of environment and plane of nutrition on plasma triglycerides in growing pigs.....132

Figure A-1.7: Effects of environment and plane of nutrition on plasma creatinine in growing pigs 133

Figure A-1.8: Effects of environment and plane of nutrition on plasma alkaline phosphatase (ALK Phos) in growing pigs 134

Figure A-1.9: Effects of heat stress on Na^+/K^+ ATPase in the Longissimus Dorsi (LD) and liver in growing pigs 135

Figure A-2.0: Effects of heat stress on AMPK phosphorylation in the Longissimus Dorsi (LD) and liver in growing pigs 136

Figure A-2.1 Effects of heat stress on adipose tissue lipogenic enzyme activity in growing pigs.....137

Figure A-2.2: Effects of environment and plane of nutrition on plasma total protein in growing pigs 140

Figure A-2.3: Effects of environment and plane of nutrition on plasma albumin in growing pigs 141

Figure A-2.4: Effects of environment and plane of nutrition on plasma creatine kinase (CK) in growing pigs.....142

Figure A-2.5: Effects of environment and plane of nutrition on plasma gamma-glutamyl transferase (GGT) in growing pigs.....143

Figure A-2.6: Effects of environment and plane of nutrition on plasma aspartate aminotransferase (AST) in growing pigs 144

Figure A-2.7: Effects of environment and plane of nutrition on plasma bicarbonate in growing pigs 145

Figure A-2.8: Effects of environment and plane of nutrition on plasma calcium in growing pigs.....146

Figure A-2.9: Effects of environment and plane of nutrition on plasma magnesium in growing pigs 147

Figure A-3.0: Effects of environment and plane of nutrition on plasma sodium in growing pigs 148

Figure A-3.1: Effects of environment and plane of nutrition on plasma potassium in growing pigs.....149

Figure A-3.2: Effects of environment and plane of nutrition on plasma chloride in growing pigs 150

Figure A-3.3: Effects of environment and plane of nutrition on plasma phosphorus in growing pigs 151

Figure A-3.4: Effects of environment and plane of nutrition on intestinal permeability measures 154

Figure A-3.5: Effects of environment and plane of nutrition on nutrient transport measures.....155

Figure A-3.6: Effects of environment and plane of nutrition on intestinal morphology measures 156

LIST OF ABBREVIATIONS

ACC	=	ACETYL CoA CARBOXYLASE
ADG	=	AVERAGE DAILY GAIN
ADP	=	ADENOSINE DIPHOSHATE
ALK PHOS	=	ALKALINE PHOSPHATASE
AMPK	=	AMP-ACTIVATED PROTEIN KINASE
APP	=	APPARENT PERMEABILITY COEFFICIENT
AST	=	ASPARTATE AMINOTRANSFERASE
ATP	=	ADENOSINE TRIPHOSPHATE
BCA	=	BICINCHONINIC ACID ASSAY
BSA	=	BOVINE SERUM ALBUMIN
BUN	=	BLOOD UREA NITROGEN
BW	=	BODY WEIGHT
CK	=	CREATINE KINASE
DMI	=	DRY MATTER INTAKE
ELISA	=	ENZYME-LINKED IMMUNOSORBENT ASSAY
FAS	=	FATTY ACID SYNTHASE
FFA	=	FREE FATTY ACIDS
FI	=	FEED INTAKE
FITC	=	FLUORESCEIN ISOTHIOCYANATE
GLUT 1	=	GLUCOSE TRANSPORTER 1
GTT	=	GAMMA-GLUTAMYL TRANSFERASE
GIT	=	GASTROINTESTINAL TRACT
G-3-PDH	=	GLYCEROL-3-PHOSPHATE DEHYDROGENASE
G-6-PDH	=	GLUCOSE-6-PHOSPHATE DEHYDROGENASE
HCO_3	=	BICARBONATE
HDL	=	HIGH DENSITY LIPOPROTEIN
HRP	=	HORSERADISH PEROXIDASE
HS	=	HEAT STRESS
HSL	=	HORMONE SENSITIVE LIPASE
HSP 70	=	HEAT SHOCK PROTEIN 70
LBP	=	LIPOPOLYSACCHARIDE BINDING PROTEIN
LD	=	LONGISSIMUS DORSI
LDL	=	LOW DENSITY LIPOPROTEIN
LPL	=	LIPOPROTEIN LIPASE
LPS	=	LIPOPOLYSACCHARIDE
MLCK	=	MYOSIN LIGHT CHAIN KINASE
NADH	=	NICOTINAMIDE ADENINE DINUCLEOTIDE
NADPH	=	NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE
NEFA	=	NON-ESTERIFIED FATTY ACIDS
PEPT1	=	PEPTIDE TRANSPORTER 1
PFTN	=	PAIR-FED THERMAL NEUTRAL
ROS	=	REACTIVE OXYGEN SPECIES

RR	=	RESPIRATION RATE
RT	=	RECTAL TEMPERATURE
TER	=	TRANSEPITHELIAL ELECTRICAL RESISTANCE
SGLT1	=	SODIUM GLUCOSE COTRANSPORTER 1
ST	=	SKIN TEMPERATURE
THI	=	TEMPERATURE-HUMIDITY INDEX
TN	=	THERMAL NEUTRAL
ZO-1	=	ZONULA-OCCLUDEN-1

ABSTRACT

Heat stress (HS) negatively affects pig performance variables and is thus a costly industry issue. It is unknown whether or not HS directly or indirectly (via reduced feed intake) is responsible for the suboptimal production. To account for differences in nutrient intake, we utilized an ad-libitum thermal neutral control group and a pair-fed thermal neutral control group of pigs. In these experiments, pigs in HS conditions had increased body temperatures, reduced feed intake, and lighter body weights compared to controls. Presumably, this production difference may also includes a difference in body composition as HS pigs have increased circulating insulin levels, decreased basal lipolysis, and increased adipose tissue lipogenesis compared to bioenergetic controls. In addition, HS pigs had increased markers of protein catabolism. Heat stress pigs also had compromised intestinal integrity, but this appears to be due to be confounded by reduced nutrient intake, as pair-fed controls had similar intestinal dysfunction characteristics. In conclusion, heat stress directly and indirectly (via reduced feed intake) affects post-absorptive metabolism and intestinal integrity and both variables probably contribute to decreased growth parameters in young pigs.

CHAPTER 1
LITERATURE REVIEW

Climate Change

Although there is no consensus regarding the extent or cause of climate change, there is little doubt global warming is occurring (Bernabucci et al., 2010). Climate change affects global temperatures, weather patterns, and sea level. Deforestation as well as green house gas emissions (i.e. CO_2, N_2O, and CH_4) contribute to rising temperatures (U.S. EPA, 2010). According to NASA's Goddard Institute for Space Studies Surface Temperature Analysis (GISTEMP), 2009 was the second warmest year since monitoring began in 1880 and the last 10 years have been the warmest decade on record. More recently, GISTEMP indicates 2010 has officially been the warmest year on record. Data suggests that temperatures are rising at a rate of 0.2℃ per decade and this number is likely to increase in the future (US EPA, 2010).

Another effect of climate change is severe weather. The National Research Defense Council (2010) stated that the southwestern USA experienced extreme drought in 2007 and the entire United States experienced moderate drought that same year. Coinciding with extreme drought, the USA has also observed an increase in other types of severe weather, including higher winds, stronger storms, and a possible increase in hurricane frequency (NRDC, 2010). This also includes an increase in the number of US heat wave days (Hayhoe et al., 2004). Collectively, this information indicates the world's future climate is likely to be accompanied by higher temperatures and more extreme weather. Increased numbers of heat-wave

days increases the likelihood of heat-related illness in both humans and animal species and necessitates a better understanding of how heat stress affects biological systems.

Human Concerns

Humans can become ill or even die due to heat stress-related pathologies. In 2003, nearly 15,000 people died during a two week heat-wave in France (Kovats et al., 2006) and over all it is estimated that 50,000 Europeans died during the same heat-wave (Kosatsky, 2005; Patz et al., 2005). For weather to be considered a heat-wave, temperatures must exceed 32.2℃ (90℉) for three consecutive days (National Weather Service, 2000). High temperatures alone can be deadly, but in many areas high humidity contributes significantly to the heat index.

Certain segments of the population are at a higher risk for heat stress due to working conditions, age, or predisposing health conditions. Athletes, soldiers, the elderly, firefighters, agricultural workers, diabetics, and children are at a higher risk for heat-related illnesses (Bouchama et al., 2007). Table 1.1 describes those at highest risk for heat-related illness and why they are more susceptible to warm temperatures (adapted from Barrow and Clark, 1998). Often human and animal deaths occur in areas not accustomed to high temperatures and without proper cooling mechanisms (fans, air conditioning, and water). O'Neill and colleagues (2009) note that those individuals acclimated to higher temperatures (such as people living in arid, semi-arid and tropical areas) respond differently to a heat-load. This includes a physiological acclimation response as well as behavioral adaptations

(spending less time outside or utilizing home air conditioners). People living in areas where summer temperatures are constantly elevated have a decreased risk of mortality, compared to those living in milder climates that might experience temporary elevated temperatures in the summer months (Basu, 2009).

Within the health care community, there are inconsistencies in diagnosing heat-related illness because no current definition of heat-related mortality exists (Ostro et al., 2009). This causes confounding variables in epidemiological studies designed to evaluate weather-related mortality because some causes of death are listed as cardiovascular disease or cerebral damage when the underlying cause of death is heat stress (Basu, 2009). This may be due to the fact that heat stress (especially if untreated) can cause a variety of serious health problems including cardiac failure and central nervous system damage (Caspani et al., 2004).

No standard definition or effective treatment exists for heat-stressed patients, so methods of alleviating this condition are aimed primarily at prevention. After several major heat waves in the United States and Europe, communities have increased preventative measures to aid people during times of excessive heat. This includes increasing air conditioned areas, planting trees (to provide shade), and educating people on heat-related illnesses (O'Neill et al., 2009).

Table 1.1 [1]Conditions with increased risk of heat-related illness

Physiological conditions	**Younger age**
-Fever	-Decreased ability to sweat
-Dehydration	-Decreased cardiac output at a give metabolic rate
-Excessive exercise	-Slower acclimation
-Chronic illnesses	-More heat produced for same level of activity
-Cardiac conditions	-Greater core temperature required to initiate sweating
-Uncontrolled diabetes	
-Extensive skin disease and/or damage	**Older age**
-Autonomic nervous system disorders	-Decreased vasodilatation
-Hyperthyroidism	-Decreased maximum heart rate, resulting in decreased maximum cardiac output
-Previous Heat-stroke	-Decreased fitness level
Additional factors	-Decreased thirst response
-Urban setting	-Decreased mobility resulting in increased difficulty of easily obtaining fluids
-Lack of access to air conditioning	
-Sleep deprivation	
-Wearing heavy clothing	
-Moving to a new climate	

[1]Information from: Holowatz et al., 2010; Sanders, 2010; and Shendell et al., 2010

Present Standard Operating Procedures

Heat-exhaustion treatment is limited for patients admitted to hospitals. The most common strategy is to first remove a patient from heat exposure and to cool with ice via conduction or by evaporative cooling via wetting the skin coupled with rehydration (Caspani et al., 2004). Internal cooling can also be accomplished by gastric or rectal flushing with cold water (Glazer, 2005). The most effective cooling method for patients diagnosed with classical or exertional heat stroke, is placing the patient in ice water while massaging the arms and legs to aid in circulation. Despite the intensive cooling, over 30% of heat-stroke patients admitted to the emergency room die (LoVecchio et al., 2007) and anywhere from 20-65% of patients suffer from acute circulatory failure which is often fatal (Bouchama and Knochel, 2002). Some

reports have noted an even higher mortality rate (between 30-80%) in those affected by heat-stroke and nearly 50% of those deaths are in the elderly population (Nixdorf-Miller et al., 2006).

There are few medications known to help effectively treat heat-stroke patients, but some drugs are effective at preventing shivering and seizures as well as metabolic heat production. One example is dantrolene sodium (a skeletal muscle relaxant) which decreases the amount of heat produced by muscles during the contraction phase (Bouchama et al., 2007). This is often used in treatment of malignant hyperthermia (not environmental hyperthermia) and acts on the sarcoplasmic reticulum by inhibiting release of Ca^{2+} for the contraction cycle (Bouchama et al., 2007).

Heat-related illnesses can lead to multi-organ damage and the cause of death varies depending on geographic area, and whether death occurred at home or in a hospital. People at a higher risk (such as the elderly) and those that live alone or lack social contact are more likely to succumb at home from heat-stroke which results in a diagnosis made post-mortem. Therefore, the cause of death could be listed as a secondary reason (i.e. cardiac failure) when the primary source was heat-stroke (Mastrangelo et al., 2006). Due to the fact that heat-stroke affects multiple organ systems, hospital staff often monitors heart, liver and kidney function and aim to protect those organs during treatment. This can be accomplished by interfering with the neurotoxic cascade response and blocking neuronal ischemic damage. Circulatory damage and cerebral ischemia can be corrected using some medications

such as aminopyrine (to treat fever), hyperbaric oxygen, and shengmai san powder (to treat low blood pressure; Yan et al., 2006). However, the most prevalent practices target prevention and not treatment. For example, firefighters are allowed minimal exposure to burning edifices and people are advised to stay inside during times of intense temperatures.

Animal Agriculture

Annual economic losses to global animal agriculture due to heat stress surpass billions of dollars. In the United States, summer-induced decreased production is well-documented in every aspect of animal agriculture. Nationally, the swine industry loses $113 million for sows and $202 million for growing-finishing swine. This combined costs over $300 million each year due to heat stress (St-Pierre et al., 2003). The fiscal losses are still observed despite recent advances in cooling systems, barn management, and other heat abatement strategies.

Third world countries are likely to experience greater effects of climate change for a number of reasons. Areas such as China, Africa, and India's populations are growing at an alarming rate (Godfray et al., 2010), and this obviously comes with an increased need for food supply. A majority of human and animal populations lie in warm tropical and sub-tropical areas and will be greatly affected by rising temperatures (Collier et al., 2006). Strategies such as zone-cooling (air-conditioning), and shades with insulated roofing are not cost efficient and systems using extensive water or electricity may not be practical or sustainable in many areas (Nienaber and Hahn, 2007). In places which lack sufficient infrastructure,

wallowing areas can be used to cool swine. However, this comes with a risk of negatively influencing water supplies and may compromise animal health (Nienaber and Hahn, 2007).

In addition to growth, animal reproduction is very sensitive to heat stress as decreased fertility is often reported during the warm summer months. The southwestern US experiences a high number of days open which is a measure of fertility encompassing many traits such as parity and season of calving in dairy cows. This also costs the dairy industry excess money and increased waiting time for breeding. Particularly, the state of Arizona, a producer of cattle (finishing beef cattle and dairy cows) experiences between 30-50 excess days open yearly (Oseni et al., 2003). Arizona is a state characterized by a hot, dry desert climate with summer temperatures averaging 30℃ in May, and 35℃ in July and August (Silva et al., 2010). Temperature humidity indices (THI) during July and August are significantly higher due to the monsoon season and increased humidity. For dairy cows, heat stress is classified at a THI ≥ 72 (Armstrong, 1994) and in Arizona the THI reaches 72 in early May. Even with the use of evaporative cooling systems, Arizona dairy's experience THI values well above the heat stress threshold and this leads to decreased milk production (Bohmanova et al., 2007). The average decrease in milk production in Arizona during the summer months is approximately 500 kg/cow per season (Nienaber et al., 1999). Little information is available about heat stress effects on swine reproduction and economics in Arizona because there are virtually no hog barns in the state (St-Pierre et al., 2003).

Iowa is the country's largest producer of finishing hogs and sows with a monthly inventory of over 8 million finishing swine and over 800,000 sows (St-Pierre et al., 2003). Iowa has the largest number of farrowings in the country, and heat stress causes a loss of 5.2 additional days open annually. This equates to economic losses of almost $5 million combined for both sows and finishing hogs due to heat stress alone. With a maximum THI in July of 80.2, including an average of 70% relative humidity, animals are at risk for severe heat exposure. Both Arizona and Iowa incur losses in body weight gain which amount to approximately 2-3 kg/head/year due to heat stress (St-Pierre et al., 2003).

Pigs as Biological Models

The pig is an excellent model for biological research because it physiologically resembles humans in many ways. Most commonly, pigs are used to study cardiovascular disease because their heart is similar to the human heart. More specifically, the coronary artery system is the same as in humans (Granada et al., 2009). Scientists are able to induce elevated LDL levels, high cholesterol, and eventually end stage atherosclerosis in domestic pigs (Suzuki et al., 2011). Porcine models are increasingly used for other types of diseases involving obesity, dermatology, and diabetes's among others (Nafikov and Beitz, 2007). Swine have emerged as a potential model for diabetes and metabolic syndrome research because they do not possess brown adipose tissue as adults, and the Ossabaw pig is an excellent model for insulin resistance (Spurlock and Gabler, 2008).

For ethical reasons, animal models are often used to study the effects of heat

stress in humans because it is possible to manipulate the environmental conditions they experience and some of the physiological responses are fairly universal across species (Aigner et al., 2010). Although primates are the closest genetically related species, the cost and ethical implications for usage in research makes them difficult subjects to obtain. A common species utilized in heat-stroke research is rodents as they can mirror the full spectrum of human heat-stroke symptoms (Chen et al., 2006), but cows, chickens, and sheep are also used. Finding a species that is cost effective, and has enough blood volume and tissue to utilize for research is a difficult task. Rodents are cost effective and readily available, but pose limitations due to their small size and physiological differences (i.e. brown adipose tissue). The amounts of blood that can be repeatedly obtained from rodents, combined with small sample size from tissue harvesting also contribute to these limitations. On the other hand, cows are a much larger species to work with but are limited by extensive cost and physiological differences (i.e. a rumen; Damanhouri and Tayeb, 1992). Pigs are an effective model for heat stress and most of this research has centered on reproduction and genetic aspects with primary goals focusing on animal production and agriculture applications (not human applications).

Swine are particularly susceptible to heat stress because they possess little to no functional sweat glands (Curtis, 1983). In addition, pigs maintain more subcutaneous fat compared to other species and this prevents effective heat dissipation (Mount et al., 1979). Due to inadequate sweat glands, pigs depend on panting as their primary mechanism of heat dissipation (Patience et al., 2005),

especially if they don't have access to a wallowing area. The normal body temperature of the pig is 39.2℃ (102.5℉) and at am bient temperatures above 22℃ heat stress indicators such as increased respiration rates, and rectal temperatures are observed (Huynh et al., 2005). Nienaber and Hahn (2007) suggest that fast growing animals near market weight are at increased risk of severe heat stress because of increased metabolic heat due to genetic selection for enhanced lean tissue accretion rates. A 2.1% increase in lean tissue correlates with a metabolic heat production increase of 18.7% (Brown-Brandl et al., 2004).

Pigs respond to warm temperatures by increasing respiration rate, maximizing their surface area by laying on the ground, as well as increasing water intake. According to Marple and co-workers (1974), severe physiological changes can be observed in pigs with a rectal temperature reaching 41.5℃ (106.7℉). This temperature can be potentially fatal, especially in finishing hogs and lactating sows which have decreased ability to dissipate heat. Table 1.2 shows normal rectal temperature of various livestock species.

Table 1.2 Normal rectal temperature (RT) of various species

Species	RT, ºF	RT, ºC
Avian	107.6	42.0
Bovine	101.0	38.3
Canine	102.0	38.9
Caprine	103.1	39.5
Equine	100.0	37.8
Feline	101.5	38.6
Leporine	101.5	38.6
Ovine	102.3	39.1
Porcine	102.5	39.2
Rodents	99.1	37.3

Adapted from Campbell et al., 2003; and O'Brien, 2008

Heat Stress Effects on Physiology

Heat-Related Illness

Elevated temperatures can cause a range of physiological conditions depending on the severity (mild or severe) of heat stress. The most severe form of heat illness in the human is classified as heat stroke and this stage of hyperthermia is when people are at the highest risk of dying. Table 1.3 shows the different forms of heat-related illnesses and associated symptoms. Increased temperature also causes elevated respiration rates, heart rate, and blood pressure in humans. The cardiovascular system is able to mitigate the effects of increased temperature until heat-stroke sets in, which causes a decrease in blood pressure and the mechanisms of heat dissipation are no longer effective (Kregel et al., 1988).

Table 1.3 Symptoms of heat-related illnesses[1] from mild (1) to severe (5)

1. Heat edema -Mildest form of heat illness caused by transient peripheral vasodilatation from the heat and orthostatic pooling during prolonged sitting or standing **2. Heat cramps** -Painful spasms of skeletal muscle of arms, legs or abdomen -Increased body temperature -Thirst -Sweating -Tachycardia **3. Heat syncope** -Dizziness -Results from inadequate cardiac output and postural hypotension -Fainting -T_c 40℃	**4. Heat exhaustion** -Excess sweating -Heat cramps -Nausea/vomiting -Headache -Hypotension -T_c 40.6 ℃ **5. Heat stroke** -T_c 41℃ (hyperpyrexia) -Heat exhaustion plus: -Dehydration/hemoconcentration -Endotoxemia -Hyperglycemia -Lactic acidosis -Cardiovascular abnormalities -Increased cortisol/corticosterone -Renal failure -Hepatocellular necrosis -Hyperventilation -Arrhythmia

[1]Adapted from Leon, 2007; Aggarwal et al., 2008; and Sanders 2010

Heat Dissipation

Under normal physiological conditions metabolic heat production due to muscle contractions, biochemical reactions, ion pump activity, etc…is offset by the body's ability to dissipate heat by radiation, convection and conduction (Roth et al., 2009). Heat is normally transferred to the atmosphere when ambient temperatures are below normal body temperature (i.e. a temperature gradient exists), but when ambient temperatures exceed the body's thermal-neutral zone (i.e. a reverse energy gradient), the only heat-loss mechanism is evaporative cooling via sweating (Roth et al., 2009). Generally, heat can be absorbed from the environment (via conduction, convection, or radiation) or generated metabolically due to increased growth,

feeding, lactation, and other parameters. On the other hand, heat can be removed from the body (via radiation, conduction, convection, or evaporation) by means of urination, defecation, and milk removal. These increments of heat gains/losses are balanced within an animal to maintain euthermia (Fuquay, 1981). Heat stress occurs when the body can no longer maintain thermoregulatory homeostasis.

In some species such as pigs, rodents, and dogs, panting is the major mechanism by which heat is dissipated. Panting increases respiration rate and decreases tidal volume which increases ventilation and evaporation in the oral cavity and esophagus (Hales and Webster, 1967). Some mammals such as horses and humans rely on other evaporative mechanisms such as sweating for cooling the core temperature (Fuller et al., 2000). Sweating is a more effective mechanism of heat dissipation compared to panting because panting requires muscle contraction which in itself produces heat (Fuller et al., 2000). This has implications for certain species and their susceptibility to heat stress. Pigs have little to no functional sweat glands which means they rely almost entirely on panting for heat dissipation (Fuquay, 1981). This may help partly explain why pigs are more susceptible to heat compared to other species (Patience, 2005).

Feed intake

Voluntary feed intake is influenced by a number of factors and is very sensitive to environmental conditions. Feed intake in lactating sows decreases when the temperature exceeds 22℃ (Gourdine et al., 2 006) and heat stress also causes decreased milk yield and body weight during lactation (Renaudeau et al.,

2001). Feed intake decreases up to 50% during heat stress and this is often considered as the primary reason for negative effects on agriculture production parameters (DeShazer et al., 2009). This decrease in nutrient intake explains a majority of weight loss, or lack of weight gain observed in heat-stressed production animals (Kouba et al., 2001). Pigs decrease feed intake to maintain thermoregulation as eating and the process of digestion, absorption and assimilation increases metabolic heat production. It is thought that reduced nutrient intake is an attempt to offset the effect of environmental heat. Table 1.4 shows the effects of heat stress on feed intake along with severity of heat-load.

Blood Flow

Other physiological responses to heat stress include a redistribution of blood flow from the viscera to the skin to aid heat dissipation (Rowell, 1983). Due to the need for heat dissipation as a cooling mechanism, the body re-routes blood to the surface of the skin. Heat stress causes reduced venous pressure, and reduced heart, liver, splanchnic and thoracic blood volume. This diversion of blood to the periphery is accompanied by increased cardiac output and this causes decreased blood volume (Crandall et al., 2010).

Evidence also indicates reduced blood flow to the gastrointestinal tract (GIT) during heat stress. Reduced GIT blood flow can cause tissue hypoxia which depletes ATP stores and can potentially cause intracellular acidosis and changes ion pump activity (Hall et al., 1999). Depletion of ATP and acidosis can jeopardize tight junctions of the intestinal epithelium and this can result in bacterial and associated

endotoxins and pathogens translocation across the membrane and into circulation. Heat stress can also cause increased tight junction permeability by increasing oxidative stress, and membrane damage. The result of bacterial translocation can lead to severe illness, endotoxemia or death as septicemia is very difficult to treat (Lambert, 2009).

Table 1.4 Effects of heat stress on pig feed intake in varying environmental conditions

↓DMI[a]	HS Pattern[b]	Max ℃	Min ℃	Humidity Controlled	Reference
19%	Diurnal	35	27	Yes	1
15%	Constant	29	29	No	2
30%	Constant	31	31	Yes	3
37%	Constant	32	32	No	4
25%	Constant	33	33	No	5
18%	Constant	30.3	30.3	No	6
49%	Constant	29	29	No	7
21%	Constant	40.5	40.5	No	8
15%	Diurnal	35	22.5	Yes	9
48%	Constant	35	35	No	10

[a]Dry Matter Intake
[b]Heat Stress

1. Becker et al., 1992
2. Le Bellego et al., 2002
3. Renaudeau et al., 2007
4. Spencer et al., 2003
5. Collin et al., 2001a
6. McGlone et al., 1988
7. Renaudeau et al., 2001
8. Kim et al., 2009
9. Lopez et al., 1991
10. Collin et al., 2001b

Gastrointestinal Tract Function

Intestinal Macronutrient Transport

The main functions of the GIT is to aid in the digestion and absorption of nutrients as well as maintain immunity and barrier function (Burkey et al., 2009). The GIT contains an epithelial barrier which normally prevents passage of unwanted luminal contents while allowing passage of ions, nutrients, and water (Yu et al.,

2010). The two ways which this occurs are transcellular and paracellular transport. Transcellular transport is primarily powered by ATP hydrolysis, while paracellular transport involves passive diffusion (Johnson, 2006). The regulation of passive diffusion depends on the ability of tight junctions to allow only beneficial molecules to cross, while preventing harmful molecules such as pathogens and bacteria (Blikslager et al., 2007).

The three major macronutrients (carbohydrates, fatty acids, and amino acids) are transported in and out of the intestine by different mechanisms and transporters. Animal cells can only transport carbohydrates in the form of monosaccharides so complex sugars must first be broken down. Glucose and galactose are transported across the apical membrane of enterocytes in the small intestine via the apical Na^+/glucose co-transporter (SGLT1) while fructose is transported by the transporter GLUT 5 (Shirazi-Beechey et al., 2011). Glucose transporter GLUT 2 is then responsible for moving these simple molecules across the basolateral membrane into circulation (Shirazi-Beechey, 1995).

Dietary proteins are transported similarly to sugars in that they are broken down into amino acids and smaller peptides, both of which depend on active and passive transport across the intestinal epithelium (Spencer, 1969). Sodium-dependent transporters aid in moving amino acids across the apical membrane specific for acidic, neutral, and basic amino acids. The basolateral side of the enterocyte also contains transporters to move amino acids into the blood stream (Silk et al., 1985). Small peptides can also be transported under certain

circumstances via the PepT1 transporter. This transports di- and tri-peptides to the enterocyte where they are broken down into free amino acids and exported into circulation (Daniel, 2004).

Lipids undergo a different process for transport into circulation. Prior to transport and absorption, triglycerides are broken down into monoacylglycerols and free fatty acids (FFA). Fatty acids are then absorbed in the intestine and transported across the apical membrane of enterocytes (Mansbach and Gorelick, 2007). Once the fatty acids are in the enterocyte, they travel to the endoplasmic reticulum and are re-synthesized into complex triglycerides. Fatty acids in the enterocyte are incorporated into triacylglycerol phospholipids, and cholesteryl esters (Johnson et al., 2006). These new complex molecules are used to synthesize chylomicrons which are then transported to peripheral tissues (Iqbal and Mahmood Hussain, 2009).

Tight Junctions

The intestinal barrier is held together by protein complexes called tight junctions. These junctions seal the perimeters of polarized epithelial cells and form a paracellular barrier which aids in absorption and transport (Hossain and Hirata, 2008). The claudin proteins as well as occludin are located in tight junctions and may be associated with zona occludins (ZO) which act as adaptor proteins (Miyoshi and Takai, 2008). A specific zona occludin, ZO-1 is a protein that is strictly associated with tight junctions (Anderson et al., 1988). Cell adhesion molecules associate with peripheral membrane proteins on the plasma membrane via adaptor

proteins which attach to the actin cytoskeleton (Anderson and Van Itallie, 2009). Actin filaments regulate tight junction barrier and contraction of these filaments ultimately leads to tight junction openings, aiding in cell motility and absorption (Yang et al., 2007). Myosin light chain kinase (MLCK) phosphorylates the regulatory light chain of type 2 myosin. Contraction of the actin cytoskeleton is regulated by phosphorylation of MLCK (Turner, 2006). It is thought that increased MLCK may initiate intestinal permeability (Yang et al., 2007).

Intestinal Permeability Measurement

Measurements of intestinal barrier function and integrity can be accomplished by measuring the membrane resistance. Epithelia exhibit unique properties such as polarity and tightness (Brown and Stow, 1996). This tightness property can be measured as transepithelial electrical resistance (TER) which is generated by membrane tight junctions. A lower resistance indicates higher permeability. Epithelial tissues also generate a voltage across the epithelium because these tissues transport ions. Essentially this voltage indicates active transport across the membrane similar to how the Na^+/K^+ pump operates by utilizing ATP to exchange ions in and out of a cell (Gordon et al., 1989). By eliminating osmotic and electrochemical gradients, the movement of ions across the membrane is the result of active transport (Johnson et al., 2006). However, Ussing Chambers can measure both types of transport.

In animal models, macromolecule permeability can also be used to determine leaky gut by using fluorescently labeled dextrans such as fluoroisothiocyanate

(FITC)-dextrans, FITC-labeled LPS, (Tomita et al., 2004). This is done by treating these molecules into the mucosal side of intestinal tissue and measuring serosal concentration over time. As the labeled molecule is fluorescent, a spectrophotometer can detect concentration based on detection of a fluorescent signal. The final calculated apparent permeability coefficient represents the amount of lipopolysaccharide (LPS) or dextran passage through the intestinal epithelium. Another more conventional index of barrier function is to measure plasma LPS as presence of LPS in the blood indicates passage from the intestine into circulation (Lambert, 2009).

Metabolism

Carbohydrate Metabolism

Mature swine obtain a majority of their glucose from dietary sources (similar to other monogastric species; McMillin, 1990). Glucose use for ATP production occurs by two main processes. One is the glycolytic pathway and a second is the pentose phosphate pathway. Glycolysis is the major pathway for glucose oxidation (80-90%) in most tissues while the pentose phosphate pathway accounts for the remaining 10-20% (Wamelink et al., 2008). Glycolysis converts glucose into two pyruvate molecules and generates 2 ATP in the process. Under normal aerobic conditions, glucose is oxidized to CO_2 and pyruvate is converted to acetyl CoA which enters the Krebs cycle (Berg et al., 2007). Excess glucose can also stored in the form of glycogen (in muscle and hepatic tissue) which can be broken down for energy production (Heppner et al., 2010).

Glucose homeostasis is maintained via intestinal glucose absorption, hepatic glucose production, as well as glucose uptake and usage by peripheral tissues (Scheepers et al., 2004). The liver regulates glucose metabolism differently because it is involved in maintaining glucose homeostasis within the body. The liver is also able to store glucose in the form of glycogen which can be utilized to release glucose via glycogenolysis (Berg et al., 2007). Glucose metabolism is largely regulated by the hormones insulin and glucagon as insulin signals a "fed" state and inhibits glycogen breakdown while stimulating ATP production (Heppner et al., 2010). Glucagon is a pancreatic hormone which primarily is responsible for raising blood glucose levels by stimulating glycogenolysis and hepatic gluconeogenssis (Baile et al., 1983).

Lipid Metabolism

Adipose tissue is the primary site for fatty acid synthesis in swine and glucose is the main carbon source for lipogenesis in porcine adipose tissue (Houpt et al., 1979). Sources of NADPH for biosynthesis of fatty acids come from the pentose phosphate pathway (Berg, 2007). The amount of triglycerides stored in adipose tissue depends on the balance between de novo fatty acid synthesis, fatty acid uptake, esterification, and lipolysis (Chilliard, 1993). Key enzymes involved in de novo fatty acid synthesis include acetyl Co-A carboxylase, fatty acid synthase (FAS), glucose-6-phosphate dehydrogenase (G-6-PDH), and glycerol-3-phosphate dehydrogenase (G-3-PDH; Zhao et al., 2010).

Release of adipocyte non-esterified fatty acids (NEFA) occurs as the result of

adipose tissue lipolysis and re-esterification (Chilliard, 1993). Endothelial lipoprotein lipase is an important enzyme which hydrolyzes circulating triglycerides in chylomicrons and VLDL and converts them into LDL and free fatty acids which are available for tissue uptake (Zhao et al., 2010). Hormone sensitive-lipase is the intra-adipocyte key enzyme involved in lipolysis as it functions to hydrolyze the first fatty acid from a triacylglycerol, freeing one fatty acid and a diacylglycerol (Berg et al., 2007).

Protein Metabolism

Dietary amino acids are used to synthesize protein for muscle growth, membrane glycoproteins, and act as precursors for synthesis of DNA/RNA, and enzymes involved in numerous biochemical processes (Young, 1976). Protein synthesis is in part regulated by the amount of amino acids and ATP available. Protein biosynthesis occurs in a multistep process after transcription initiation. Messenger RNA contains a code for specific polypeptide sequences and acts as a template for synthesis of amino acid chains at the ribosome. This ultimately leads to formation of a complex protein structure (Berg et al., 2007). The liver catabolizes amino acids to incorporate into protein which supplies peripheral tissues (Bergen, 1974). Protein turnover is the balance between protein anabolism and catabolism. Anabolism occurs as amino acids from the diet are incorporated into proteins, or bio-synthesized in the body. Catabolism occurs via cell breakdown to create amino groups which synthesize urea, further protein synthesis, or carbon skeletons for synthesis of fatty acids or glucose. Nitrogen excretion can be an estimate of protein

balance as nitrogen is excreted during protein catabolism or recycling (Berg et al., 2007).

Insulin

Insulin decreases blood glucose concentrations primarily by increasing glucose uptake in adipose tissue and muscle (Heppner et al., 2010). In muscle and adipose tissue, insulin signaling causes the glucose transporter GLUT 4 to translocate to the plasma membrane where it facilitates glucose uptake (Hadley, 2000). In muscle, it affects protein metabolism by increasing the synthesis of proteins while at the same time decreasing protein breakdown (Hadley, 2000). Insulin facilitates glucose storage as glycogen in the liver while inhibiting gluconeogenesis. In adipose tissue, insulin stimulates lipoprotein lipase, in order to hydrolyze lipoprotein triglycerides and allow NEFA's to enter the adipocyte. Overall, insulin acts anabolically to divert nutrients to muscle and adipose tissue for synthesis (Brockman, 1986). Figure 1.1 summarizes insulin's effect on metabolism.

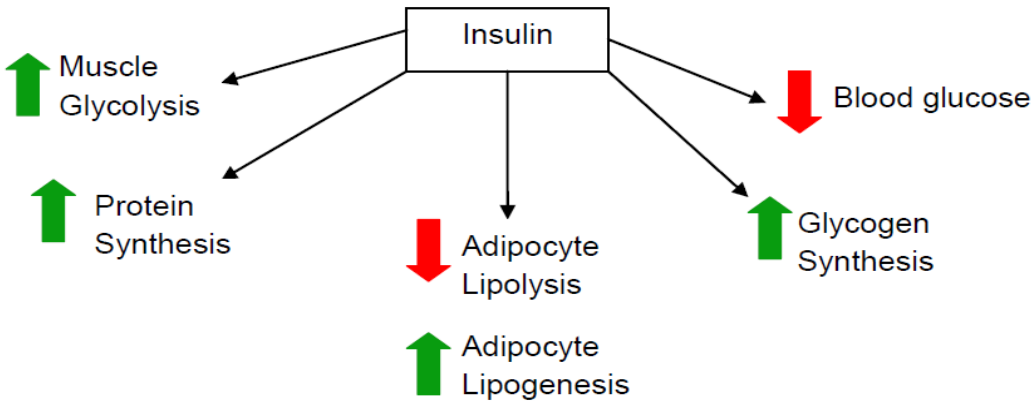

Figure 1.1 Partial list of insulin's effects on whole body metabolism in a healthy animal.

AMP-Activated Kinase

AMP-activated Kinase (AMPK) is the primary gauge of cellular energy requirements (Hardie, 2008) and is activated when AMP levels increase (i.e. energy status is low). It then acts to inhibit enzymes involved in ATP-requiring reactions in order to conserve cellular energy (Kemp et al., 1999). When AMPK is activated, fatty acid oxidation and glucose uptake are stimulated (Goodyear and Kahn, 1998). In the cell, AMPK can exist in a T state (less active) and an R state (more active) but also exists as dephospho-AMPK and phospho-AMPK indicating that overall AMPK can exist in four forms (Hardie and Carling, 1997). Phosphorylation by upstream kinases activates AMPK by phosphorylating the α subunit at Thr-172 (Kemp et al., 2003). Conversely, AMPK is deactivated upon dephosphorylation (Steinberg and Kemp, 2009). The liver is largely unaffected by changes in AMPK concentrations, because ATP concentrations in the liver remain relatively constant (Viollet et al., 2009). Only severe stress stimuli such as intensive exercise or fasting can cause hepatic ATP levels to decrease (Hardie, 2004). In the case that AMPK is activated in the liver, glucose output can be inhibited (Zhou et al., 2001).

Na^+/K^+ ATPase

The sodium potassium pump is essential for all animal cells because it is primarily responsible for maintaining cellular ion balance. This ATPase is in the family of P-type ATPases which generate a charge gradient across the membrane (Morth et al., 2011). The Na^+/K^+ ATPase pumps three Na^+ ions out of, and two K^+ ions into the cell while hydrolyzing one ATP and is necessary for maintaining cell

osmolarity (Bers and Despa, 2009). Skeletal muscle contains a large K^+ and Na^+ pool, thus it can easily cause changes in plasma ion concentrations (Benziane and Chibalin, 2008). Due to this, muscle contains a large amount of Na^+/K^+ pumps (Clausen, 2010). In the intestine, the Na^+/K^+ pump moves Na^+ into the blood and this creates an intracellular gradient which allows for transport of glucose and amino acids (Cant et al., 1996). Adrenaline can affect Na^+/K^+ transport as epinephrine binds β-receptors which activate adenylate cyclase, thus increasing intracellular cyclic AMP concentration. Then cyclic AMP activates protein kinase A which phosphorylates a subunit of the Na^+/K^+ ATPase pump leading to an increased affinity for Na^+ and increased extracellular K^+ (Clausen, 2010).

Heat Stress Effects on Gastrointestinal Tract

Measures of intestinal permeability

Elevated body temperatures can damage the intestinal epithelial barrier and increase permeability to toxins such as endotoxins or LPS (Lambert, 2009). Heat stress effects on intestinal permeability have been studied on a number of models including humans, primates, in vitro, and rodents. Temperature exceeding 41℃ cause a marked decrease in transepithelial resistance (TER) in Caco-2 cell lines which correlated with a direct increase in paracellular permeability (Dokladny et al., 2006). These results were also observed in a rodent model of heat stress at a core temperature of 41.5℃ where TER decreased 60% below baseline after heat stress (Prosser et al., 2004). Increased permeability to a FITC-labeled dextran has been detected at core temperatures of 42.5℃ in rats (Lamb ert, 2002). Similarly, rats

heated to 42℃ for 30 minutes exhibited an increase in FITC-Dextran 24 h post hyperthermia (Singleton and Wischmeyer, 2006). Yang and colleagues (2007) found a decrease in TER in T84 monolayer cells at 41 and 43℃. This group also found a 3-fold increase in horseradish peroxidase (HRP) flux indicating increased permeability at 43℃.

Table 1.5 Effects of heat stress on intestinal morphology measures in various species

Species	Villous Height	Crypt Depth	Reference
Pigs	Decreased	Decreased	1
Pigs	Decreased	Decreased	2
Chickens	No Change	No change	3
Quails	Decrease	N/A	4
Rats	Decrease	Decrease	5
Fowl	Decrease	N/A	6
Pigs	No change	No change	7

1. Yu et al., 2010
2. Liu et al., 2009
3. Quinteiro-Filho et al., 2010
4. Sandikci et al., 2004
5. Sengupta and Sharma, 1993
6. Mitchell and Carlisle, 1992
7. Song et al., 2011

Tight Junction Proteins

Little data is available regarding how or if heat stress effects tight junction protein expression. Early studies indicated that heat stress decreased ZO-1 protein expression while occludin expression was increased at 41℃ in Caco-2 cell lines and this indicates some dysfunction of intestinal barrier proteins (Dokladny et al., 2006, 2008). The increase in occludin expression was blunted when cells were heated and given a heat-shock protein inhibitor (Dokladny et al., 2006). This suggests that heat-shock proteins may be necessary for up-regulation of occludin. In T84 monolayer cells, temperatures of 41 and 43℃ caused upre gulation of myosin light chain kinase (MLCK), correlating with an increase in permeability (Yang et al.,

2007). Despite little information available regarding expression, physiological changes in morphology have been determined using another method. Tight junctions have been analyzed via transmission electron microscopy in rat jejunum after 90 min of heat stress at temperatures varying from 41-42.5℃. Results indicate that tight junctions were intact and not damaged by increased temperatures; but confounding variables may have affected these results (Lambert et al., 2002). Intestinal morphology has been shown to change during heat stress in pigs (Liu et al., 2009; Yu et al., 2010). Table 1.5 shows the effects of heat stress on intestinal morphology.

Circulating Endotoxin

It is hypothesized that heat stress increases circulating endotoxin concentrations in a variety of species (Lambert, 2009). One study indicated that differences in endotoxemia following heat stress were not observed in heat-stressed rodents however an increase in gram negative bacteria in duodenal samples was detected, indicating some bacterial movement (DuBose et al., 1983). In other rat models, portal blood LPS concentration increased at temperatures of 41.5℃ (Hall et al., 2001). Similarly, Singleton and Wischmeyer (2006) found elevated plasma LPS concentrations in anesthetized rats 24 h post hyperthermia. Increased plasma LPS levels have also been detected in classical heat-stroke patients and this was correlated with an increase in core temperature. Even after patients were cooled, LPS concentrations remained elevated above controls, indicating some level of intestinal permeability may remain for a longer period of time (Bouchama et al.,

1991). Endotoxin has been found to enter circulation at core temperatures at or above 40℃ and concentration of LPS in the circulation increases with increased core temperature (Gathiram et al., 1988). Table 1.6 shows a list of species where leaky-gut is induced during heat stress.

Table 1.6 Effects of heat stress on intestinal permeability in various species

Species	Technique	Response	Reference
Rats	Plasma LPS[a]	Increase	1,7,10
Humans	Plasma LPS	Increase	2
Primates	Plasma LPS	Increase	3
Dairy Goats	Plasma LPS	Increase	8
Chickens	Plasma LPS	Increase	11
Rats	Ussing Chambers - TER[b]	Decrease	4
Caco-2 Cells	Ussing Chambers - TER	Decrease	5
Rats	Ussing Chambers - FITC[c]	Increase	6,7
T84 Cells	Ussing Chambers - TER	Decrease	9
T84 Cells	Ussing Chambers - HRP[d] flux	Increase	9

[a]Lipopolysaccharide
[b]Trans-epithelial Electrical Resistance
[c]Fluorescein Isothiocyanate-labeled Dextran
[d]Horseradish Peroxidase

1. Hall et al., 2001
2. Bouchama et al., 1991
3. Gathiram et al., 1988
4. Prosser et al., 2004
5. Dokladny et al., 2006
6. Lambert, 2002
7. Singleton and Wischmeyer, 2006
8. Wang et al., 2010
9. Yang et al., 2007
10. Lim et al., 2007
11. Cronje, 2007

Heat Stress Effects on Metabolism

Insulin

Studies on insulin and hyperthermia are relatively recent but demonstrate interesting and perplexing results. Heat stress affects the insulin response in a unique way compared to other types of physical stress. Under most stress conditions, catecholamine production (such as epinephrine) inhibits insulin release

(Katsuhiko et al., 1982) in order to increase gluconeogenesis/glycogenolysis (to provide glucose for extra-hepatic tissues), and adipose tissue lipolysis (to provide fatty acids for skeletal muscle) to meet energy requirements (Brockman, 1986). However, during a stressful heat-load, insulin is actually increased in most species, including a model of porcine malignant hyperthermia (Hall et al., 1980), rodents (Doi et al., 1982), and cows (Wheelock et al., 2010; O'Brien et al., 2010) despite a decrease in feed intake, reduction in blood glucose, loss of body weight and decrease in a calculated energy balance. In addition, the insulin response to a glucose bolus during heat stress is not altered compared to a thermal-neutral environment although the response is weaker in a pair-fed thermal neutral model (Itoh et al., 1998; O'Brien et al., 2010; Rhoads et al., 2010). Glucagon is also decreased in malignant hyperthermic pigs (Hall et al., 1980) and this also leads to decreased availability of glucose and lipids for energy production. In a porcine model of endotoxemia (another type of stressor), insulin infusion decreased plasma glucagon and TNF-α suggesting that insulin may have anti-inflammatory effects (Brix-Christensen et al., 2004).

Interestingly, it appears that the immune system influences parameters of glucose homeostasis. For example, despite reduced feed intake, experimentally induced mastitic cows have increased circulating insulin levels (Waldron et al., 2006). In addition, LPS IV infused steers have immediate and extreme hyperinsulinemia (i.e. > 30 fold; Rhoads et al., 2009b) and this occurs with only a mild decrease in plasma glucose levels. As a consequence, it appears that heat-

induced leaky-gut may be mediating some of the inexplicable changes in post-absorptive metabolism (Baumgard and Rhoads, 2011).

Glucose Metabolism

Heat-stroke patients often experience hyperglycemia which could indicate an increase in glucose production, or a decrease in tissue uptake (Al-harthi et al., 1990). This has also been observed in a porcine model of terminal heat stress (Marple et al., 1974). The increase in production may come from an increase in glycogenolysis and/or gluconeogenesis (Rowel et al., 1968). It has been demonstrated that during an increased heat-load, dietary carbohydrates are not able to reduce glucose production by the liver (Angus et al., 2001) and hepatic glucose production increases (Fink et al., 1975). An increase in glycogen utilization may be due to enhanced anaerobic metabolism as lactate accumulation occurs in the muscle of heat-stressed humans (Young et al., 1985). Heat stress effects on blood glucose are not entirely conclusive as decreases in blood glucose levels have been observed in a variety of species including rats (Mitev et al., 2005), ruminants (O'Brien, et al., 2010) and chickens (Rahimi, 2005). The reasons for the inconsistencies may be due to the type of experimental model, plane of nutrition, and timing/severity of heat stress.

Lipid Metabolism

Stress hormones such as epinephrine normally induce lipolysis and increase circulating NEFA concentrations. Catecholamines are lipolytic and also inhibit insulin (Carey, 1998). However, during heat stress, mobilization of adipose tissue is

not observed in a number of species including malignant hyperthermic pigs, cows and rodents (Frascella et al., 1972; Hall et al., 1980; Rhoads et al, 2009a). In a porcine model of terminal heat stress, animals exhibited an increase in NEFA concentrations; however this model represented a severely acute bout of heat stress (Marple et al., 1974).

Heat stress also decreases fatty acid oxidation in humans (Jentjens et al., 2002). A heat load seems to favor retention of body fat as heat-stressed piglets have a greater percentage of body fat compared to piglets in thermal neutral conditions (Collin et al., 2001a). It is interesting to note the relationship between carbohydrate and lipid metabolism during heat stress. Oxidizing fatty acids are less efficient at producing ATP (~13% less) than that of glucose (Baumgard et al., 2007) which may help explain why more carbohydrates are utilized and adipose tissue is not mobilized during a heat-load.

Protein Metabolism

Protein metabolism is affected by heat stress as muscle breakdown may occur. Tissue break down causes an increase in blood urea nitrogen (BUN) levels and this is often used as an indicator of muscle catabolism. Other indicators of muscle breakdown include increased plasma Nt-methylhistidine, creatinine, and creatine. Increases in these parameters have been observed previously during a heat-load in humans (Febbraio, 2001), poultry (Yunianto et al., 1997), and cows (Kamiya et al., 2006). Heat-stressed pigs also have an increase in plasma creatine kinase (CK) which is a measure of protein breakdown (Aberle et al., 1974) as it

converts creatine and ATP to phosphocreatine and ADP. As insulin promotes protein accretion (Allen, 1988), it is interesting that protein catabolism is increased during a heat-load. Reasons for this are not clear however some hypothesize that protein is broken down in order for the liver to utilize the carbon in amino acids for gluconeogenesis (Brockman, 1986). Table 1.7 summarizes the effects of heat stress on various plasma metabolites mentioned in this chapter.

AMP-Activated Kinase (AMPK)

AMPK may be especially sensitive to heat stress as this type of stress increases ATP requirements while at the same time decreasing ATP production via oxidative phosphorylation (Corton et al., 1994). In skeletal muscle, AMPK can be activated by muscle contraction which decreases the ATP/AMP ratio by utilizing available ATP for the contraction cycle (Rasmussen et al., 1998). In rodents, AMPK was differentially regulated in muscle as it increased in type 1 skeletal muscle but decreased in type 2, perhaps indicating that one type of muscle may be more affected by heat stress (Sanders, 2010). Also in rodents, liver AMPK remained unchanged, indicating the liver's ability to compensate for increased energy demands (Sanders, 2010). Heat stress has also been shown to increase tissue hypoxia and oxidative stress (Sato et al., 1991) and these physiological states also increase cellular AMPK concentrations (Mu et al., 2001; Fryer et al., 2002). Therefore, although direct studies of heat-stress effects on AMPK are difficult to find, comparisons may be made by observing secondary effects.

Na^+/K^+ ATPase

Very little research has been conducted regarding how heat stress affects Na^+/K^+ ATPase pump activity (Francesconi et al., 1997). One of few known studies in heat-stressed broiler chickens produced a significantly higher sodium pump activity in kidney and brain tissue but total ATPase activity (including all pumps) was inhibited (Chen et al., 1994). Other related stress types such as exercise, and elevated thyroid hormone lead to increased pump activity in humans (Benziane and Chibalin, 2008). Increased thyroid hormone has been correlated specifically with increased active Na^+/K^+ transport in human skeletal muscle (Iannello et al., 2007). Oxidative stress which has been associated with an increased heat-load (Ozturk and Gumuslu, 2004) may decrease sodium pump activity. In ox brain, gerbil brain, and dog kidney, H_2O_2 infusion induced a decrease in pump activity, which was associated with an increase in reactive oxygen species (ROS) (Dobrota et al., 1999).

Table 1.7 [a]The effects of heat stress on plasma metabolites in various species

Metabolite	Species	Response	Reference
Glucose	Humans	Increase	1, 27, 28
	Rats	Decrease	2
	Rats	Increase	16, 26
	Pigs	Increase	3,4
	Chickens	Decrease	5
	Cows	Decrease	6, 7, 8, 9, 10
Insulin	Cows	Increase	10
	Pigs	Increase	3
	Rats	Increase	25
	Steer	Increase	11
	Humans	No change	12
LPS[b]	Rats	Increase	13
	Humans	Increase	14
	Primates	Increase	15
NEFA[c]	Rats	Decrease	16, 17, 18
	Chickens	Fluctuates	19
	Pigs	Decrease	2
	Sheep	Decrease	20
	Cows	Decrease	6, 7, 9, 10, 20, 21
BUN[d]	Cows	Increase	10,11
	Humans	Increase	23
Cholesterol	Cows	Decrease	8, 22
	Humans	Unchanged	24

[a]Adapted from: Wheelock 2008; Sanders 2010
[b]Lipopolysaccharide
[c]Non-esterified fatty acids
[d]Blood urea nitrogen

1. Febbraio, 2001
2. Mitev et al., 2005
3. Hall et al., 1980
4. Prunier et al., 1997
5. Yalcin et al., 2009
6. Rhoads et al., 2007
7. Rhoads et al., 2009a
8. Shehab-El-Deen et al., 2010
9. Shwartz et al., 2009
10. Wheelock et al., 2010
11. O'Brien et al., 2010
12. Kappel et al., 1997
13. Hall et al., 2001
14. Bouchama et al., 1991
15. Gathiram et al., 1988
16. Frascella et al., 1977
17. Burger et al., 1972
18. Frankel, 1968
19. Bobek et al., 1997
20. Sano et al., 1983
21. Abeni et al., 2007
22. Fuquay, 1981
23. Fukumoto et al., 1988
24. Francesconi et al., 1976
25. Torlinska et al., 1987
26. Simon, 1953
27. Monteleone and Keefe, 1969
28. Al-harthi et al., 1990

Heat Stress Effects on Cytokines

Cytokines are chemical messengers primarily released by macrophages, T and B cells, as well as other immune cells (Kelker et al., 1985). These messengers can be either pro- or anti-inflammatory, and both types may be activated in the presence of heat-stroke. Bacterial infections, as well as heat stress or other types of physiological stress can induce cytokine release (Bouchama et al., 1991; Drexler, 1995). A few major cytokines found to be associated with heat-stroke are: IL-1β, IL-6, IL-10, IFNγ, and TNFα. These cytokines are commonly elevated in patients admitted to hospitals with heat-stroke symptoms (Leon, 2007). Increased concentrations of IL-6 tend to be correlated with elevated mortality rates and IFNγ in serum is increased in over 50% of heat-stroke patients (Bouchama et al., 1993).

Cytokines also pay a role in gut permeability during times of increased stress. Increased permeability occurs due to elevated nitric oxide levels induced by cytokine stimulation. As mentioned previously, “leaky” gut leads to increased endotoxin concentrations in the blood and this elicits an immune response, thus releasing cytokines (Yan et al., 2006). These cytokines induce fever, or may actually cause hypothermia in the CNS. In the periphery, cytokine release causes systemic inflammation, leading to multi-organ damage, organ failure, and ultimately death (Leon, 2007).

CHAPTER 2
THE EFFECTS OF HEAT STRESS AND NUTRITIONAL STATUS ON METABOLISM IN GROWING PIGS

Abstract

Heat stress (HS) negatively affects pig performance variables and is thus a costly industry issue. However, whether HS directly or indirectly (via reduced feed intake) is responsible for the suboptimal production is not known. Crossbred gilts (n=48; 35±4 kg BW) were housed in constant climate controlled rooms in individual pens and exposed to 1) thermal neutral (TN) conditions (20℃; 35-50% humidity) with ad libitum intake (n=18), 2) HS conditions (35°C; 20-35% humidity) with ad libitum intake (n=24) or 3) pair-fed (PF in TN conditions [PFTN], n=6: to eliminate confounding effects of dissimilar feed intake[FI]) and sacrificed at 1, 3, or 7 d of environmental exposure. Individual rectal temperature (T_r), skin temperature (T_s), respiration rates (RR) and FI were determined daily. Pigs exposed to HS had an increase (P<0.01) in T_r (39.3 vs. 40.8℃) and a doubling in RR (54 vs.107 bpm). HS pigs had an immediate (d 1) decrease (47%; P<0.05) in FI which continued through d 7; by design the PFTN controls nutrient intake pattern mirrored the HS group. TN pigs had 1.14 kg BW ADG throughout the experiment, while HS pigs lost 2.7 kg BW after 1 d but had gained 0.3 and 1.65 kg on d 3 and 7, respectively. By d 7, PFTN pigs had lost 2.47 kg BW. HS pigs had reduced (8.9%; P<0.05) plasma glucose compared to TN pigs. BUN concentrations were elevated (36%; P<0.05) in HS pigs after 24 h but return to TN control levels by d 3. Plasma cholesterol increased (P<0.05) in HS pigs at 24 h (25%) and d 3 (22%) but were similar to TN and PFTN pigs by d 7. HS pigs had an immediate and sustained increase (50%; P<0.05) in

plasma creatinine compared to both TN and PFTN controls. Plasma alkaline phosphatase (ALK) was progressively decreased (P<0.05) during HS, and was 50% lower in HS compared to TN and PFTN pigs on d 7. HS pigs had a 24 h increase (67%; P<0.01) in plasma NEFA levels compared to TN pigs, but NEFA levels were similar to TN pigs on d 3 and 7. PFTN pigs on d 7 had increased (110%; P<0.05) NEFA concentrations. Plasma insulin was increased (49%; P<0.05) in d 7 HS pigs PFTN controls. HS pigs tended (P<0.11) to have increased FAS activity compared to PFTN pigs. G6PDH activity decreased (P<0.05) in HS pigs compared to TN and PFTN pigs. Irrespective of day, HS pigs tended (P=0.06) to have increased LD Na^+/K^+ ATPase activity (52%). Liver Na^+/K^+ ATPase activity was not different between the TN and HS pigs, however, PFTN pigs had decreased pump activity compared to the HS and TN pigs (23%; P=0.06).

Introduction

While the causes remain debatable, there is little doubt global warming is occurring (Bernabucci et al., 2010). In fact, ambient temperatures in North America are predicted to raise 2-3℃ by 2100 (Luber and McGee hin, 2008). Humans and animals are sensitive to increasing temperatures and can become ill or even die due to heat stress related illnesses. For example, in 2003 more than 50,000 Europeans died during a two week heat-wave (Kosatsky, 2005; Patz et al., 2005) and human mortality is likely to increase as global warming continues and the occurrence and severity of heat-waves become more frequent (Hayhoe et al., 2004).

Animal agriculture is also severely affected by heat stress as the United

States swine industry loses over $300 million annually and global loses are in the billions (St-Pierre et al., 2003). The heat-induced economic burden is due to increased morbidity, mortality, suboptimal growth, inefficient nutrient utilization, poor sow performance, decreased carcass value and carcass processing problems (St-Pierre et al., 2003). The deleterious effects of climate change on pig production variables are likely to increase in the future as genetic selection for lean tissue accretion enhances the animal's sensitivity to heat stress (Nienaber and Hahn, 2007). Consequently, climate change threatens the global protein food supply chain and compromises the competiveness of the US hog industry (Godfray et al., 2010).

A prerequisite to developing mitigation strategies to reduce heat-related human illness/death and maximize animal performance during the warm summer months, is a better understanding of how environmental-induced hyperthermia affects post-absorptive metabolism and nutrient partitioning. Heat-stressed animals decrease feed intake (presumably to minimize heat production) and prior research suggests this nutrient intake reduction is responsible for decreased pig performance parameters (DeShazer et al., 2009). However, post-absorptive changes in nutrient partitioning in some heat-stressed models do not reflect normal metabolic changes observed in thermal neutral animals on a similar plane of nutrition. For example, despite marked reductions in nutrient intake, heat-stressed ruminants have increased basal and stimulated circulating insulin concentrations (Itoh et al., 1998; O'Brien et al., 2010; Wheelock et al., 2010). The altered insulin variables may also explain why heat-stressed animals do not mobilize adipose tissue triglycerides

despite being in a catabolic state (Rhoads et al., 2009a; Shwartz et al., 2009; O'Brien et al., 2010). Insulin and effective insulin action appears to play a key role in the heat stress response and this is seemingly conserved amongst species. For example, rodent data suggests that experimental-induced diabetics are more sensitive to heat stress and exogenous insulin increases survival time during a severe thermal load (Niu et al., 2003; Najemnikova et al., 2007). In addition, diabetic humans have a higher risk of suffering from heat-related illnesses (Shuman, 1972; Semenza et al., 1999; Shendell et al., 2010). The changes in insulin action may explain why growing rodents (Schmidt and Widdowson, 1967; Katsumata et al., 1990), poultry (Geraert et al., 1996; Yunianto et al., 1997) and pigs (Heath et al., 1983; Nienaber et al., 1987; LeBellego et al., 2002) have more carcass lipid (insulin is a potent lipogenic stimulant) than their thermal neutral contemporaries.

Much of the heat stress research has been conducted on rodent, poultry, and ruminant models (Doi et al., 1982; Yalcin et al., 2009; Rhoads et al., 2009a) and less is known about how heat stress affects metabolism in humans and swine. Pigs are an agriculturally important species and are frequently used as a model for humans. Pigs and humans share many similarities including: body weight, digestive tract physiology (Guilloteau et al., 2010), cardiovascular system, renal morphology (Miller and Ullrey, 1987) and both have a substantial amount of subcutaneous adipose tissue. However, a key difference (with regards to heat stress) between the two species is the mechanism of heat dissipation. Pigs have little or no functional sweat glands, thus rely heavily upon convection and panting for heat dissipation (Curtis,

1983), while humans are effective sweaters and rely primarily on evaporative cooling (Glazer, 2005).

Study objectives were to metabolically characterize a porcine model of heat stress and differentiate between the direct and indirect effects (mediated by reduced nutrient intake) of a thermal load on production parameters and post-absorptive metabolism in growing pigs. We hypothesized that heat directly (independent of reduced nutrient intake) effects post-absorptive metabolism and this altered energetic homeostasis may explain the phenotypic change observed (increased carcass lipid) in heat-stressed animals.

Materials and Methods

Animals and Treatment

Iowa State University Institutional Animal Care and Use Committee approved all procedures involving animals. Female crossbred gilts (n = 48, 35 ± 4 kg BW) were selected by body weight and housed in individual pens (with individual feeders and waters) in one of two rooms (24 pens/room) at thermal-neutral conditions. Animals were allowed to acclimate to their environment for 5 d at the Iowa State University Swine Nutrition farm prior to start of the experiment. Pigs were assigned to one of three environmental treatments: 1) thermal-neutral (TN) conditions (20℃; 35-50% relative humidity) with ad libitum feed intake, 2) heat stress (HS) conditions (35℃; 20-35% relative humidity) with ad libitum in take or 3) TN conditions but pair-fed (PFTN) to mirror the nutrient intake of the HS pigs. To evaluate the temporal response to environment, pigs in the TN (n=18) and HS (n=24) conditions were

sacrificed at 1, 3 and 7 d post initiation of environment treatment. The PFTN pigs (n=6) were only sacrificed at 7 d post initiation of nutrient restriction.

After environmental initiation, reduced feed intake in the HS pigs was calculated daily based on the percentage decrease from each animal's average feed intake prior to HS; the amount offered to PFTN pigs was reduced by that amount. The PFTN group lagged one day behind the 7 d heat stress pigs in order to calculate and implement feed intake reductions. Pair-feeding was used to eliminate confounding effects of dissimilar feed and nutrient intake. Individual animal feed intake was determined daily at 0800 h. Pair-fed pigs were fed calculated amounts thrice daily at (0700, 1200, and 2000 h) in an attempt to reduce large post-prandial shifts in carbohydrate and lipid metabolism.

Regardless of environmental treatment, all animals were fed the same diet throughout the duration of the experiment. Samples were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories. Feed was analyzed in duplicate for proximates of crude protein (LECO), crude fat, moisture, ash, and crude fiber. A standard protein hydrolysate package for amino acid analysis of: Asp, Thr, Ser, Glu, Pro, Gly, Ala, Met, Val, Ile, Leu, Tyr, Phe, His, Lys, Arg was also performed (Table 2.1).

Table 2.1 Ingredients (DM basis) and chemical composition of diet

Ingredient	% DM	kcal
Corn	61.65	1555
Soy Bean Meal	20.65	1536
Dried Distillers Grains (DDGS)	15.00	1552
HOI-45-30[a]	2.15	778
MonCal21%	0.33	
Lysine	0.11	
Methionine	0.03	
Threonine	0.03	
Copper	0.05	
Chemical Analyses, W/W%		
Aspartic Acid	1.73	
Serine	0.81	
Glutamic Acid	3.19	
Proline	1.10	
Glycine	0.76	
Alanine	1.02	
Valine	0.89	
Isoleucine	0.76	
Leucine	1.72	
Tyrosine	0.64	
Phenylalanine	0.92	
Histidine	0.49	
Arginine	1.14	
Crude Protein	17.51	
Moisture	10.89	
Crude Fat	3.68	
Ash	4.39	

[a]Vitamin Pre-mix

Each room's temperature and humidity were monitored by a data recorder (Lascar® model EL-USB-2-LCD, Erie, PA) which recorded environmental data every 30 min. Each room's ambient temperature was controlled but humidity was not governed. All pigs were monitored continuously for signs of distress. Body temperature parameters were obtained four times daily (0800, 1200, 1600, and 2000 h). Rectal temperatures were recorded with a digital thermometer (Top care®, Waukegan, IL), skin temperatures at the shoulder and ham were recorded with an

infrared temperature gun (Extech® instruments Model 42505, Waltham, MA) and respiration rates (breaths/min) calculated with a stopwatch. Carcass stored heat was calculated by: T_r℃ x specific heat of tiss (0.8℃) x BW (kg) as previously described by (Silanikove, 2000). Heat-stressed pigs were removed from the room and cooled down with cool water if rectal temperature exceeded 41.0℃ (105.8℉). Cooling time was standardized at 10 min, at which point the animals were returned to their respective pens. There were not sufficient data to conduct statistical analysis at 1600 h for daily body temperatures.

Body weights were recorded on all animals at the beginning of the experiment and immediately preceding sacrifice. Jugular vein blood was obtained (10 mL BD® vacutainers containing 143 U.S.P units of sodium heparin) while the animals was nose-snared and immediately sacrificed using the captive bolt technique followed by exsanguination. Blood was also obtained after sacrifice from the hepatic portal vein (using a 22G 5 mL syringe; BD® LEUR-LOK™, Franklin Lakes, NJ) and placed into sodium heparin vacutainers. Blood harvested from the jugular and hepatic portal vein was centrifuged at 1300 x g to obtain plasma, alliquotted into five 1.5 mL microcentrifuge tubes and stored at -20℃ for later an alysis.

All tissues were harvested within 12 min of death and included: muscle (longissimus dorsi, psoas major, red and white semitendinosus), liver (left lobe and caudette lobe), pancreas and adipose tissue from the nape of the neck. Tissue samples were snap-frozen in liquid nitrogen and stored at -80℃ until later analyses.

Total Protein Determination

Total protein concentration of all tissues and plasma was measured for various lab analyses and concentration was determined in triplicate using a commercially available assay kit (Pierce® BCA microplate protein assay kit, Pierce, Rockford, IL). Bovine serum albumin (BSA) and MQ H_2O combinations were used as standards and samples were analyzed in triplicate. After addition of standards, MQ H_2O was added to all non-standard wells and then samples were vortexed and added to the plate. Finally, 160 µL of the working color reagent (50 parts Solution A to 1 part Solution B) was added to all wells. The plate was incubated for 1 h at room temperature and then read at 562 nm using a Synergy 4 microplate reader (Bio-Tek, Winooski, VT). Specific dilutions and sample quantities for each tissue will be mentioned along with their related analysis in later sections.

Metabolite Assays

Blood obtained from the jugular vein prior to sacrifice was centrifuged and one plasma aliquot was analyzed at the Iowa State Veterinary Diagnostic Laboratory for a routine blood panel (large animal complete panel consisting of blood urea nitrogen, creatinine, glucose, total protein, albumin, creatine kinase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, total bilirubin, calcium, phosphorus, magnesium, sodium, potassium, chloride, and total carbon dioxide) as well as triglycerides, cholesterol and non-esterified fatty acids. All plasma samples were analyzed on a Vitros® 5.1 FS chemistry analyzer (Ortho Clinical Diagnostics, Rochester, NY) which simultaneously measures plasma

samples in duplicate for all the aforementioned diagnostic tests.

Plasma insulin concentrations were determined enzymatically using a commercially available kit validated for use in our laboratory. Plasma samples were analyzed in duplicate using an ELISA kit solid phase two-site enzyme immunoassay based on the sandwich technique, (Mercodia Porcine Insulin ELISA, ALPCO Diagnostics, Salem, NH). The assay contained 5 calibrators as well as internal serum pools with low, intermediate, and high porcine insulin concentrations. The assay was conducted in 96-well microplates and read at 450 nm using a Synergy 4 microplate reader (Bio-Tek, Winooski, VT). Inter- and intra-assay coefficients for the plasma insulin assay were 4.25, 4.1% respectively.

Adipose Tissue Enzyme Activity

Adipose tissue was freeze-ground in liquid nitrogen. Approximately 500 mg of adipose tissue was homogenized in 5 mL of lysis buffer consisting of: 0.3 M sucrose, 30 mM trizma base pH 7.4, 1 mM EDTA pH 8.0, and 1 mM Dithiothreitol (DTT). Samples were centrifuged at 3,300 xg for 10 min at 4℃ (Ingle et al., 1973). Following centrifugation, protein supernatant was collected for each sample and stored at -80℃ for later analysis in 1.5 mL microcentri fuge tubes. Activity of glucose-6-phosphate dehydrogenase (G6PDH), fatty acid synthase (FAS), and glycerol-3-phosphate dehydrogenase (G3PDH) were determined by reading the absorbance at 340 nm every 30 sec for 7 min at 25℃ on Synergy 4 microplate reader (Bio-Tek, Winooski, VT). Enzyme activity was calculated from the change in absorbance at 340 nm and expressed as nmol NADPH per min per mg protein. For

G6PDH activity (n=48), protein was added to 100 mL of reaction buffer (125 mM tris pH 7.5, 1 M KCl, 50 mM $MgCl_2$, 0.25 mM NADP+, 5 mM glucose-6-phosphate) and absorbance was read at 340 nm (Deutsch, 1983). For G3PDH activity (n=24), protein lysate was added to 100 mL of reaction buffer (125 mM Triethanolamine-HCl pH 7.5, 2.5 mM EDTA, 0.5 mM NADH, 1.1 mM dihydroxyacetone phosphate, 0.125 mM b-mercaptoethanol) and absorbance was read at 340 nm (Gamou et al., 1990). Activity of G3PDH was only analyzed for d 7 animals. For FAS activity (n=48), protein was added to 100 mL of reaction buffer (50 mM potassium phosphate pH 6.8, 2.5 mM b-mercaptoethanol, 0.26 mM NADPH, 0.2 mM acetyl CoA, 0.2 mM malonyl CoA) and absorbance was read at 340 nm (Ingle et al., 1973). Reagents for enzyme activities were obtained from Sigma-Aldrich (St. Louis, MO). Absorbance values were used in the Beer-Lambert equation to calculate a final activity value. All three assays were conducted in the linear range with respect to amount of read time as well as amount of enzyme.

Na^+/K^+ ATPase Activity

Longissimus dorsi (LD) muscle and whole liver tissue was freeze-ground in liquid nitrogen and stored at -80℃ for later use. Prior to the start of the assay, 500 mg was homogenized in 4 mL of sucrose buffer (pH 7.4) consisting of: 50 mM sucrose, 1 mM Na_2EDTA, and 20 mM tris base and centrifuged at 1000 xg for 10 min for protein extraction. Protein extracts were separated into 5 aliquots: two for water, two for ouabain, and one for BCA protein analysis. Proteins with either MQ H_2O or 20 mM Ouabain were pre-incubated for 15 min with Na^+/K^+ ATPase reaction

buffer (pH 7.0; 2000 mM NaCl, 100 mM KCl, 50 mM $MgCl_2$ and 250 mM HEPES) and then incubated for 45 min after addition of fresh 105 mM ATP to start the reaction. After 45 min the reaction was terminated using ice-cold 50% trichloroacetic acid. Samples were centrifuged at 1500 xg for 10 min to obtain the final product which was present in the supernatant (Fuller et al., 2003). Lastly, samples were analyzed for the presence of inorganic phosphate using the Molybdovanadate method (Ueda and Wada, 1970) and assessed in triplicate at a wavelength of 400 nm using a Synergy 4 microplate reader (Bio-Tek, Winooski, VT). Specific Na^+/K^+ ATPase activity was determined by the difference in P_i production from ATP in the presence of absence of ouabain (specific Na^+/K^+ ATPase inhibitor). Unspecific phosphate hydrolysis was correlated by measuring P_i freed in the absence of protein suspension. The inter- and intra-assay coefficients for LD, and liver phosphorus determination were 4.2, 4.3 and 3.9, 3.8%, respectively.

Western Blot

Longissimus dorsi tissue samples (~500 mg tissue in 5 mL extraction buffer) were homogenized and proteins were extracted and analyzed for protein content and frozen at -80℃ for later use. The extraction b uffer consisted of: 10 mM sodium monophosphate, 10 mM sodium diphosphate and 2% SDS. Aliquots from each sample were standardized to a common concentration. Samples were placed into a new 1.5 mL tube containing 50 µL of loading dye and SDS extraction buffer. Load checks were run on all samples using 15% polyacrylamide gel (acryl-amide:N,N'-bis-methylene acrylamide =100:1) consisting of 0.1% SDS (wt/vol), 0.05% TEMED

(vol/vol), 0.05% APS (vol/vol) and 0.5 M Tris-HCL pH 8.8 (Melody et al., 2004). Gels were then stained with Coomasse brilliant blue (100 mg brilliant blue/100 mL destain). After staining, gels were washed with destain (consisting of 40% methanol, 7% glacial acetic acid, and water) over night (Sasse and Gallagher, 2009).

Tissue homogenates (40 µg) were separated by SDS (10%) polyacrylamide gel electrophoresis (SDS-PAGE) with the same recipe as mentioned prior for load checks. Gels were run under reducing conditions and transferred to PVDF membranes. Prior to blocking, both gels and membranes were stained with Ponceau S stain (0.1% Ponceau S in 5% acetic acid) to ensure that proteins were transferred correctly (Sasse and Gallagher, 2008). Membranes were washed with distilled water to remove stain before proceeding. Membranes were blocked for 1 h in 5% non-fat dry milk (NFDM) in PBST. Membranes were then blocked in primary antibody with 10 mL PBST overnight. After blocking in primary antibody, membranes were washed 3 X for 10 min each with PBST (1X PBS, 0.1% Tween-20) then incubated with secondary antibody for one hour. Finally, membranes were washed 3 X for 10 min each with PBST (Huff-Lonergan et al., 1996). For detection, Amersham™ ECL Plus™ was used (50 µL of reagent A and 2 mL of reagent B) and membranes were incubated for 5 min in the dark before being imaged. Band densities were quantified by densitometry using FOTO Analyst® Luminary/FX® (Fotodyne Inc, Hartland, WI) and TotalLab Quant (Total Lab®, Newcastle Upon Tyne, UK). One control pig was used on all gels and bands were standardized to the density of the control animal.

Table 2.2 Primary and secondary antibody dilution and source information for Western blot analysis

Protein[a]	Tissue[b]	1°dilution	1°source	2°source	2°dilution
HSP 70	LD	1:1000	Mouse monoclonal	Anti-mouse[c]	1:10,000

[a]Heat shock protein 70 (HSP70)
[b]Longissimus Dorsi (LD)
[c]Anti-mouse from Cell Signaling Technology, Danvers, MA

AMP-Activated Protein Kinase (AMPK)

Phosphorylation of AMP-activated protein kinase (AMPK) was measured in longissimus dorsi muscle (LD), and liver samples from all animals using a commercially available solid-phase sandwich ELISA kit (Pathscan© Phospho-AMPK α Thr172, Cell Signaling Technology). Tissue (200 mg) was homogenized in 2 mL of lysis buffer consisting of: 20 mM tris-HCL (pH 7.5), 150 mM NaCl, 1mM Na_2EDTA, 1 mM EGTA, 1% triton, 20 mM sodium pyrophosphate, 25 mM sodium fluoride, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, and 1 µg/mL leupeptin. Samples were centrifuged at 2500 xg for 10 min and separated into two aliquots; one for the ELISA, and one for BCA protein analysis.

Prior to sample analysis, a BCA protein assay was conducted in order to determine total protein content and dilution needed for the samples. Samples required dilution if the protein content was greater than 1 mg/mL. Diluent provided with the kit was used to dilute samples in the 96-well plate, and the plate was incubated overnight at 4℃. The plate was washed 4 X with 1 X wash buffer (provided by the kit) 200 µL each time for each well. After each wash, the plate was physically struck against paper towels in order to remove any residual solution left in the wells. Next, 100 µL of the detection antibody was added to each well. The plate

was then incubated for 1 h at 37℃. After incubation , the plate was washed again as previously described, and 100 µL HRP-linked secondary antibody was added and incubated for 30 min at 37℃. The plate was then wash ed a final time and 100 µL of TMB substrate was added to each well to initiate the reaction and incubated for 10 min at 37℃. Finally, 100 µL of stop solution was add ed to each well and the plate was shaken for a few seconds. At this time the plate was read at 450 nm using a Synergy 4 microplate reader (Bio-Tek, Winooski, VT). Specific phosphorylation activity was based on endogenous levels of AMPK α when phosphorylated at Thr172. Values were calculated by standardizing samples based on protein content. Total protein content was determined from the BCA method (5.83 dilution factor for LD and liver). Total Absorbance was calculated in duplicate by factoring in sample dilution (1:1 for LD, 1:4 for liver). The final value was calculated as arbitrary units of total absorbance/total protein (mg/mL).

Statistics

All data were statistically analyzed using the PROC MIXED procedure of SAS version 9.1 (SAS Inst. Inc. Cary NC). Data are reported as LSmeans and considered significant if $P < 0.05$. Data were evaluated using two distinct models. For daily measurements (body temperatures, respiration rates and feed intake) each animal's respective parameter was analyzed using repeated measures with an auto-regressive covariance structure and day as the repeated effect. The model included environment, day, and the interaction. Analysis also tested differences between all three environments (TN, PFTN, HS) on d 7.

Results

Irrespective of day or time of day, pigs exposed to HS had an increase (P<0.01) in rectal temperature (39.3 vs. 40.8℃) compared to TN pigs and this increase was maintained throughout the duration of the experiment (Table 2.3). Rectal temperatures in PFTN pigs were decreased (P<0.05) compared to TN pigs by d 1 and this difference in body temperature was maintained throughout all 7 d (Figure 2.1). Skin-temperatures at the shoulder and ham were increased (P<0.05) during heat stress compared to TN and PFTN pigs (37.5 vs. 43.3℃ and 37.3 vs. 43.1℃, respectively). Skin temperature variables did not differ (P>0.1) in PFTN vs. TN pigs (Table 2.3). Independent of time of day, compared to TN and PFTN pigs, HS animals had an immediate increase (P<0.05) in respiration rate on d 1 (49 vs. 136 bpm). Respiration rates decreased (P<0.05) in HS pigs on d 2 and 3, but remained elevated (P<0.05) compared to TN and PFTN pigs (55 vs. 115 bpm) throughout d 7 (Figure 2.1). Respiration rates did not differ between PFTN and TN pigs (Table 2.3).

TN pigs consumed 1.96 kg/d of feed and this did not differ (P>0.1) over time (Figure 2.2). Compared to TN pigs, HS pigs had an immediate decrease (47%; P<0.05) in FI by d 1 and this extent of decrease continued through d 7 (Table 2.4). By design, PFTN pig's nutrient intake pattern mirrored intake of the HS group (Figure 2.2). During the experiment, TN pigs had an ADG of 1.14 kg/d and had body weight gains of 1.27, 3.12, and 7.76 kg at 1, 3, and 7 d respectively (Table 2.4; Figure A-1.3). HS pigs lost 2.7 kg of BW at 1 d post-HS initiation but had gained

0.03 and 1.65 kg BW by d 3 and 7 respectively (Table 2.4; Figure 2.3). The PFTN pigs lost 2.47 kg BW by d 7 (Table A-1.1; Figure 2.3).

Overall, HS pigs had reduced (P<0.05) plasma glucose levels compared to TN controls and this is primarily due to differences at d 3 and 7 (Table 2.5; Figure A-1.4). At d 7, analysis of all three environments indicates no treatment differences in plasma glucose levels (Figure 2.4). Compared to TN controls, HS pigs had an immediate (d 1) and marked (P<0.05; 67%) increase in blood NEFA levels. NEFA levels in HS pigs decreased on d 3 and d 7 and were similar to TN controls on both time points (Table 2.5; Figure A-1.4). By d 7, circulating NEFA concentrations in PFTN pigs were increased 137% compared to TN and HS pigs (Figure 2.4). Blood urea nitrogen levels in HS pigs increased 35% (P<0.05) on d 1 compared to TN pigs, but had reduced (18%; P<0.05) BUN levels compared to TN controls on d 3 and 7 (Table 2.5; Figure A-1.4). On d 7, BUN levels in PFTN pigs were similar to both TN and HS pigs (Figure 2.4). Plasma cholesterol increased (P<0.05) in HS pigs at 1 d (25%) and d 3 (22%; Table 2.5; Figure A-1.5) but were similar to TN and PFTN pigs by d 7 (Figure A-1.5). HS pigs had an immediate and sustained increase (50%; P<0.05) in plasma creatinine compared to both TN and PFTN controls (Table 2.5; Figure A-1.7). Plasma insulin levels were decreased (P<0.05; 0.05 ng/mL) in HS pigs compared to TN pigs (P<0.05; Table 2.5). On d 7, plasma insulin values were lowest in PFTN pigs and increased in HS and TN pigs (0.06^{a}, 0.12^{b}, and 0.17^{c} ng/mL, respectively; Figure 2.4).

There was a treatment by day effect (P=0.05) for LD HSP 70 expression as it

was increased 151% in HS pigs at d 1 and temporally decreased (but was still elevated 75% compared to TN pigs) by d 7 (Figure 2.6). Overall, HS pigs tended (P=0.06) to have increased (52%) LD Na^+/K^+ ATPase activity compared to TN pigs, although differences were largest (72%) at d 1 and became smaller with time (46 and 40% on d 3 and 7, respectively; Table 2.6). Analysis of just d 7 indicates there were no differences in LD Na^+/K^+ ATPase activity between TN, HS, and PFTN controls (Figure 2.5; Table A-1.3). Overall, the hepatic Na^+/K^+ ATPase activities did not differ between TN and HS pigs (Table 2.6). Analysis of all three environments on d 7 indicates PFTN pigs had a reduced (29%; P<0.05) hepatic Na^+/K^+ ATPase activity compared to both TN and HS pigs (Figure 2.5; Table A-1.3).

There were no overall differences between TN and HS pigs on LD phosphorylated-AMPK (Table 2.6) and d 7 analyses indicated similar phosphorylated-AMPK levels between all three treatments (Figure 2.5). Liver phosphorylated-AMPK levels also did not differ between TN and HS pigs at any time point (Table 2.6). However, PFTN pigs tended (P=0.08) to have reduced (24%) hepatic phosphorylated AMPK levels compared to TN and HS pigs (Figure 2.5).

Irrespective of day, adipose tissue FAS enzyme activity was decreased (30%; P<0.01) in HS pigs compared to TN controls (Table 2.7). FAS enzyme activity on d 7 tended (P=0.06) to decrease (35%) in HS pigs compared to TN pigs, but was increased 77% in HS compared to PFTN pigs (Figure 2.7). Irrespective of day, adipose tissue G-6-PDH activity was decreased (16%; P<0.01) in HS pigs compared to TN controls (Table 2.7). On d 7, activity of G-6-PDH decreased (24%; P<0.05) in

HS pigs compared to TN and PFTN controls (Figure 2.7). There were no treatment affects on the activity of adipose tissue G-3-PDH on d 7 (Figure 2.7).

Discussion

Heat stress is a health (human and animal) issue which reduces domestic animal production parameters and negatively affects the global agriculture economy. Determining how HS detrimentally affects energetic homeostasis in growing pigs may allow for future nutritional or pharmaceutical interventions to ameliorate the negative effects of increased environmental temperatures. An immediate effect of HS is a decrease in feed intake and this reduced appetite is presumably a strategy to minimize metabolic heat production. Caloric restriction has obvious effects on metabolism, therefore in order to differentiate between the direct and indirect effects (mediated by reduced nutrient consumption) of HS, we utilized a thermal neutral ad-libitum and a pair-fed thermal neutral model to eliminate the confounding effects of dissimilar feed intake.

Our HS protocol resulted in marked hyperthermia as all body temperature indices in the HS pigs were elevated compared to both TN and PFTN controls. The HS regimen was constant and the lack of a cyclical or diurnal pattern of ambient temperature prevented the pigs from returning to euthermia during the cooler hours of the night. Consequently, our heat stress protocol more closely resembles tropical or semi-tropical regions or southern regions of the USA. The PFTN pigs had a reduced body temperature (0.6℃) compared to the TN co ntrols and this likely represents a reduced heat increment of feeding. On a thermal energy basis, the

PFTN pigs had on average 195 kcal less total heat stored than TN pigs and this represents 6% of the difference in energy intake (TN pigs consumed 6577 kcal ME/d while the HS and PFTN pigs consumed 3456 kcal ME/d).

Heat stress conditions caused an immediate decrease in feed intake which remained lower (46%) than TN controls throughout the experiment. By design, PFTN pigs had a similar pattern of reduced feed intake as the HS group. Decreased feed intake during heat stress was expected as this is a highly conserved response amongst species (Collin et al., 2001b; DeShazer et al., 2009; Baumgard and Rhoads, 2011). The extent of intake decrease in the current experiment is higher than typically reported in the literature (McGlone et al., 1988; Becker et al., 1992; Le Bellego et al., 2002) and indicates our heat-load was severe and probably more reflective of intense heat stress. However, similar decreases in feed intake during heat stress have been observed in young pigs (48%; Collin et al., 2001b) and lactating sows (49%; Renaudeau et al., 2001), and demonstrates our model was experimentally and commercially relevant.

Both HS and PFTN conditions decreased pig body weight gain, while TN pigs continued to grow. Body weight loss in HS pigs was most severe at 1 d which represented an acute heat-load and by d 3 and 7 of HS, weight gain remained lower than TN pigs but higher than the PFTN pigs. Actually, by d 7 there was a 4.12 kg body weight difference between the HS and PFTN pigs. Although the chemical composition of that body weight difference is not known, the literature suggests that pigs and other species gain more adipose tissue while heat-stressed (Heath, 1983).

If we assume this is all adipose tissue, the 4.12 kg equals more than 37 Mcal of energy. However, it is likely that the 4.12 kg of body weight represents a combination of 1) a decrease in basal metabolic rate in HS pigs, 2) a difference in adipose accretion rates and 3) an increase in activity energy expenditure in the PFTN pigs (the PFTN pigs were visibly anxious while the HS pigs were much less active). Regardless, based upon gross changes in body weight, reduced nutrient intake in HS pigs appears to fully explain the decrease in live/gross carcass weight and this agrees with previous results in pigs (Safranski et al., 1997) and cattle (O'Brien et al., 2010).

Heat stress decreased circulating glucose concentrations compared to TN pigs and this appears to be due to decreased feed intake as differences between environments (HS vs. TN vs. PFTN) were not detectable at d 7. Our current results are similar to findings in heat-stressed pigs (Becker et al., 1992), and cows (Itoh et al., 1998; Ronchi et al., 1999; Shwartz et al., 2009). In contrast with our results, previous studies indicated an increase in blood glucose concentrations in poultry (Bobek et al., 1997), rabbits (Marder et al., 1990), pigs (Prunier et al., 1997) and humans (Fink et al., 1975). Reasons for the discrepancies in blood glucose in a myriad of studies may be due to the severity (both extent and acute vs. chronic) of the heat-load, physiological state, and nutritional status. Pigs fed a typical high carbohydrate diet primarily obtain circulating glucose from intestinal absorption (McMillin, 1990), but the contribution from gluconeogenesis will increase depending upon the extent and severity of nutrient restriction (Campbell, 1999) In addition,

circulating glucose (or any metabolite) is a combination of both pool entry (intestinal absorption or hepatic output) and pool removal (i.e. myocyte and adipocyte uptake) and thus the plasma concentration has severe limitations to understanding glucose dynamics/turnover.

Animals on a lowered plane of nutrition are typically hypoinsulemic (Berg et al., 2007) and this is a highly conserved post-absorptive effect amongst species. Our PFTN model had reduced plasma insulin compared to HS and TN pigs on d 7. Both HS and PFTN pigs were nutrient restricted (~50%) and while both had decreased circulating insulin compared to the TN pigs, the HS animals had 100% higher insulin levels than the PFTN pigs. This is unusual as they were both on a inadequate/suboptimal plane of nutrition. In addition, HS animals are thought to be in a catabolic state and epinephrine and cortisol both inhibit pancreatic insulin secretion (Katsuhiko et al., 1982). This peculiar insulin response agrees with a previous malignant hyperthermic pig model (Hall et al., 1980) as well as heat-stressed rodents (Torlinska et al., 1987), growing steers (O'Brien et al., 2010) and lactating cows (Wheelock et al., 2010). Increased insulin appears to be an important survival adaptation to elevated temperatures as diabetic rats (Frascella et al., 1977; Niu et al., 2003) and humans (Shuman, 1972; Bouchama et al., 2007) are more susceptible to heat stress. Compared to the PFTN controls, the HS pigs had reduced NEFA levels (discussed below) and it has recently been demonstrated that high NEFA levels blunt pancreatic insulin secretion (Boden and Shulman, 2002) and the differential NEFA levels between the two energetically similar models may help

explain differences in basal insulin levels.

Undernourished growing animals alter metabolism and mobilize adipose tissue and this is a classic glucose sparing mechanism to maximize skeletal protein accretion (Randle, 1998). As expected, after 7 d the PFTN pigs had increased plasma NEFA levels. However, HS pigs only had increased plasma NEFA concentrations at d 1, but had similar levels as TN pigs at d 3 and 7. This temporal pattern is strikingly similar to what is observed in heat-stressed chickens (Bobek et al., 1997). Reasons for the immediate increase in NEFA and subsequent decline are ill-defined but may correlate with the temporal pattern of stress hormones (i.e. cortisol, catecholamines) which have been shown to increase during acute heat stress (Beede and Collier, 1986), but have not been thoroughly characterized during chronic heat stress (especially in the pig model). Regardless, this eventual decrease in circulating NEFA also agrees with previous research demonstrating decreased NEFA levels in malignant hyperthermic pigs (Hall et al., 1980), rodents (Sano et al., 1983) and cattle (Rhoads et al., 2009a; Schwartz et al., 2009). Despite agreeing with heat stress literature, this is somewhat unexpected as heat-stressed animals are on a lowered plane of nutrition, losing body weight, and in a calculated negative energy balance. This is especially surprising as plasma stress hormones (i.e. cortisol and epinephrine) are increased during a heat-load, and both normally stimulate adipose tissue mobilization (Beede and Collier, 1986). Insulin is a potent lipolytic inhibitor and the aforementioned increase in basal insulin may explain the lack of a NEFA response during heat stress (Baumgard and Rhoads, 2011). This

mechanism (minimal adipose mobilization) may have evolved in order to survive a heat load as β-oxidation of fatty acids may produce more metabolic heat compared to oxidation of carbohydrates (Baumgard and Rhoads, 2007). This hypothesis is supported by evaluating the metabolic effects that occur during hypothermia. Animals in a cold environment increase NEFA and blood glucose levels and this has been observed in cold-stressed cows (Tamminga and Schrama, 1998), humans (Hurley and Haymes, 1982) and rodents (Doi et al., 1982). These alterations during hypothermia may be due to decreased plasma insulin (Doi et al., 1982) and demonstrate metabolic responses in a physiological state which are opposite of what occurs during heat stress.

Increased insulin levels may also help explain why heat-stressed animals (rodents, poultry, pigs) have more carcass lipid than bioenergetically expected (Heath, 1983; Prunier et al., 1997; Ronchi et al., 1999). Pigs on a high carbohydrate/ low fat diet primarily accrue adipose tissue via de novo fatty acid synthesis (as opposed to pre-formed fatty acid uptake) and FAS and G-6-PDH are two key enzymes involved with this process. In agreement with the circulating insulin levels, adipose FAS activity was almost double in the HS pigs compared to the PFTN controls. Interestingly, G-6-PDH was decreased in the HS pigs (compared to TN and PFTN controls), but this enzyme is multifunctional and one of its functions is to minimize oxidative damage (Kletzien et al., 1994). Decreased G-6-PDH activity may indicate a decrease in cellular NADPH which can lead to reactive oxygen species (ROS) and apoptosis (Mailloux and Harper, 2010). Consequently, it

appears HS pigs may be prone to oxidative stress (at least in adipose tissue) and this agrees with a variety of previous reports (Hall et al., 2001). Regardless of the ambiguous G-6-PDH data, the FAS results coupled with the plasma NEFA data suggest that both increased lipogenesis and blunted lipolysis contribute to increased carcass adipose tissue in heat-stressed pigs.

A response to malnutrition is skeletal muscle mobilization and circulating BUN and creatinine can be used as gross proxies of proteolysis. Compared to TN pigs, BUN levels were elevated after 24 h of HS, but returned to basal levels by 3 and 7 d and no differences were observed between the three environments on d 7. Although these results do not agree with previous models which demonstrated a sustained BUN increase in HS ruminants (O'Brien et al., 2010; Wheelock et al., 2010), this may be due to differential nutrient utilization in ruminants. Plasma creatinine in our model was also increased during HS compared to both TN and PFTN pigs. Creatinine can be an indicator of muscle catabolism as it is produced from the breakdown of creatine phosphate. Creatine phosphate is converted to creatine (and vice a versa) by creatine kinase during protein break down (Berg, 2007). Elevated plasma creatinine agrees with previous data in a baboon model of severe heatstroke (Bouchama et al., 2005) as well as in heat-stressed cows (Srikandakumar et al., 2003). The increased creatinine levels in HS may indicate skeletal muscle catabolism and this is different from the PFTN pigs which appear to conserve muscle mass during nutrient restriction. The lack of a sustained response of BUN also agrees with our plasma creatine kinase (CK) data which did not differ between

environmental treatments. However, it has previously been demonstrated that CK is increased in HS poultry (Yuinato et al., 1997) and cows (Schneider et al., 1988; Kamiya et al., 2006). In addition, Nt-methyl-histidine is another good indicator of muscle proteolysis and is increased during a heat-load in poultry (Yunianto et al., 1997) and lactating cows (Kamiya et al., 2006).

Severely heat-stressed animals presumably have an increase in energy expenditure (Brody, 1945; Klieber, 1961). Cellular ion pumping has a large energetic cost (Hardie, 2008) and it is the biggest component of the basal metabolic rate (Ianello et al., 2007). The Na^+/K^+ ATPase pump maintains cellular osmolarity and utilizes ATP to generate ion gradients and is responsible for 20-25% of the total O_2 required by muscle (Adeola et al., 1989). The reason for increased pump activity during HS (Chen et al., 1994) is not known, but catecholamines (Clausen and Hansen, 1977) and insulin (Erlij and Grinstein, 1979) increase pump action and both are elevated in HS animals (Frascella et al., 1977; O'Brien et al., 2010; Figure 2.4). As we hypothesized, the LD from HS pigs had increased Na^+/K^+ ATPase activity and this supports the idea that HS animals have increased basal energy requirements.

In contrast to skeletal muscle, hepatic Na^+/K^+ ATPase activity did not differ between HS and TN pigs. However, PFTN pigs had reduced Na^+/K^+ ATPase activity and this may be due to increased circulating blood NEFA levels, as fatty acids are thought to decrease pump activity in some tissues (Ianello et al., 2007). The exact reasons why there appears to be differential effects of heat stress on the Na^+/K^+ pump between tissues is of interest and needs to be evaluated further.

AMPK is a primary cellular energy gauge (Hardie, 2008) and is activated by (amongst others) a decreased ATP/AMP ratio (Kemp et al., 1999). Activated AMPK (via phosphorylation) stimulates cellular glucose uptake (via GLUT-4) and fuel (fatty acids and glucose) oxidation (Goodyear and Kahn, 1998). Based upon recent data (Sanders et al., 2010) we speculated that HS pigs would have increased LD levels of phosphorylated AMPK, but both the HS and PFTN pigs had similar levels as the TN pigs. This is surprising as the HS pigs had increased LD Na^+/K^+ ATPase activity and this is a large ATP utilizing system. However, we only measured phosphorylated AMPK and a better indicator of cellular AMPK status would include the ratio of total to phosphorylated AMPK (Kemp et al., 1999). Regardless, it initially appears that the intra-cellular energy levels of the LD may be adequate during HS.

Similar to the LD AMPK data, hepatic levels of phosphorylated AMPK did not differ between TN and HS pigs. In contrast, the PFTN pigs had a reduction in liver phosphorylated AMPK and this may indicate an energy conservation strategy. However, why there are differences in hepatic activated AMPK between the HS and PFTN controls is not known (both in similar nutritional states) but this supports other energetic data that suggests the heat-stressed liver somehow protects itself from metabolic and oxidative stress (Sanders et al., 2010).

Conclusions

Heat-stressed animals enlist energy-sparing mechanisms in order to reduce metabolic heat production. In order to do this, animals may preferentially utilize carbohydrates instead of lipids during a heat-load. Muscle proteolysis is increased

during heat stress, presumably to provide carbons for energy utilization. Also during heat stress, insulin is increased which reduces blood glucose, inhibits adipose lipolysis, and increases adipose lipogenesis. Elevated insulin levels may be a metabolic adaptation to preferentially oxidize carbohydrates as oxidation of fatty acids produces more metabolic heat. If this is indeed the case, the liver would need to maintain energy homeostasis in order to support hepatic gluconeogenesis, and glycogenolysis. This is supported by decreased NEFA levels, increased fatty acid synthesis (FAS), and decreased blood glucose concentrations. In addition, hepatic gluconeognesis would be inhibited by a rise in cellular AMP. Measures of hepatic energy homeostasis indicate that the liver may be able to compensate for increased energy demands during a longer period of heat stress.

Table 2.3 Effect heat stress (HS) and plane of nutrition on body temperature parameters in growing pigs

	Environment				*P*		
Parameter	TN[1]	HS	PFTN[2]	SEM	Trt	Day	T x D[3]
Rectal Temperature, ℃							
0800 h	39.1[a]	40.4[b]	38.7[c]	0.1	<0.01	<0.01	<0.01
1200 h	39.1[a]	40.7[b]	38.8[c]	0.1	<0.01	<0.01	<0.01
2000 h	39.3[a]	40.9[b]	38.7[c]	0.1	<0.01	<0.01	<0.01
Respiration, bpm							
0800 h	48.8[a]	100.2[b]	48.4[a]	1.8	<0.01	<0.01	<0.01
1200 h	50.4[a]	117.6[b]	49.3[a]	2.0	<0.01	<0.01	<0.01
2000 h	55.0[a]	115.1[b]	55.9[a]	2.6	<0.01	<0.01	<0.01
Skin Temperature, ℃							
Shoulder							
0800 h	34.6[a]	42.4[b]	33.1[a]	0.3	<0.01	<0.01	<0.01
1200 h	34.8[a]	43.1[b]	33.6[a]	0.3	<0.01	<0.01	<0.01
2000 h	37.3[a]	43.3[b]	35.6[a]	0.2	<0.01	<0.01	<0.01
Ham							
0800 h	34.0[a]	42.4[b]	32.6[a]	0.3	<0.01	<0.01	<0.01
1200 h	34.2[a]	43.1[b]	33.2[a]	0.3	<0.01	<0.01	<0.01
2000 h	37.1[a]	43.1[b]	35.4[a]	0.2	<0.01	<0.01	<0.01

[1]Thermal Neutral
[2]Pair-fed Thermal Neutral
[3]Treatment x Day Interaction
Values are an average of days 1-7 of environmental conditions
[a,b,c] $P<0.05$ represents treatment differences

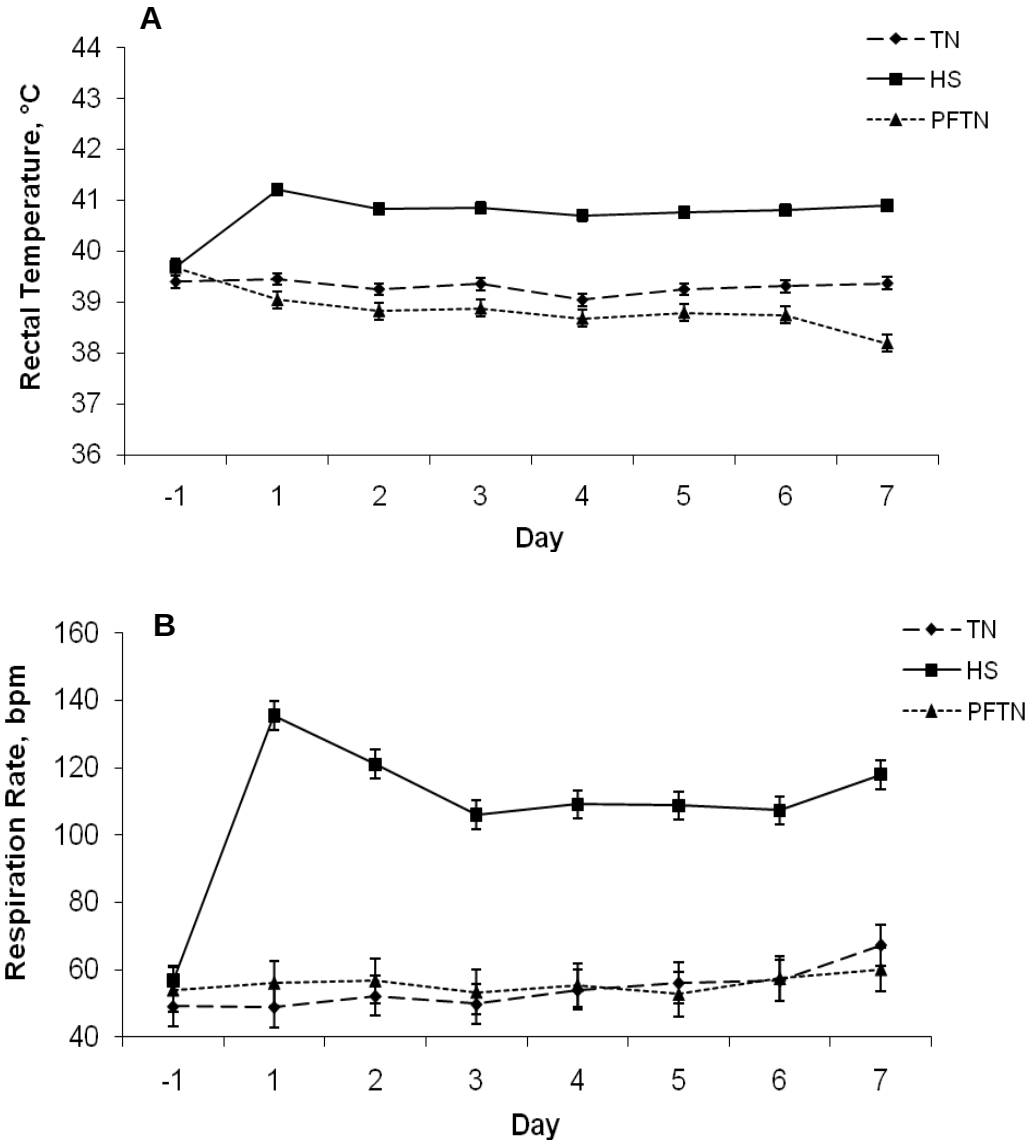

Figure 2.1: Effects of constant environment [ad-libitum intake in thermal neutral (TN; 20℃) conditions; heat stress (HS; 35℃) or pairfee ding in thermal neutral conditions (PFTN)] on (A) Rectal temperatures and (B) Respiration rates at 2000h in growing pigs.

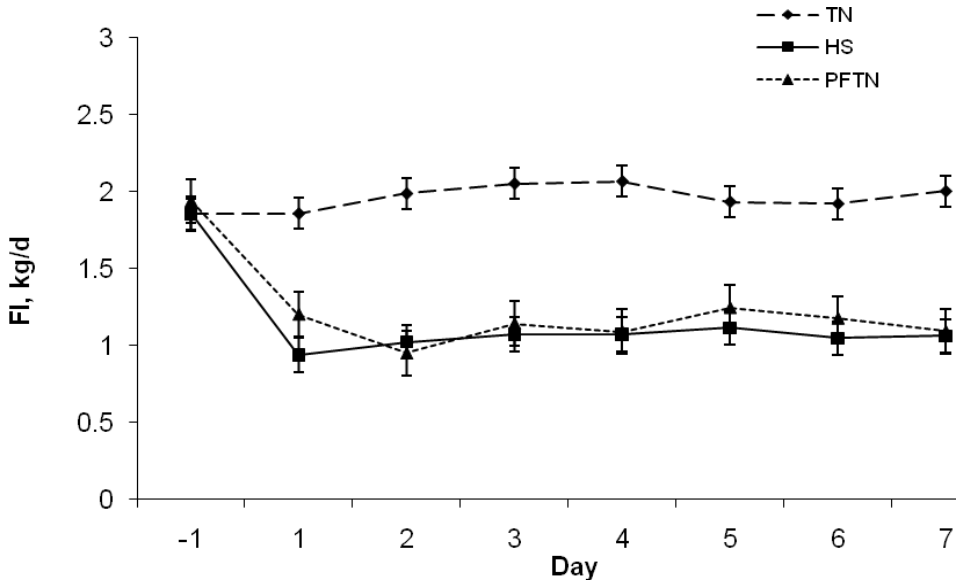

Figure 2.2: Effects of constant environment [ad-libitum intake in thermal neutral (TN; 20℃) conditions; heat stress (HS; 35℃) or pairfeeding in thermal neutr al conditions (PFTN)] on daily feed intake in growing pigs.

Table 2.4 Effects of heat stress (HS) on production parameters in growing pigs

	Day of Experiment									
	1		3		7			*P*		
Parameter	TN[1]	HS	TN	HS	TN	HS	SEM	Trt	Day	T x D[2]
ADFI[3], kg/d	1.86[a]	0.94[b]	2.05[a]	1.07[b]	2.00[a]	1.06[b]	0.11	<0.01	<0.01	<0.01
ADG[4], kg/d	1.27[a]	-2.70[b]	1.04[a]	0.01[c]	1.11[a]	0.24[c]	0.11	<0.01	<0.01	<0.01
Δ BW[5], kg	1.27[a]	-2.70[b]	3.12[c]	0.03[d]	7.76[e]	1.65[a]	0.55	<0.01	<0.01	<0.01

[1]Thermal Neutral
[2]Treatment x Day Interaction
[3]Average Daily Feed Intake
[4]Average Daily Gain
[5]Change in Body Weight
[a,b,c]$P < 0.05$

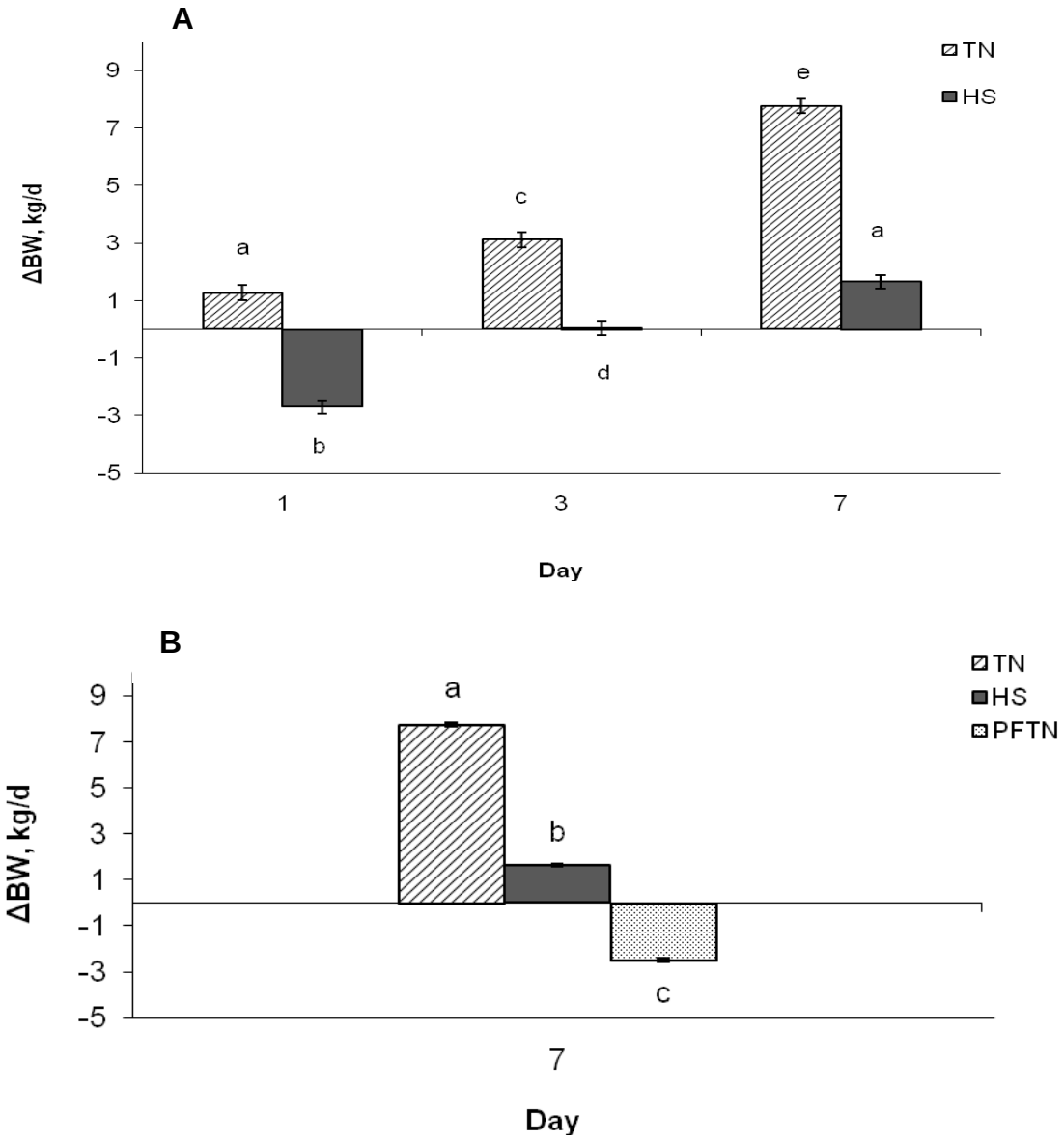

Figure 2.3: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) and heat stress (HS; 35℃) conditions on the (A) tempora l change in body weights and (B) effects of TN, HS, and pair-feeding in thermal neutral conditions (PFTN) on change in body weight at the end of the experiment. $^{a,b,c}P<0.05$

Table 2.5 Effects of heat stress (HS) on plasma energetic variables growing pigs

	Day of Experiment									
	1		3		7			*P*		
Parameter	TN[1]	HS	TN	HS	TN	HS	SEM	Trt	Day	T x D[2]
Glucose, mg/dL	110.7	108.8	116.0	104.5	116.8	105.8	4.5	0.04	0.94	0.50
BUN[3], mg/dL	10.2^{bc}	13.8^{a}	11.2^{b}	8.4^{cd}	10.0^{bc}	7.3^{d}	0.9	0.42	<0.01	<0.01
NEFA[4], mmol/L	0.09^{a}	0.27^{b}	0.06^{a}	0.09^{a}	0.07^{a}	0.09^{a}	0.03	0.02	<0.01	0.03
Insulin, ng/mL	0.09	0.05	0.13	0.09	0.17	0.12	0.02	0.01	<0.01	0.88
Triglycerides, mg/dL	44.0	50.7	41.4	47.8	37.5	47.3	5.6	0.11	0.66	0.94
Cholesterol, mg/dL	93.0^{a}	116.7^{b}	92.4^{a}	112.7^{b}	92.7^{a}	92.7^{a}	5.7	<0.01	0.07	0.07
Creatinine, mg/dL	1.03	1.50	1.06	1.37	1.0	1.51	0.08	<0.01	0.82	0.47
ALK Phos[5], IU/L	247^{a}	211^{ab}	232^{ab}	132^{c}	204^{b}	103^{c}	16	<0.01	<0.01	0.11

[1]Thermal Neutral
[2]Treatment x Day Interaction
[3]Blood Urea Nitrogen
[4]Non-esterified Fatty Acids
[5]Alkaline Phosphatase
[a,b,c]$P<0.05$

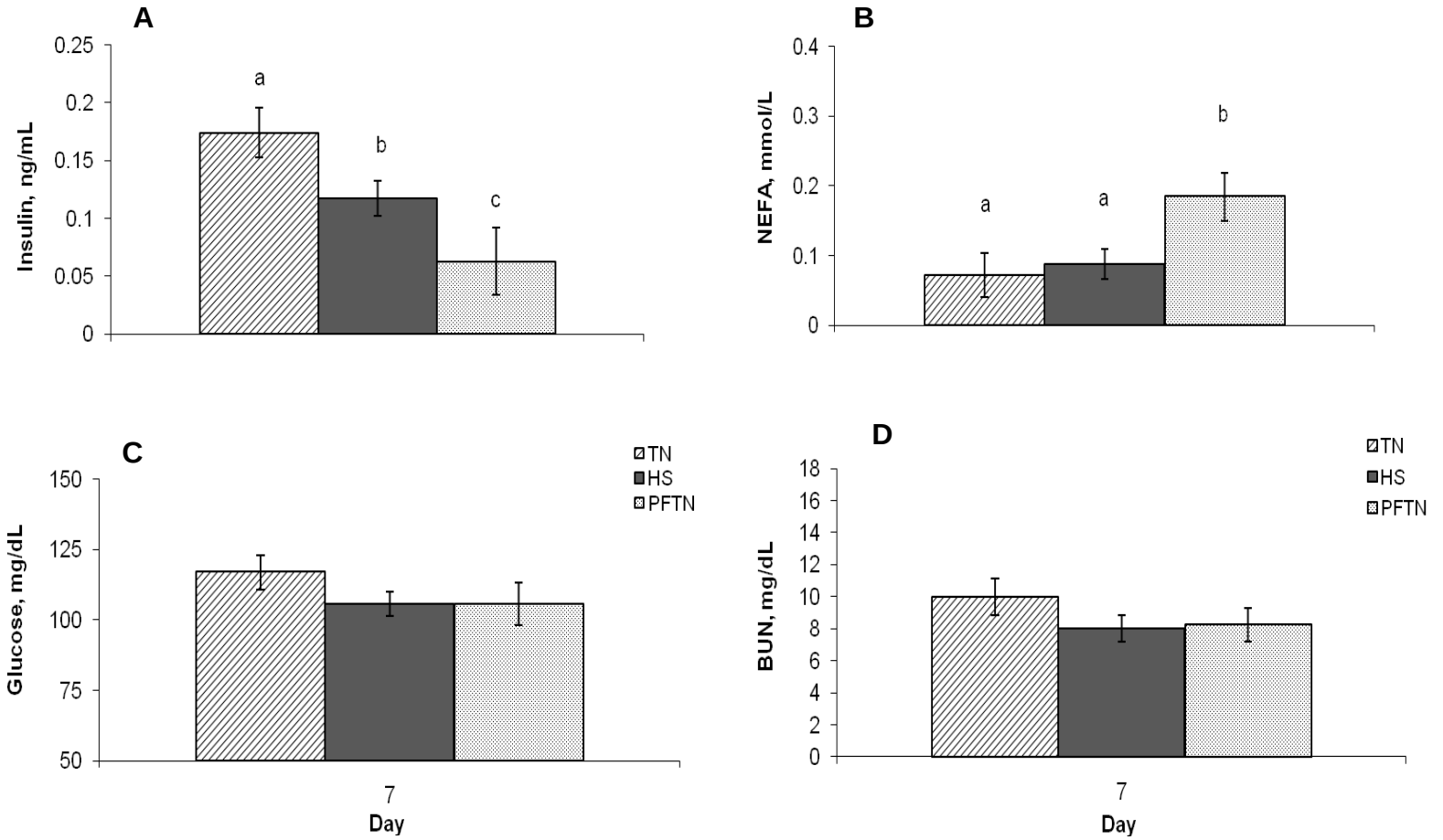

Figure 2.4: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃), heat stress (HS; 35℃) and pair-feeding in thermal neutral conditions (PFTN) on (A) Plasma insulin, (B) Plasma non-esterified fatty acids (NEFA), (C) Plasma glucose, and (D) Plasma blood urea nitrogen (BUN) at the end of the experiment in growing pigs. $^{a,b,c}P<0.05$

Table 2.6 Effects of heat stress (HS) on energetic variables of the Longissimus Dorsi (LD) and liver in growing pigs

	Day of Experiment									
	1		3		7			*P*		
Parameter	TN[1]	HS	TN	HS	TN	HS	SEM	Trt	Day	T x D[2]
LD HSP 70[3]	1.41[a]	3.54[b]	1.46[a]	2.40[c]	1.29[a]	2.27[c]	0.26	<0.01	0.03	0.05
LD ATPase[4]	122.5	210.5	139.8	205.2	135.5	189.2	41.9	0.06	0.97	0.92
Liver ATPase	107.0	105.1	101.3	100.5	104.3	88.8	9.1	0.43	0.56	0.65
LD AMPK[5]	0.24	0.36	0.27	0.25	0.26	0.30	0.05	0.24	0.73	0.25
Liver AMPK	1.18	1.30	1.03	1.05	1.29	1.32	0.10	0.51	0.05	0.87

[1]Thermal Neutral
[2]Treatment x Day Interaction
[3]Heat-shock Protein 70, Arbitrary Units
[4]Na+/K+ ATPase Activity, µmol Pi/mg protein
[5]AMP-activated Kinase Absorbance, (mg/mL)
[a,b,c]*P* <0.05

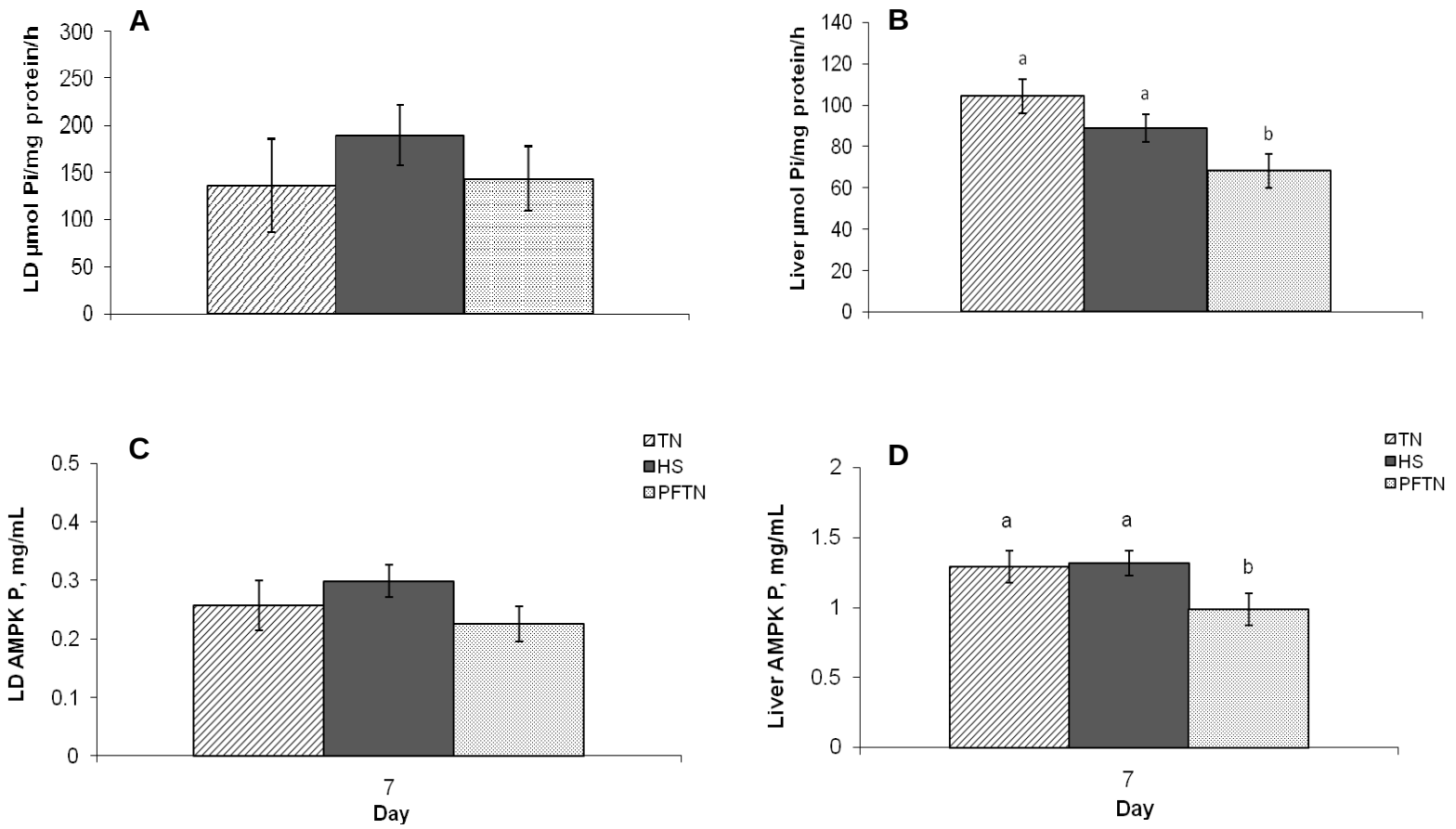

Figure 2.5: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃), heat stress (HS; 35℃) and pair-feeding in thermal neutral conditions (PFTN) on (A) Longissimus Dorsi (LD) Na^+/K^+ ATPase activity, (B) Liver Na^+/K^+ ATPase activity, (C) LD AMPK, and (D) Liver AMPK at the end of the experiment in growing pigs. $^{a,b,c}P<0.05$

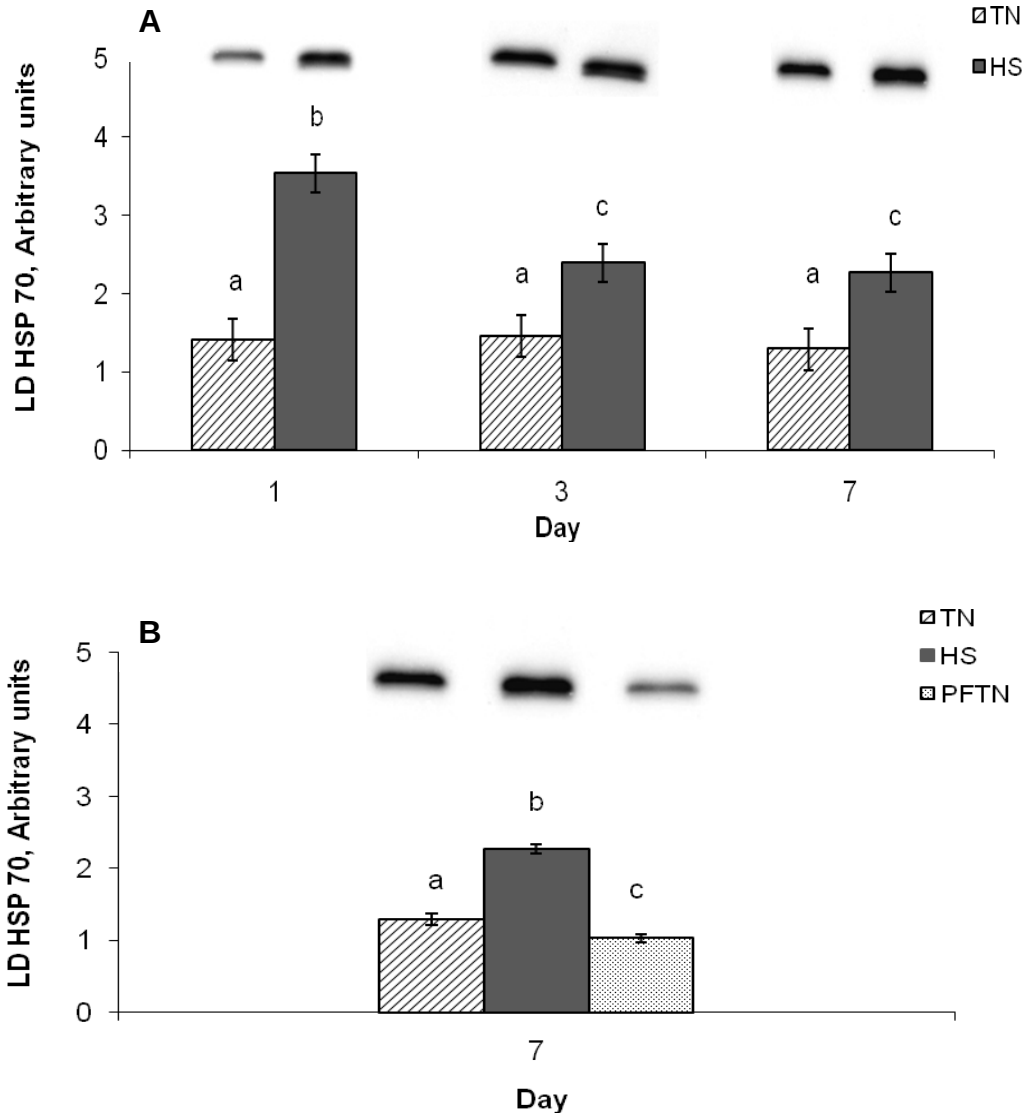

Figure 2.6: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) and heat stress (HS; 35℃) conditions on the (A) tempora l change in Longissimus Dorsi (LD) HSP 70 expression and (B) effects of TN, HS, and pair-feeding in thermal neutral conditions (PFTN) on change in LD HSP 70 expression at the end of the experiment. $^{a,b,c}P<0.05$

Table 2.7 Effects of heat stress (HS) on energetic metabolism measures in growing pigs

	Day of Experiment									
	1		3		7			*P*		
Parameter	TN[1]	HS	TN	HS	TN	HS	SEM	Trt	Day	T x D[2]
FAS activity[3]	19.89	16.65	22.42	14.35	19.88	12.89	2.56	<0.01	0.66	0.64
G6PDH ativity[4]	39.88	35.71	40.72	34.84	34.86	26.60	2.56	<0.01	<0.01	0.63

[1]Thermal Neutral
[2]Treatment x Day Interaction
[3]Fatty Acid Synthase, nmol NADPH oxidized/min/mg protein
[4]Glucose-6-phosphate Dehydrogenase, nmol NADPH produced/min/mg protein

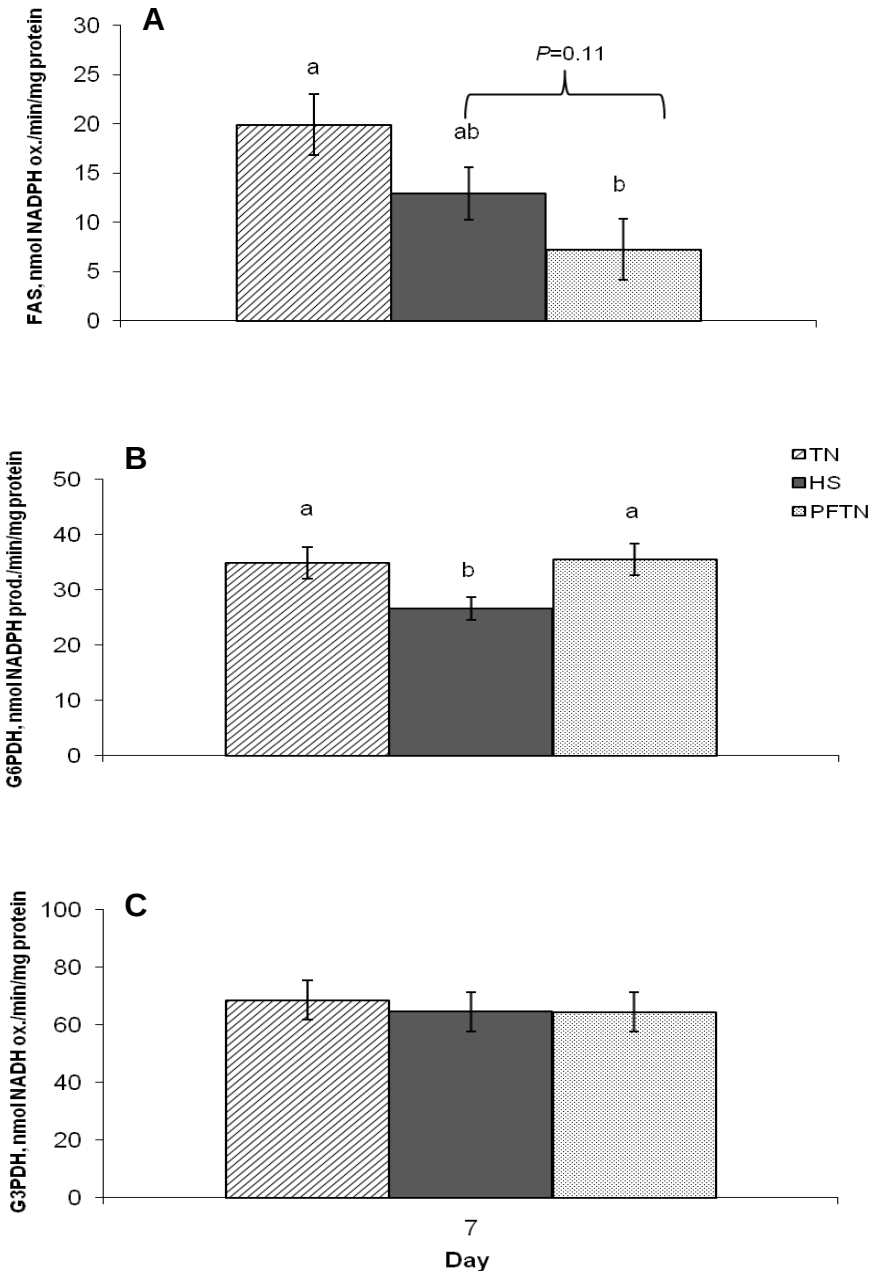

Figure 2.7: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃), heat stress (HS; 35℃) and pair-feeding in thermal neu tral conditions (PFTN) on (A) FAS enzyme activity (B) G-6-PDH enzyme activity, and (C) G-3-PDH enzyme activity at the end of the experiment in growing pigs. $^{a,b,c}P<0.05$

CHAPTER 3
THE EFFECTS OF HEAT STRESS ON INTESTINAL PERMEABILITY IN GROWING PIGS

Abstract

Heat stress can compromise intestinal integrity and induces “leaky” gut in a variety of species. This increase in intestinal permeability adds to the risk of bacterial sepsis and ultimately reduces growth performance. However, whether this occurs in pigs and the mechanisms responsible for it are ill-defined. Crossbred gilts (n=48; 35±4 kg BW) were housed in constant climate controlled rooms in individual pens and exposed to 1) thermal neutral (TN) conditions (20℃; 35-50% humidity) with ad libitum intake (n=18), 2) HS conditions (35°C; 20-35% humidity) with ad libitum intake (n=24) or 3) pair-fed in thermal neutral conditions [PFTN], n=6: to eliminate confounding effects of dissimilar feed intake [FI]). Pigs were sacrificed at 1, 3, or 7 d of environmental exposure and freshly isolated jejunum samples were mounted into modified Ussing chambers. Intestinal segments were then analyzed for transepithelial electrical resistance (TER) and intestinal fluorescein isothiocyanate (FITC)-labeled lipopolysaccharide (LPS) transport expressed as endotoxin apparent permeability coefficient (APP). Irrespective of day, plasma endotoxin concentrations increased 46% (P<0.05) in HS pigs compared to TN pigs, while TER decreased 24% (P<0.05) and endotoxin APP increased 81% (P<0.01). Furthermore, d 7 HS pigs tended (P=0.06) to have increased APP compared to PFTN controls. These data indicate that HS and PFTN conditions decreases intestinal integrity and increases endotoxin permeability. We hypothesize that these events lead to increased acute inflammation which contributes to reduced pig

performance during warm summer months.

Introduction

Although a consensus appears to have been reached on whether or not global warming is occurring, the reasons contributing to it remain intensely debated (Bernabucci et al., 2010). Data suggests that global temperatures are rising at a rate of 0.2℃ per decade and this number is likely to i ncrease in the future (US EPA, 2010). Both humans and animals are adversely affected by rising environmental temperatures. Humans can become ill or even die due to heat stress related illnesses and during the last 75 years, heat related mortality has been particularly high (Yan et al., 2006). In 2003, 15,000 people died during a two week heat-wave in France (Kovats et al., 2006), and heat-related morbidity is projected to increase if climate change is not curbed (Schar and Jendritzky, 2004). This is alarming because currently, over 30% of human heat -stroke patients admitted to hospitals die (LoVecchio et al., 2007) and some estimate this number to be near 80% (Nixdorf-Miller et al., 2006). Animal production is also affected by heat stress as the US swine industry loses over $300 million annually due to heat stress and global loses to animal agriculture are in the tens of billions of dollars (St-Pierre et al., 2003). Growing pigs and lactating sows are especially heat susceptible because they lack functional sweat glands and produce a large amount of metabolic heat (D'Allaire et al., 1996; Brown-Brandl et al., 2004).

Heat-stressed humans and animals redistribute blood to the periphery in an attempt to maximize radiant heat dissipation and vasoconstrict the gastrointestinal

tract in order to reprioritize blood flow (Lambert, 2008). Consequently, the reduced blood and nutrient flow to the intestinal epithelium compromises the integrity of the intestinal barrier (Yan et al., 2006). This “leaky gut” or enhanced intestinal permeability increases certain blood markers of endotoxemia and inflammation (Hall et al., 2001). Increased plasma LPS has been observed during sepsis, indicating that bacteria (or Gram negative bacterial cellular components) have been transported across the intestinal epithelium and into systemic circulation (Hall et al., 2001). Circulating cytokines (indicative of an immune response) are also increased during endotoxemia and they consequently lead to inflammation (Leon, 2007). Incidentally, human heat stroke patients exhibit similar increased blood markers (Bouchama et al., 1993), as do people suffering from septic shock. In addition, despite marked reductions in nutrient intake, immune stimulation and specifically LPS inexplicably increases circulating insulin levels (Waldron et al., 2006; Rhoads et al., 2009b). Therefore, these sepsis and inflammation markers may also alter metabolism and nutrient partitioning (Baumgard and Rhoads, 2011) and may explain why heat-stressed farm animals have decreased production.

Currently, besides cooling and rehydration there are no standard protocols for treating people with heat-stroke and little or no drugs are approved to medicate the environmentally-induced hyperthermic patient (Caspani et al., 2004). Surprisingly, many patients die 1-3 days following the heat insult (even though body temperatures had returned to euthermia) and reasons for this are unclear, as no current definition of heat-related mortality exists (Ostro et al., 2009). Due to the complex nature of

heat-stroke pathogenesis, accurate morbidity and mortality statistics are difficult to obtain and this introduces confounding variables as to the cause of death (Basu, 2009). A common plasma variable frequently observed in heat-stroke patients that eventually die is elevated endotoxin or lipopolysaccharide (LPS) concentrations and these are often accompanied by other associated hallmarks of sepsis and inflammation (Lambert, 2009).

Therefore, the objectives of this study were to determine if heat stress directly or indirectly increases intestinal permeability in growing pigs and that this leaky gut augmented endotoxin transport and circulating concentrations. We hypothesized that an increased heat-load causes physiological changes to the intestinal epithelium, results in compromised barrier integrity and altered intestinal function and that this may contribute to the overall severity of heat stress related illness.

Materials and Methods

Animals and Treatment

Iowa State University Institutional Animal Care and Use Committee approved all procedures involving animals. Female crossbred gilts (n = 48, 35 ± 4 kg BW) were selected by body weight and housed in individual pens (with individual feeders and waters) in one of two rooms (24 pens/room) at thermal neutral conditions. Animals were allowed to acclimate to their environment for 5 d at the Iowa State University Swine Nutrition Farm prior to start of the experiment. Pigs were assigned to one of three environmental treatments: 1) thermal-neutral (TN) conditions (20℃;

35-50% relative humidity) with ad libitum feed intake, 2) heat stress (HS) conditions (35℃; 20-35% relative humidity) with ad libitum in take or 3) TN conditions but pair-fed (PFTN) to mirror the nutrient intake of the HS pigs. Pigs in the TN (n=18) and HS (n=24) conditions were sacrificed at 1, 3 and 7 d post initiation of environment treatment. The PFTN pigs (n=6) were only sacrificed at 7 d post initiation of nutrient restriction.

After the environmental initiation, reduced feed intake in the HS pigs was calculated daily based on the percentage decrease from each animal's average feed intake prior to HS; the amount offered to PFTN pigs was reduced by that amount. The PFTN group lagged one day behind the 7 d heat stress pigs in order to calculate and implement feed intake reductions. Pair-feeding was used to eliminate confounding effects of dissimilar feed and nutrient intake. Individual animal feed intake was determined daily at 0800 h. Pair-fed pigs were fed calculated amounts thrice daily at (0700, 1200, and 2000 h) in an attempt to reduce post-prandial shifts in carbohydrate and lipid metabolism.

Regardless of environmental treatment, all animals were fed the same diet throughout the duration of the experimental period. Samples were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories. Samples were analyzed in duplicate for proximates of crude protein (LECO), crude fat, moisture, ash, and crude fiber. A standard protein hydrolysate package for amino acid analysis of: Asp, Thr, Ser, Glu, Pro, Gly, Ala, Met, Val, Ile, Leu, Tyr, Phe, His, Lys, Arg was also performed (for diet composition see table 2.1).

Each room's temperature and humidity were monitored by a data recorder (Lascar® model EL-USB-2-LCD, Erie, PA) which was set to record environmental data every 30 min. Each room's ambient temperature was controlled but humidity was not governed. All pigs were monitored continuously for signs of distress. Body temperature parameters were obtained four times daily (0800, 1200, 1600, and 2000 h). Rectal temperatures were recorded with a digital thermometer (Top care®, Waukegan, IL), skin temperatures at the shoulder and ham were recorded with an infrared temperature gun (Extech® instruments Model 42505, Waltham, MA) and respiration rates (breaths/min) calculated with a stopwatch. Heat-stressed pigs were removed from the room and cooled down with cool water if rectal temperature exceeded 41.0℃ (105.8℉). Cooling time was standardi zed at 10 min, at which point the animals were returned to their respective pens.

Body weights were recorded on all animals at the beginning of the experiment and immediately prior to sacrifice. Jugular vein blood was obtained (10 mL BD® vacutainers containing 143 U.S.P units of sodium heparin) while the animals was nose-snared and immediately sacrificed using the captive bolt technique followed by exsanguination. Blood was also obtained after sacrifice from the hepatic portal vein (using a 22G 5 mL syringe; BD® LEUR-LOK™, Franklin Lakes, NJ) and placed into sodium heparin vacutainers. Blood harvested from the jugular and hepatic portal vein was centrifuged at 1300 x g to obtain plasma, alliquotted into five 1.5 mL microcentrifuge tubes and stored at -20℃ for later an alysis.

All tissues were harvested within 12 min of death and included: whole

jejunum, whole colon, jejunum mucosal scrapings. Tissue samples were snap-frozen in liquid nitrogen and stored at -80℃ until later analyses. Two additional samples of whole jejunum and whole colon were obtained and either placed immediately into Krebs-Henseleit buffer (containing 25 mM $NaHCO_3$, 120 mM NaCl, 1 mM $MgSO_4$, 6.3 mM KCl, 2 mM CaCl and 0.32 mM NaH_2PO_4, pH 7.4) under constant aeration for transport to the laboratory and mounting into Ussing Chambers. Fresh intestinal segments were also immediately fixed in 10% formalin for later histology analysis.

Ussing Chambers

Intestinal tissue from the proximal jejunum and colon of each animal was obtained mounted into modified Ussing chambers (Physiological Instruments and DVC 1000; World Precision Instruments, New Haven, CT) for determination of intestinal integrity, nutrient and endotoxin transport. Tissue samples were pinned and placed vertically into the chambers with the mucosal membrane facing one half of the chamber and the serosal membrane facing the other half. Each side of the membrane was bathed in 4 mL of Krebs-Henseleit buffer (KHBB) and tissue was provided with a constant O_2-CO_2 mixture. Individual segments were clamped at a voltage of 0 mV and after 30 min of stabilization small and large intestine tissue was challenged (sequentially, 20 min apart) with 10 mmol/L $_D$-glucose, 10 mmol/L glutamine, and 10 mmol/L lysine for nutrient transport. Measurements of current for nutrient transport were then obtained every 10 sec and the change in maximal current used to calculate tissue conductance from Ohm's law. This was used to

determine rate of transport of the aforementioned nutrients. Transepithelial electrical resistance (TER) was calculated by averaging the current during the first 10 min of tissue stabilization (Gabler et al., 2007, 2009).

Small and large intestinal segments were also assessed for endotoxin transport using fluorescein isothiocyanate labeled lipopolysaccharide (FITC-LPS). After 20 min of stabilization, Krebs-Henseleit buffer was removed from the donor side and 20 µg/mL of FITC-LPS was added while 5 mL of KHBB was added to the acceptor side. Samples from the acceptor side were obtained in duplicate every 20 min for 120 min and read in a fluorescence spectrophotometer at 495 nm and analyzed for apparent permeability coefficient (APP). The APP was calculated (as previously described by Tomita et al., 2004) using the following formula:

$$APP = dQ/ (dt \times A \times C_0)$$

where dQ/dt is the rate of FITC-LPS transport in µg/sec which is the slope of the regression line obtained by the spectrophotometer. C_0 is the concentration in µg/mL and A is the area of the membrane in cm^2.

Total Protein Determination

Total protein concentration of all tissues and plasma was measured for various lab analyses and concentration was determined in triplicate using a commercially available assay kit (Pierce® BCA microplate protein assay kit, Pierce, Rockford, IL). Bovine serum albumin (BSA) and MQ H_2O combinations were used as standards and samples were analyzed in triplicate. After addition of standards, MQ H_2O was added to all non-standard wells and then samples were vortexed and

added to the plate. Finally, 160 µL of the working color reagent (50 parts Solution A to 1 part Solution B) was added to all wells. The plate was incubated for 1 h at room temperature and then read at 562 nm using a Synergy 4 microplate reader (Bio-Tek, Winooski, VT).

Circulating Endotoxin Assay

Plasma endotoxin concentrations were determined using a commercially available kit validated for use in our laboratory. Endotoxin concentrations were determined in triplicate using a recombinant Factor C (rFC) endotoxin assay with a 1/1000 dilution factor for porcine plasma samples (PyroGene® Recombinant Factor C Endotoxin Detection System, Lonza, Walkersville, MD). Activation of Factor C by endotoxin binding causes action upon the fluorogenic substrate in the assay producing a fluorescent signal. The procedure was conducted in 96-well microplates. Fluorescence was measured at time 0 and after 1 h incubation at 37℃. The plates were then read under fluorescence using a Synergy 4 microplate reader (Bio-Tek, Winooski, VT) with excitation/emission wavelengths of 380/440nm. All d 7 pigs (HS, TN, and TNPF, n=24) were analyzed for total protein concentration using the BCA assay in order to determine the dilution factor needed for the endotoxin assay. The assay was run first on jugular plasma and then on portal plasma. Some portal plasma samples (n=5) were unable to be used due to low or no volume obtained at time of sacrifice. The inter- and intra-assay coefficients for plasma endotoxin were 27.8, 21.8%, respectively.

Jejunum Na^+/K^+ ATPase Activity

Whole jejunum tissue was freeze-ground in liquid nitrogen and stored at -80℃ for later use. Prior to the start of the assay , 500 mg was homogenized in 4 mL of sucrose buffer (pH 7.4) consisting of: 50 mM sucrose, 1 mM Na_2EDTA, and 20 mM tris base and centrifuged at 1000xg for 10 min for protein extraction. Protein extracts were separated into 5 aliquots: two for water, two for ouabain, and one for BCA protein analysis. Proteins with either MQ H_2O or 20 mM Ouabain were pre-incubated for 15 min with Na^+/K^+ ATPase reaction buffer (pH 7.0; 2000 mM NaCl, 100 mM KCl, 50 mM $MgCl_2$ and 250 mM HEPES) and then incubated for 45 min after addition of fresh 105 mM ATP to start the reaction. After 45 min the reaction was terminated using ice-cold 50% trichloroacetic acid. Samples were centrifuged at 1500 xg for 10 min to obtain the final product which was present in the supernatant (Fuller et al., 2003). Lastly, samples were analyzed for the presence of inorganic phosphate using the Molybdovanadate method (Ueda and Wada, 1970) and assessed in triplicate at a wavelength of 400 nm using a Synergy 4 microplate reader (Bio-Tek, Winooski, VT). Specific Na^+/K^+ ATPase activity was determined by the difference in P_i production from ATP in the presence of absence of ouabain (specific Na^+/K^+ ATPase inhibitor). Unspecific phosphate hydrolysis was correlated by measuring P_i freed in the absence of protein suspension. The inter- and intra-assay coefficients for jejunum phosphorus determination were 3.8, and 3.4% respectively.

Histology

Whole jejunum samples fixed in formalin were sent to the Iowa State University Veterinary Diagnostic Laboratory for sectioning and hematoxylin and eosin staining of intestinal enterocytes. Using a microscope (Leica® DMI3000 B Inverted Microscope, Bannockburn, IL) with an attached camera (QImaging® 12-bit QICAM Fast 1394, Surrey, BC) pictures were obtained of 10 microvilli per sample section. On some occasions two pictures were needed to obtain a total of 10 microvilli. Each microvillus was measured for villus height and width, as well as crypt depth. Finally, the averages of the ten microvilli were calculated and reported as one number per pig. Images of individual villi were obtained using Q-capture Pro 6.0 (QImaging®, Surey, BC) and measurements were taken using Image-Pro Plus 7.0 (Media Cybernetics®, Bethesda, MD).

Statistics

All data were statistically analyzed using the PROC MIXED procedure of SAS version 9.1 (SAS Inst. Inc. Cary NC). Data are reported as LSmeans and considered significant if $P < 0.05$. Data were evaluated using two distinct models. For daily measurements (body temperatures, respiration rates and feed intake) each animal's respective parameter was analyzed using repeated measures with an auto regressive covariance structure and day as the repeated effect. The model included environment, day, and the interaction. Analysis also tested differences between all three environments on (TN, PFTN, HS), just d 7 variables (obtained at sacrifice).

Results

Body temperature indices and production variables are reported in Chapter two. In brief, HS pigs had a marked and sustained increase in rectal temperatures (1.5℃) and respiration rates (2X) compared to TN and PFTN pigs. HS pigs also had a large decrease in FI (46%) compared to TN pigs and by design the PFTN pigs FI mirrored that of the HS pigs. Pigs in TN conditions gained body weight (1.14 kg/d ADG) throughout the trial while the HS pigs initially lost 2.7 kg of BW at d 1 but gained 0.03 and 1.65 kg by d 3 and 7, respectively. The PFTN pigs had lost 2.47 kg of BW by d 7.

Overall, small intestinal TER was decreased (P<0.05; 24%) during HS compared to TN pigs (Table 3.1; Figure A-3.4) but this difference did not change with time and no differences were observed compared to PFTN pigs (Figure 3.2). Irrespective of day, small intestine APP increased 81% (P<0.01) during HS compared to TN pigs (Table 3.1; Figure A-3.4). Just d 7 analysis indicates HS pigs tended (P=0.06) to have increased APP (81%) compared to both TN and PFTN controls (Figure 3.2).

Irrespective of day, plasma endotoxin concentrations from the jugular vein increased 46% (P<0.05) in HS pigs compared to TN pigs (Table 3.1; Figure A-3.4). However, analysis from all environments indicates no differences on d 7 (Figure 3.2).

There were no overall differences observed between treatments, or time for small intestinal glucose and lysine transport (Table 3.2). Overall, and compared to TN pigs, glutamine transport was increased (91%; P<0.05) during HS (Table 3.2;

Figure A-3.5), but analysis of all three environments on d 7 indicates no differences in intestinal glutamine transport (Figure 3.3). There was an environment by day interaction in jejunum Na^+/K^+ ATPase pump activity as it was markedly increased in HS pigs (175%; $P<0.05$) on d 1, but returned to TN levels by d 3 and 7 (Table 3.2; Figure 3.1). Analysis of all three environments on d 7 indicates that PFTN pigs tended ($P=0.11$) to have a reduced (41%) jejunum Na^+/K^+ ATPase pump activity compared to TN and HS pigs (Figure 3.1).

Irrespective of day, small intestinal villous height was decreased (19%; $P<0.05$) in HS pigs compared to TN controls (Table 3.3; Figure A-3.6). By d 7, both HS and PFTN pigs had reduced villous height (21%; $P<0.01$) compared to TN pigs (Figure 3.5). There tended ($P=0.11$) to be a treatment by day interaction for villous width as it was increased 7% in HS pigs on d 1 and increased 13 and 19% on d 3 and 7, respectively (Table 3.3; Figure A-3.6). Analysis of all three environments on d 7 indicates HS increased width (22%; $P<0.01$) compared to both TN and PFTN pigs (Figure 3.5). There was also a treatment by day interaction ($P<0.01$) as crypt depth decreased over time in HS pigs compared to TN pigs (Table 3.3; Figure A-3.6). On d 7 (Figure 3.5), compared to TN pigs, both HS and PFTN pigs had decreased crypt depth ($P<0.05$; 292^a, 276^b, 239^c µm, respectively).

Discussion

Heat stress disrupts intestinal tight junctions and increases permeability in rodents and humans (Hall et al., 2001; Dokladny et al., 2006). As an environmental heat-load increases, blood is diverted to the skin (via coordinated peripheral

vasodilatation and gastrointestinal tract vasoconstriction). The orchestrated partitioning of blood results in the interior of the body (gut, stomach, spleen and liver) receiving reduced blood flow (Hall et al., 1999). Consequently, the intestinal epithelium can become hypoxic, acidotic, ATP depleted, experience oxidative/nitrosative stress, and ultimately apoptosis can occur (Yan et al., 2006). These insults can damage enterocytes, increase permeability, and eventually lead to endotoxemia, inflammation and organ damage (Lambert, 2004).

Interestingly, nutrient restriction alone (similar to the HS and PFTN pigs) can lead to alterations in intestinal function, transport, morphology, and may increase the risk of developing bacterial sepsis (Ferraris and Carey, 2000). Welsh and co-workers (1998) also indicate increased permeability to macronutrients in moderately and severely malnourished humans. Increased paracellular permeability may maximize nutrient absorption but this also increases the chance of bacterial translocation.

In our study, jugular LPS concentrations were increased in HS pigs and this is consistent with heat-stressed rodent models (Hall et al., 2001; Lim et al., 2009), chickens (Cronje, 2007) as well as human heat-stroke patients (Bouchama et al., 1991). This is a function of increased intestinal permeability as LPS is normally maintained within the small intestinal lumen and is typically too large of a molecule to fit though healthy intestinal tight junctions (Lambert, 2009). Normally, the liver metabolizes and clears endotoxins (Hall et al., 2001) and thus the presence of endotoxin in the systemic circulation is indicative of the liver's inability to filter a high

bacterial load (Leon, 2007). Once the LPS enters circulation, it binds to LPS-binding protein (LBP; Gaffin and Hubbard, 1996) and activates the macrophage CD14 receptor. The CD14 receptor is a glycoprotein present on the surface of immune cells such as monocytes, macrophages and neutrophils (Morrison and Ryan, 1987) and activating the CD14 receptor signals the synthesis and secretion of pro-inflammatory cytokines (TNF-α, IL-1β; Kielian and Blecha, 1995). In general, cytokines communicate and partially coordinate the immune system (Leon, 2007), but also reduce appetite and alter metabolism (i.e. increased insulin, skeletal muscle proteolysis, etc…; Gaffin and Hubbard, 1996). Some of the cytokine induced changes in metabolism are similar to characterized changes observed in heat-stressed animals (Baumgard and Rhoads, 2011).

Small intestine transepithelial electrical resistance is a measure of how "tight" the junctions are and decreased TER reflects increased permeability (Yang et al., 2007). Consistent with the circulating LPS data, HS pigs had reduced TER and this agrees with in vitro (Dokladny et al., 2006) and in vivo rodent data (Prosser et al., 2004). The reduced TER in HS pigs also agrees with the increased APP data (Table 3.1). Increased APP is a qualitative measure of FITC-LPS intestinal transport and is another indicator of "leakiness". Many of the effects HS has on intestinal integrity parameters appear to be directly mediated by reduced feed intake as the PFTN controls variables were similar to the HS pigs. Consequently, many biological effects at the intestine purportedly caused by heat stress (Lambert, 2004; Hall et al., 2001; Leon et al., 2007) appear to be indirect (mediated by reduced feed intake)

effects of environmental-induced hyperthermia. To our knowledge, this is the first trial to evaluate the effects of HS on intestinal permeability while experimentally controlling for reduced nutrient intake. However, our experimental design probably does not accurately model acute/intense HS typically observed in humans that succumb to heat-stroke (where nutrient restriction likely plays little or no role in intestinal permeability).

The intestinal epithelium is comprised of tight junctions which form the protective barrier. This barrier aids in absorption and transport of nutrients while preventing translocation of potentially harmful molecules (i.e. toxins; Hossain and Hirata, 2008). Organization of intestinal tight junctions is important in maintaining cell polarity and structure (Anderson and Van Itallie, 1995). Epithelial cells also consist of an actin cytoskeleton which forms a large part of the epithelial barrier. Contraction of the actin cytoskeleton helps maintain cell motility and is regulated by myosin light chain kinase (MLCK; Yang et al., 2007). Disrupted tight junctions are thought to be a major cause of increased intestinal permeability (Lambert, 2009). Phosphorylation of myosin light chain by MLCK has been observed during heat stress, suggesting that heat-induced increased contraction leads to opening of tight junctions (Yang et al., 2007). In vitro, HS also causes alterations in the distribution of the protein ZO-1 (i.e. diffusion to cytosol; Ikari et al., 2005). Similarly, ZO-1 protein expression is also decreased, indicating tight junction disruption (Dokladny et al., 2006). Heat stress also up-regulates occludin gene expression (Dokladny et al., 2008) which is an important tight junction protein for maintaining barrier function and

increased expression may indicate a protective response.

Heat-stressed animals reduce feed intake in an attempt to minimize basal heat production and this induces a malabsorptive state. Consequently, it has been hypothesized (Mitchell and Carlisle, 1992) that the intestine adapts in order to optimize/maximize nutrient absorption and minimize the nutrient imbalance. Heat-stressed animals appear to shift post-absorptive fuel selection and increase their reliance on glucose metabolism while deemphasizing fatty acid oxidation (Baumgard and Rhoads, 2011). Both the nutrient absorption optimization and post-absorptive metabolic change theories are supported by in vivo data indicating heat stress induced increased in galactose and glucose absorption ability (Mitchell and Carlisle, 1992; Garriga et al., 2006). In addition, in vivo experiments demonstrate increased renal glucose re-absorption (also via SGLT-1) ability (Sussman and Renfro, 1997). Mitchell and Carlisle (1997) also reported that heat-stressed poultry have an increased ability to absorb methionine. In contrast to poultry data, our results indicate that HS pigs do not have an increased ability to intestinally transport glucose or lysine. However, HS pigs did have an increase in the transport glutamine, especially during the acute stages of heat stress (Table 3.2). Glutamine is of interest because it is a primary energy source for intestinal cells such as enterocytes (Singleton and Wischmeyer, 2006) and it is not clear whether or not the observed changes reflect increased transport or increased glutamine oxidation. Regardless, glutamine appears to be playing a key role in intestinal integrity because dietary glutamine improves the intestinal integrity of malnourished children

(Lima et al., 2005) and this may be because it up-regulates heat-shock proteins (Sikora and Grzesiuk, 2007).

In the intestine, the Na^+/K^+ ATPase is responsible for maintaining cell osmolarity and creating a gradient which allows for transport of glucose and amino acids (Cant et al., 1996). Assuming that the GI tract contributes 20% of total O_2 consumption, intestinal Na^+/K^+ ATPase may contribute anywhere from 29-62% of GI energy expenditure and anywhere from 5.7 to 12.4% of total body energy expenditure in ruminant animals (Mcbride and Kelley, 1990). During heat stress cells become more permeable to sodium, and thus require more sodium pump activity to maintain osmolyte homeostasis (Gaffin and Hubbard, 1996) and this probably explains the increase in jejunum Na^+/K^+ ATPase. Decreased intestinal pump activity in PFTN pigs may be explained by decreased nutrient intake as decreased $Na^{+/}K^+$ ATPase activity in the small intestine has previously been reported in fasting rats (Murray and Wild, 1980). In addition, insulin stimulates Na^+/K^+ ATPase (Clausen, 2010) and the PFTN pigs were hypoinsulemic.

Intestinal villi function to increase surface area for nutrient absorption. Morphological changes in the small intestine were observed in our study. Previous studies looking specifically at HS effects on morphology have shown varying results. A study in heat-stressed chickens did not find any differences in villus height or crypt depth (Quinteiro-Filho et al., 2010). However, a study on pigs heat-stressed to 40℃ observed decrease villus height in jejunum, as well as duodenum (Yu et al., 2010). In addition, crypt depth in jejunum tissue was significantly decreased during heat

stress. Similarly, another heat stress study by the same investigators demonstrated shorter villus height in duodenum and jejunum tissue as well as shallower crypt depth (Liu et al., 2009). Shortened height of intestinal villi and crypts indicates damage to the intestinal epithelium (Figure 3.4c). Damage to the epithelium (i.e. epithelial sloughing and necrosis; Figure 3.4b) is thought to contribute largely to increased permeability (Lambert, 2002). This damage appears to occur before other organ damage because the GI tract receives reduced blood flow first. Also, recovery of the GI tract takes longer than other regions of the body (Gaffin and Hubbard, 1996). Damage to the intestinal epithelium may also affect digestion and absorption of nutrients (Liu et al., 2009).

Conclusions

Heat stress compromises intestinal integrity, but this appears to be due to heat-induced reductions in nutrient intake. This increased permeability is likely due to alterations in intestinal morphology and decreased function of intestinal tight junctions. However, this heat stress model may not accurately represent a severely acute bout of heat stress and may not be a good indicator of what occurs in human heat-stroke patients.

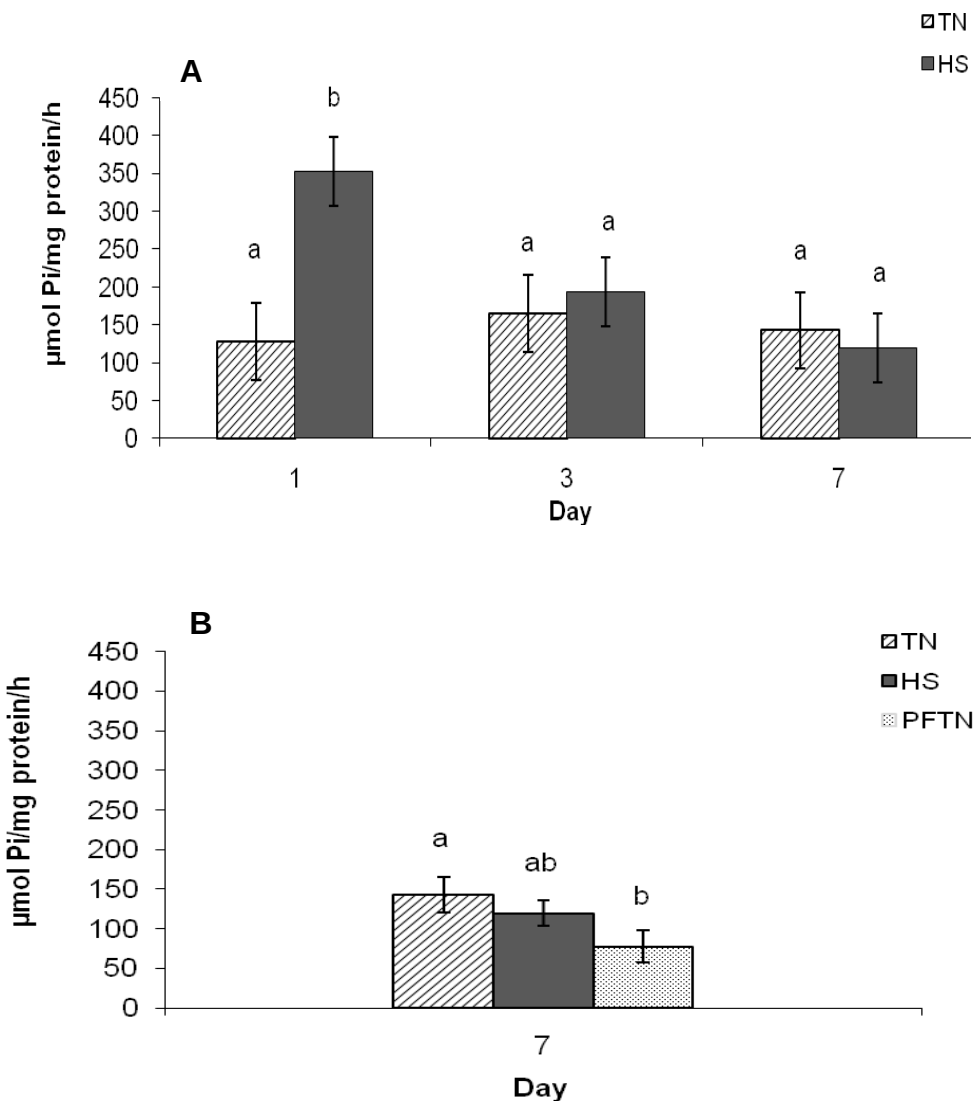

Figure 3.1: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) and heat stress (HS; 35℃) conditions on the (A) tempora l change in Jejunum Na^+/K^+ ATPase activity (B) effects of TN, HS, and pair-feeding in thermal neutral conditions (PFTN) on change in Jejunum Na^+/K^+ ATPase at the end of the experiment. $^{a,b,c}P<0.05$

Table 3.1 Effects of heat stress (HS) on intestinal permeability variables in growing pigs

	Day of Experiment									
	1		3		7			*P*		
Parameter	TN[1]	HS	TN	HS	TN	HS	SEM	Trt	Day	T x D[2]
Plasma LPS[3]	23.8	36.8	25.5	38.3	27.2	36.6	5.3	0.01	0.94	0.92
TER[4]	991	777	1028	794	897	648	135	0.04	0.55	0.99
APP[5]	1.71	2.62	1.92	3.42	1.01	2.43	0.55	<0.01	0.25	0.85

[1]Thermal Neutral
[2]Treatment x Day Interaction
[3]LPS – Lipopolysaccharide, EU (endotoxin units)
[4]TER – Transepithelial Electrical Resistance, Ω x cm^2
[5]APP – Apparent Permeability Coefficient for FITC-LPS, µg/mL/min/cm^2

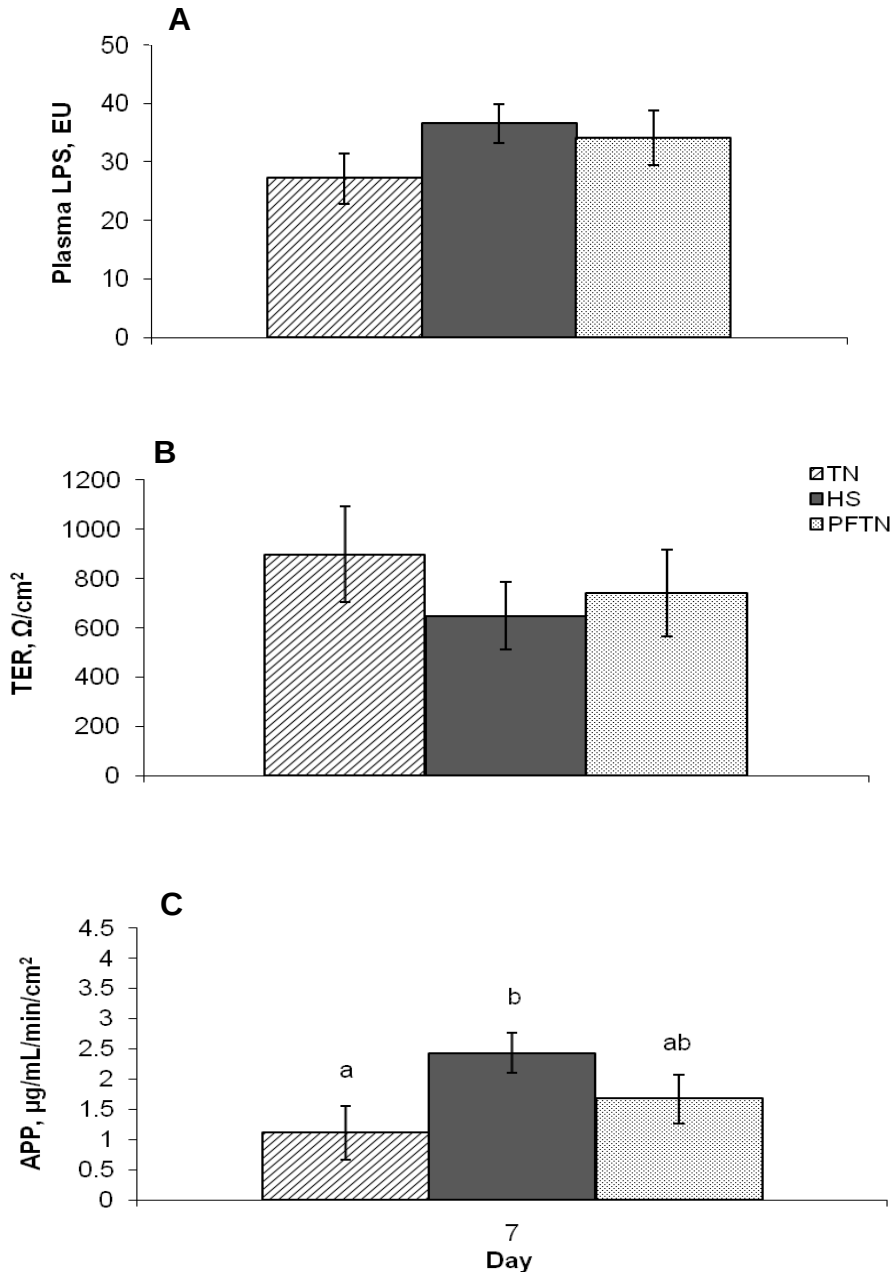

Figure 3.2: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃), heat stress (HS; 35℃) and pair-feeding in thermal neu tral conditions (PFTN) on (A) Plasma LPS (B) APP, and (C) TER at the end of the experiment in growing pigs. [a,b,c]*P*<0.05

Table 3.2 Effects of heat stress (HS) on intestinal nutrient transport in growing pigs

	Day of Experiment									
	1		3		7			*P*		
Parameter	TN[1]	HS	TN	HS	TN	HS	SEM	Trt	Day	T x D[2]
Glucose[3]	1.56	2.05	1.65	2.11	1.67	1.33	0.43	0.56	0.62	0.53
Lysine	0.82	1.39	0.95	1.10	0.87	1.36	0.44	0.28	0.98	0.90
Glutamine	0.33	0.94	0.31	0.46	0.38	0.54	0.16	0.02	0.31	0.27
Na^+/K^+ ATPase[4]	128.1[a]	352.9[b]	164.9[a]	193.0[a]	142.7[a]	119.7[a]	47.9	0.06	0.08	0.03

[1]Thermal Neutral
[2]Treatment x Day Interaction
[3]Small Intestinal Nutrient Transport, μA/cm^2
[4]Jejunum Na^+/K^+ ATPase, μmol Pi/mg protein/h
[a,b,c]*P* <0.05

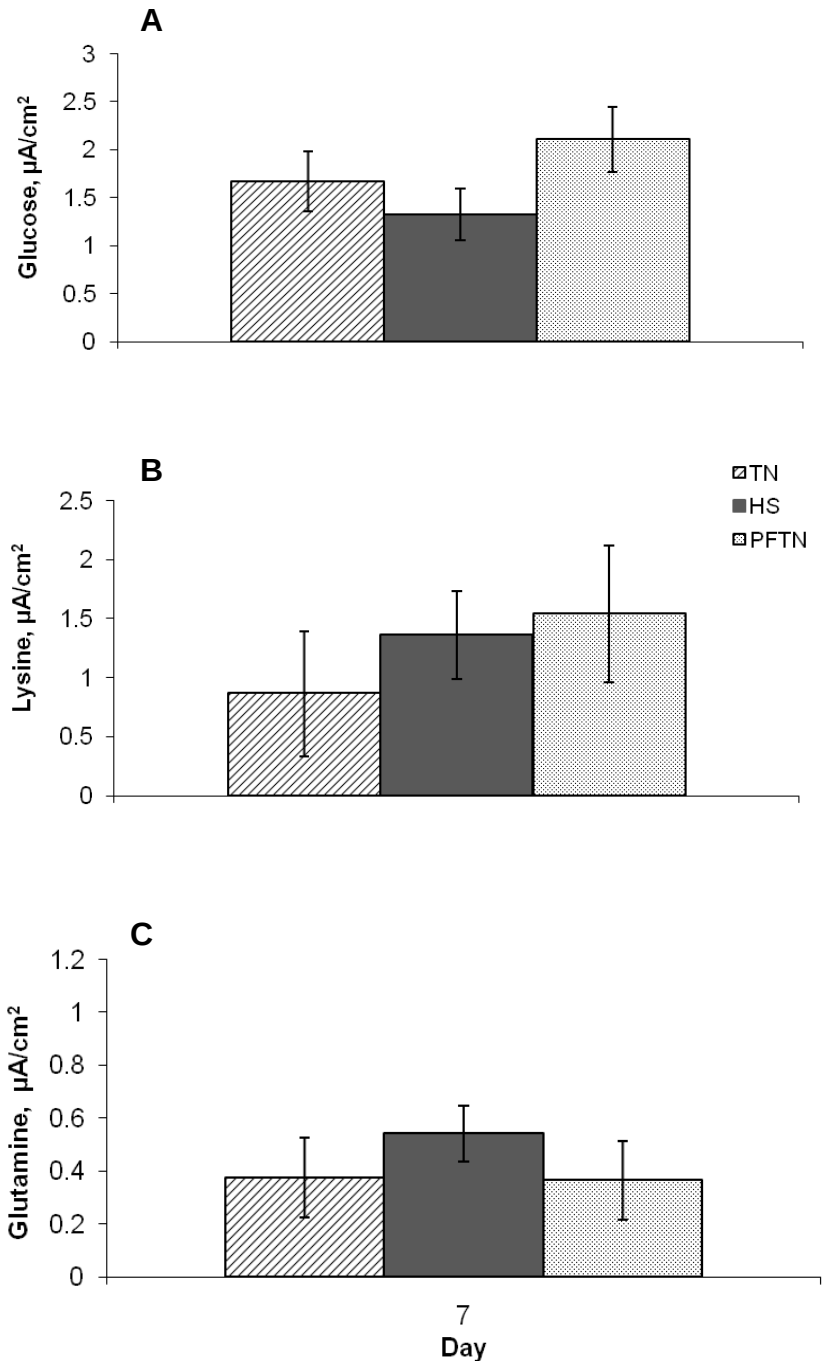

Figure 3.3: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃), heat stress (HS; 35℃) and pair-feeding in thermal neu tral conditions (PFTN) on (A) Glucose transport (B) Lysine transport, and (C) Glutamine transport at the end of the experiment in growing pigs. $^{a,b,c}P<0.05$

Table 3.3 Effects of heat stress (HS) on intestinal morphology in growing pigs

	Day of Experiment									
	1		3		7			*P*		
Parameter	TN[1]	HS	TN	HS	TN	HS	SEM	Trt	Day	T x D[2]
Villous height, µm	500	426	497	397	503	385	14	<0.01	0.35	0.32
Villous width, µm	150[a]	161[b]	146[a]	165[b]	151[a]	178[c]	4	<0.01	0.05	0.11
Crypt depth, µm	285[ab]	304[b]	292[b]	302[b]	292[b]	276[a]	6	0.32	0.07	0.02

[1]Thermal Neutral
[2]Treatment x Day Interaction
[a,b,c]*P* <0.05

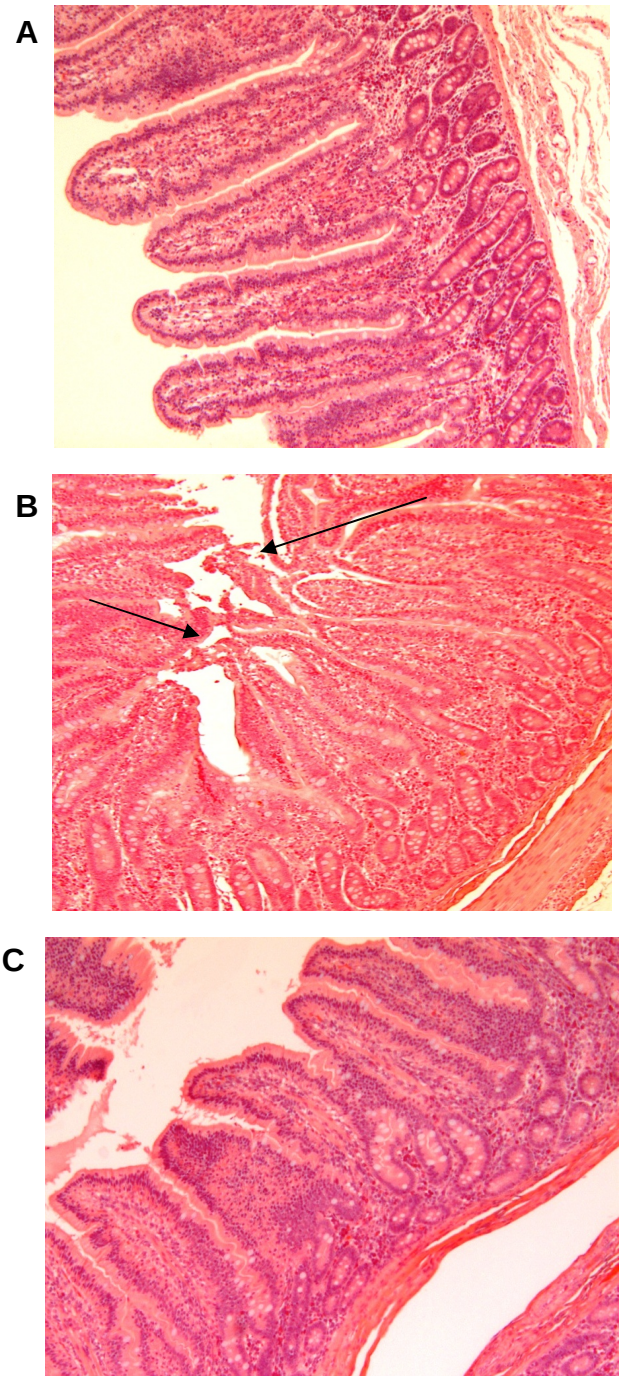

Figure 3.4: Morphological alterations of porcine small intestine in (A) thermal neutral conditions (TN; 20℃), (B) heat stress conditi ons (HS; 35℃), or (C) pair feeding in thermal neutral conditions (PFTN). The arrows in panel (B) indicate damage to intestinal villi after heat stress.

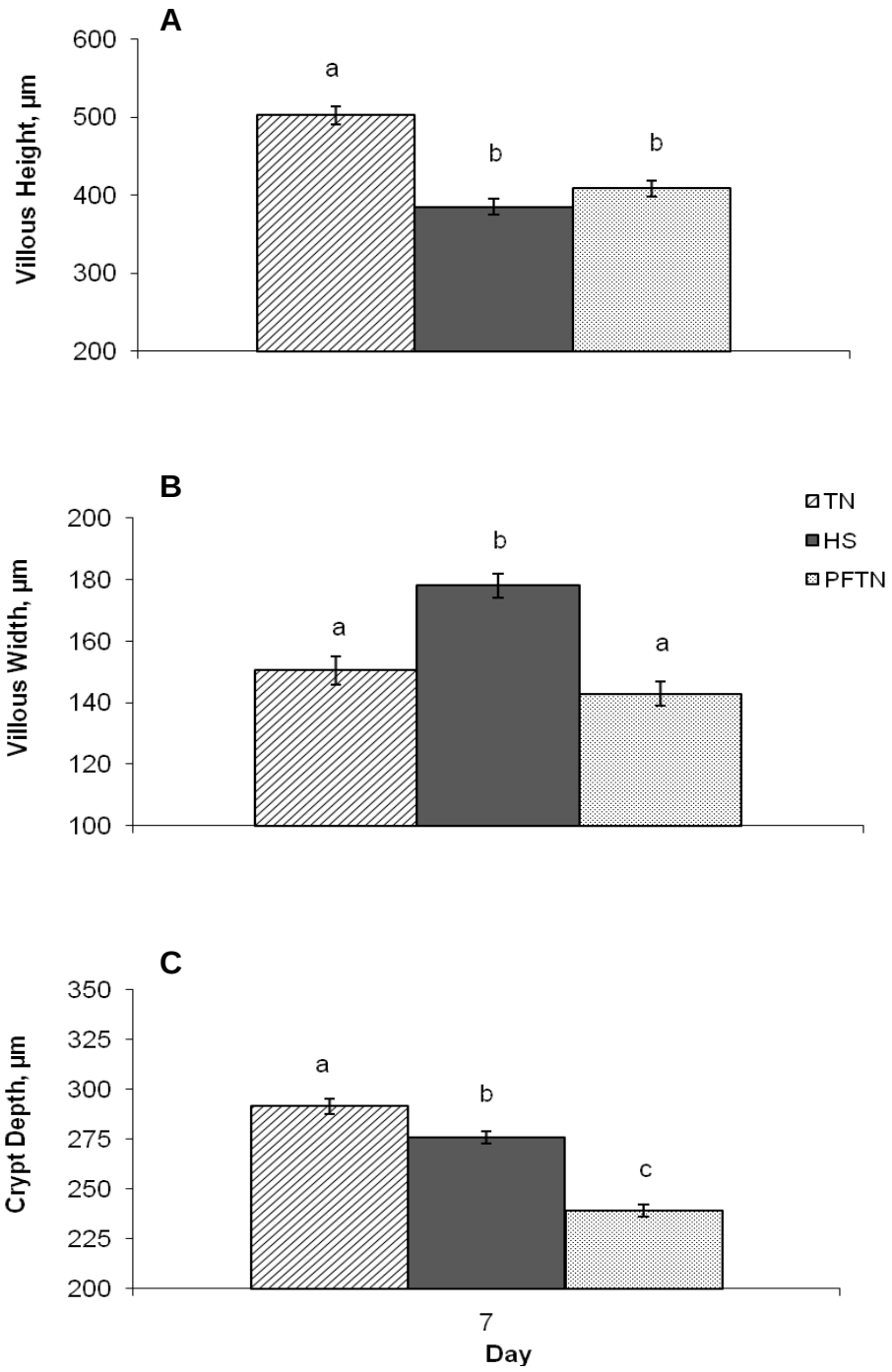

Figure 3.5: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃), heat stress (HS; 35℃) and pair-feeding in thermal neu tral conditions (PFTN) on (A) Villus height (B) Villus width, and (C) Crypt depth at the end of the experiment in growing pigs. [a,b,c]*P*<0.05

CHAPTER 4
INTEGRATIVE SUMMARY

Heat stress negatively impacts all areas of animal agriculture, and jeopardizes human health. The global economic impact of heat stress is immense (much higher than all other variables that contribute to suboptimal production combined) and this value is likely to increase with the elevated threat of global warming. Despite recent advances in heat abatement strategies, heat stress continues to cause increased days on feed, numerous health problems and mortality, reduced growth, and reduced reproductive performance.

Although heat stress has been researched extensively in pigs, much of the literature is focused on applied and practical aspects of pork production and not on metabolism and molecular mechanisms. Many heat stress studies in pigs have not thoroughly differentiated between the direct and indirect effects (i.e. reduced FI) of heat. Utilizing a pair-fed thermal neutral model is necessary in order to accurately determine if environmental heat is independently causing reduced production or if reduced nutrient intake (caused by heat stress) is partially responsible. Utilizing this type of experimental design will likely allow for the development of more accurate and useful mitigation strategies in the future.

In the current study, the reduction in feed intake due to heat stress was responsible for the decreased body weight gain in growing pigs. Heat stress directly and indirectly (via reduced feed intake) affected physiology, metabolism, and performance in growing pigs. Heat stress directly altered aspects of post-absorptive

metabolism by increasing circulating insulin concentrations. This elevated insulin inhibited lipolysis (illustrated by reduced circulating NEFA levels) and increased lipogenesis (via FAS enzyme activity). An increased heat load also directly altered protein metabolism by increasing muscle proteolysis. The reasons behind these alterations in whole-body energetics are not clear, but likely represent an evolutionary adaptation for survival.

Reduced feed intake, independent of hyperthermia also causes alterations in intestinal barrier function, and can also lead to "leaky gut". This appeared to be the case in longer term heat stress. In the current study, alterations in intestinal permeability indeed appeared to be due to the heat-induced reduced feed intake in growing pigs. However, various differences in intestinal morphology indicated that some damage may also be caused by heat alone. It is not clear if this is the case in acute hyperthermia, as is the case with many heat stroke patients. However, it is clear from the current study, that increased permeability due to either cause, is negatively affecting animal health and production.

Increased intestinal permeability and infection may also have effects on metabolism. Stress can lead to production of pro-inflammatory cytokines such as interleukins or TNFα and an increase in LPS in systemic circulation. This affects metabolism and increases inflammation and can ultimately can lead to insulin resistance (Laugerette et al., 2011). Interestingly, it appears that the immune system influences parameters of glucose homeostasis. For example, despite reduced feed intake, experimentally induced mastitic cows have increased

circulating insulin levels (Waldron et al., 2006). In addition, LPS IV infused steers have immediate and extreme hyperinsulinemia (i.e. > 30 fold; Rhoads et al., 2009b) and this occurs with only a mild decrease in plasma glucose levels. As a consequence, it appears that heat-induced leaky-gut may be mediating some of the inexplicable changes in post-absorptive metabolism (Baumgard and Rhoads, 2011).

The role of heat shock proteins also seems to influence mediators of infection and inflammation (Dokladny et al., 2010). These are molecular chaperones and are up-regulated during times of elevated heat and provide protection. Heat shock proteins have previously been shown to mediate responses to endotoxin induced cytokine production (Dokladny et al., 2001), and HSP 70 may interfere with NFκB transcription, thus disrupting the inflammatory response (Shi et al., 2006). This is further evidence that intestinal permeability may be linked with other mechanisms and may provide new targets for heat shock therapy.

Areas that require further investigation include enzyme activity levels of other lipogenic enzymes as well as investigate what directly causes increased circulating insulin, and how this may affect other areas of metabolism, as well as intestinal barrier function. Data obtained in the current studies provides novel information regarding how heat stress affects growing pigs. This will allow for future studies to further characterize a porcine model of heat stress, as well as enable new methods to ameliorate the negative effects of a heat load.

LITERATURE CITED

Abeni, F., L. Calamari, and L. Stefanini. 2007. Metabolic conditions of lactating Friesian cows during the hot season in the Po valley. 1:Blood indicators of heat stress. Int. J. Biometeorol. 52:87-96.

Aberle, E.D., R.A. Merkel, J.C. Forrest, and C.W. Alliston. 1974. Physiological responses of stress susceptible and stress resistant pigs to heat stress. J. Anim. Sci. 38:954-959.

Adeola, O., L.G. Young, B.W. McBride, and R.O. Ball. 1989. In vitro Na^+,K^+-ATPase (EC 3.6.1.3)-dependent respiration and protein synthesis in skeletal muscle of pigs fed at three dietary protein levels. Brit. J. Nutr. 61:453-465.

Aggarwal Y., B.M. Karan, B.N. Das, and R.K. Sinha. 2008. Prediction of heat-illness symptoms with the prediction of human vascular response in a hot environment under resting condition. J. Med. Syst. 32:167-176.

Aigner, B., S. Renner, B. Kessler, N. Klymiuk, M. Kurome, A. Wunsch, and E. Wolf. 2010. Transgenic pigs as models for translational biomedical research. J. Mol. Med. 88:653-664.

Al-harthi, S.S., O. Kamar, S.A. Al-Mashhadani, and A.A. Saddique. 1990. Metabolite and hormonal profiles in heatstroke patients at Mecca pilgrimage. J. Intern. Med. 228:343-346.

Allen, R.E. 1988. Muscle growth and development. Page 142-162 in Designing foods. Animal product options in the marketplace. Natl. Acad. Press, Washington, DC.

Anderson, J.M., B.R. Stevenson, L.A. Jesaitis, D.A Goodenough, and M.S. Mooseker. 1988. Characterization of ZO-1, a protein component of the tight junction from mouse liver and madin-darby canine kidney cells. J. Cell Biol. 106:1141-1149.

Anderson, J.M. and C.M. Van Itallie. 2009. Physiology and function of the tight junction. Cold Spring Harb. Perspect. Biol. 1:a002584.

Angus, D.J., M.A. Febbraio, D. Lasini, and M. Hargreaves. 2001. Effect of carbohydrate ingestion on glucose kinetics during exercise in the heat. J. Appl. Physiol. 90:601-605.

Armstrong, D.V. 1994. Heat stress interaction with shade and cooling. J. Dairy Sci. 77:2044-2050.

Baile, C.A., M.A. Della-Fera, and C.L. McLaughlin. 1983. Hormones and feed intake. Proc. Nutr. Soc. 42:113-127.

Barrow, M.W. and K.A. Clark. 1998. Heat-related illnesses. Am. Family Physician. 58:749-756.

Basu, R. 2009. High ambient temperature and mortality: a review of epidemiologic studies from 2001 to 2008. Environ. Health. 8:40.

Baumgard, L.H. and R.P. Rhoads. 2007. The effects of hyperthermia on nutrient partitioning. Proc. Cornell Nutr. Conf. p.93-104.

Baumgard, L.H. and R.P. Rhoads. 2011. Effects of environment on metabolism. Chapter 6. Environmental Physiology. Ed: R.J. Collier. John Wiley and Sons, Inc (in press).

Becker, B.A., C.D. Knight, F.C. Buonomo, G.W. Jesse, H.B. Hedrick, and C.A. Baile. 1992. Effect of a hot environment on performance, carcass characteristics, and blood hormones and metabolites of pigs treated with porcine somatotropin. J. Anim. Sci. 70:2732-2740.

Beede, D.K. and R.J. Collier. 1986. Potential nutritional strategies for intensively managed cattle during thermal stress. J. Anim. Sci. 62:543-554.

Benziane, B. and A.V. Chibalin. 2008. Frontiers: Skeletal muscle sodium pump regulation: a translocation paradigm. Am. J. Physiol. Endocrinol. Metab. 295:E553-E558.

Berg, J.M., J.L. Tymoczko, and L. Stryer. 2007. Biochemistry. 6th ed. W.H. Freeman Custom Publishing, New york, NY.

Berg, W.G. 1974. Protein synthesis in animal modes. J. Anim. Sci. 38:1079-1091.

Bers, D.M. and S. Despa. 2009. Na/K-ATPase – an integral player in the adrenergic fight-or-flight response. Trends Cardiovasc. Med. 19:111-118.

Bernabucci, U., N. Lacetera, L.H. Baumgard, R.P. Rhoads, B. Ronchi, and A. Nardone. 2010. Metabolic and hormonal acclimation to heat stress in domesicated ruminants. Animal. 4:1167-1183.

Blikslager, A.T., A.J. Moeser, J.L. Gookin, S.L. Jones, and J. Odle. 2007. Restoration of barrier function in injured intestinal mucosa. Physiol. Rev. 87:545-564.

Bobek, S., A. Sechman, E. Wieczorek, D. Wronska-Fortuna, K. Koziec, and J. Niezgoda. 1997. Reverse 3, 3', 5'-triiodothyronine (rT3) enhances hyperglycemia and lipemic effects of heat-stress in chickens. Horm. Metab. Res. 29:252-254.

Boden, G. and G.I. Shulman. 2002. Fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction. Eur. J. Clin. Invest. Suppl 3: 14-23.

Bohmanova J., I. Misztal, and J. B. Cole. 2007. Temperature-humidity indices as indicators of milk production losses due to heat stress. J. Dairy Sci. 90:1947–1956.

Bouchama, A., R.S. Parhar, A. el-Yazigi, K. Sheth, and S. al-Sedairy. 1991. Endotoxemia and release of tumor necrosis factor and interleukin 1 alpha in acute heatstroke. J. Appl. Physiol., 70: 2640–2644.

Bouchama, A., S. al-Sedairy, S. Siddiqui, E. Shail, and M. Rezeig. 1993. Elevated pyrogenic cytokines in heatstroke. Chest. 104:1498–1502.

Bouchama, A. and J.P. Knochel. 2002. Heat stroke. N. Engl. J. Med. 346:1978-1988.

Bouchama, A., G. Roberts, F. Al Mohanna, R. El-Sayed, B. Lach, S. Chollet-Martin, V. Ollivier, R. Al Baradei, A. Loualich, S. Nakeeb, A. Eldali, and D. De Prost. 2005. Inflammatory, hemostatic, and clinical changes in a baboon experimental model for heatstroke. J. Appl. Physiol. 98:697-705.

Bouchama, A., M. Dehbi, and E. Chaves-Carballo. 2007. Cooling and hemodynamic management in heatstroke: practical recommendations. Crit. Care. 11:R54-R63.

Brix-Christensen V., S.K. Andersen, R. Andersen, A. Mengel, T. Dyhr, N.T. Andersen, A. Larsson, O. Schmitz, H. Ørskov, and E. Tønnesen. 2004. Acute hyperinsulinemia restrains endotoxin-induced systemic inflammatory response: an experimental study in a porcine model. Anesth. 100:861-870.

Brockman, R.P., L.P. Milligan, W.L. Grovum, and A. Dobson (Ed.). 1986. Pancreatic and adrenal hormonal regulation of metabolism. Control of digestion and metabolism in ruminant. Prentice-Hall, Englewood Cliffs, NJ: p.405.

Brody, S. 1945. *Bioenergetics and Growth*. Hafner Publishing Co.,New York.

Brown D.A and J.L. Stow. 1996. Protein trafficking and polarity in kidney epithelium: from cell biology to physiology. Physiol. Rev. 76:245-97.

Brown-Brandl, T.M., R.A. Eigenberg , G.L. Hahn, J.A. Nienaber, T.L. Mader, D.E. Spiers, and A M. Parkhurst. 2005. Analyses of thermoregulatory responses of feeder cattle exposed to simulated heat waves. Int. J. Biometerol. 49:285-296.

Brown-Brandl, T.M., J.A. Nienaber, H. Xin, and R.S. Gates. 2004. A literature review of swine heat production. Trans. ASAE. 47:259-270.

Burger, F.J., J.P. Du Plessis, E.U. Bieler, and P.J. Lategan. 1972. Further studies on the chemical changes in the blood and tissues of rats during hyperthermia S. Afr. Med. J. 41:718-721.

Burkey, T.E., A. Skjolaas, and J.E. Minton. 2009. Board-invited review: Porcine mucosal immunity of the gastrointestinal tract. J. Anim. Sci. 87:1493-1501.

Campbell, M.K. 1999. Biochemistry. 3rd ed. Saunders College Publishing, New York, NY.

Campbell, J.R., M.D. Kenealy, and K.L. Campbell. 2003. Animal Sciences: The biology, care, and production of domestic animals. McGraw Hill, San Francisco, CA.

Cant, J.P., B.W. McBride, and W.J. Croom Jr. 1996. The regulation of intestinal metabolism and its impact on whole animal energetic. J. Anim. Sci. 74:2541-2553.

Caspani, M.I., M. Savioli, S. Crotti, P. Bruzzone, and I. Gattinoni. 2004. Heat stress: characteristics, pathophysiology and avoidable mistakes. Minerva Anestesiol. 70:617-24.

Chen, C.L., S. Sangiah, H. Chen, J.D. Roder, and Y. Shen. 1994. Effects of heat stress on Na+, K (+)-ATPase, Mg (2+)-activated ATPase, and Na (+)-ATPase activities of broiler chickens vital organs. J. Toxicol. Environ. Health. 41:345-356.

Chen, S., K. Niu, and M. Lin. 2006. Cerebrovascular dysfunction is an attractive target for therapy in heat stroke. Clin. Exper. Pharmacol. Physiol. 33:663-672.

Chilliard, Y. 1993. Dietary fat and adipose tissue metabolism in ruminants, pigs, and rodents: a review. J. Dairy Sci. 76:3897-3931.

Clausen T. and O. Hansen. 1977. The effect of catecholamines on Na-K transport and membrane potential in rat soleus muscle. J Physiol 270: 383–414.

Clausen, T. 2010. Hormonal and pharmacological modification of plasma potassium homeostasis. Fundament. Clin. Pharmacol. 24: 595–605.

Collier, R.J., G.E. Dahl, and M.J. VanBaale. 2006. Major advances associated with environmental effects on dairy cattle. J. Dairy Sci. 1244-1253.

Collin, A., J. van Milgen, S. Dubois, and J. Noblet. 2001a. Effect of high temperature and feeding level on energy utilization in piglets. J. Anim. Sci. 79:1849-57.

Collin, A., J. van Milgen, and J. Le Dividich. 2001b. Modeling the effect of high, constant temperature on food intake in young growing pigs. J. Anim. Sci. 72:519-527.

Corton, J.M., J.G. Gillespie, and D.G. Hardie. 1994. Role of the AMP-activated protein kinase in the cellular response. Curr. Biol. 4:315-324.

Crandall, C.G. and J. Gonzalez-Alonso. 2010. Review: Cardiovascular function in the heat- stressed human. Acta. Physiol. 199:407–423.

Cronje, P.B. 2007. Gut health, osmoregulation and resilience to heat stress in poultry. Aust. Poult. Sci. Symp. 19:9-13.

Curtis, S.E. 1983. Environmental Management in Animal Agriculture. Ames; Iowa State University Press.

D'Allaire, S., R. Drolet, and D. Brodeur. 1996. Sow mortality associated with high ambient temperatures. Can. Vet. J. 37:237-239.

Damanhouri, Z.A. and O.S. Tayeb. 1992. Animal models for heat stroke studies. J. Pharmacol. Toxicol. Methods 28:119-127.

Daniel, H. 2004. Molecular and integrative physiology of intestinal peptide transport. Annu. Rev. Physiol. 66:361-384.

DeShazer, J.A., G.L. Hahn and H. Xin. 2009. Chapter 1: Basic principles of the thermal environment and livestock energetics. In *Livestock Energetics and Thermal Environment Management*. J.A. DeShazer ed.

De Braganca, M.M. and A. Prunier. 1999. Effects of low feed intake and hot environment on plasma profiles of glucose, nonesterified fatty acids, insulin, glucagon, and IGF-1 in lactating sows. Domest. Anim. Endocrinol. 16:89-101.

Deutsch, J. 1983. Glucose-6-phosphate dehydrogenase, p. 190-197 in H. V. Bergmeyer (ed.), Methods in enzymatic analysis, vol. 3. Verlag Chemie, Weinheim, Germany.

Dobrota, D., M. Matejovicova, E.G. Kurella, and A.A. Boldyrev. 1999. Na/K-ATPase under oxidative stress: molecular mechanisms of injury. Cell. Molec. Neurobiol. 19:141-149.

Doi, K., T. Ohno, and A. Kuroshima. 1982. Role of endocrine pancreas in temperature acclimation. Life Sci. 30:2253-2259.

Dokladny, K., D. Ye, J.C. Kennedy, P.L. Moseley, and T.Y. Ma. 2008. Cellular and molecular mechanisms of heat stress-induced up-regulation of occluding protein expression: regulatory role of heat shock factor-1. Am. J. Pathol. 172:659-670.

Dokladny, K., P.L. Moseley, and T.Y. Ma. 2006. Physiologically relevant increase in temperature causes an increase in intestinal epithelial tight junction permeability. Am. J. Physiol. Gastrointest. Liver Physiol. 290:G204-G212.

DuBose, D.A., K. Basamania, L. Maglione, and J. Rowlands. 1983. Role of bacterial endotoxins of intestinal origin in rat heat stress mortality. J. Appl. Physiol. Respirat. Environ. Exercise Physiol. 54:31-36.

Drexler, A.M. 1995. Tumor necrosis factor: its role in HIV/ AIDS. STEP Perspect. 7:13–15.

Erlij F. and S. Grinstein. 1976. The number of sodium ion pumping sites in skeletal muscle and its modification by insulin. J .Physiol. 259:13-31.

Febbraio, M.A. 2001. Alterations in energy metabolism during exercise and heat stress.Sports Med. 31:47-59.

Ferraris, R.P. and H.V. Carey. 2000. Intestinal transport during fasting and malnutrition. 20:195-219.

Fink, W.J., D.L. Costill, and P.J. Van Handel. 1975. Leg muscle metabolism during exercise in the heat and cold. J. Appl. Physiol. 34:183-190.

Frankel, H.M. 1968. Serum lipid changs during progressive hyperthermia. Life Sci. 21:1203-1207.

Francesconi, R.P., J.S. Willis, S.L. Gaffin, and R.W. Hubbard. 1997. On the trail of potassium in heat injury. Wilderness Environ. Med. 8:105-110.

Francesconi, R.P., B.J. Fine, and J.L. Kobrick. 1976. Heat and simulated high altitude: effects on biochemical indices of stress and performance. Avait. Space Environ. Med. 47:548-552.

Frascella, D.W., I.S. Kampa, and B.H. Francis. 1977. Metabolic effect of high environmental temperature on non-diabetic and diabetic rats. Horm. Metab. Res. 9:374-378.

Fryer, L.G. A.Parbu-Patel, and D. Carling. 2002. The anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. J. Biol. Chem. 277:25226-25232.

Fukumoto, T., T. Tanaka, H. Fujioka, S. Yoshihara, T. Ochi, and A. Kuroiwa. 1988. Differences in composition of sweat induced by thermal exposure and by running exercise. Clin. Cardiol. 11:707-709.

Fuller, A., S.K. Maloney, P.R. Kamerman, G. Mitchell, and D. Mitchell. 2000. Absence of selective brain cooling in free-ranging zebras in their natural habitat. Exp. Physiol. 85:209-217.

Fuller, W., V. Parmar, P. Eaton, J.R. Bell, and M.J. Shattock. 2003. Cardiac ischemia causes inhibition of the Na/K ATPase by a labile cytosolic compound whose production is linked to oxidant stress. Cardiovasc. Res. 57:1044-1051.

Fuquay, J.W. Heat stress as it affects animal production. 1981. J. Anim. Sci. 52:164-174.

Gabler, N.K., J.S. Radcliffe, J.D. Spencer, D.M. Webel, and M.E. Spurlock. 2009. Feeding long-chain n−3 polyunsaturated fatty acids during gestation increases

intestinal glucose absorption potentially via the acute activation of AMPK. J. Nutr. Biochem. 20: 17–25.

Gabler, N.K., J.D. Spencer, D.M. Webel, and M.E. Spurlock. 2007. In utero and postnatal exposure to long chain (n-3) PUFA enhances intestinal glucose absorption and energy stores in weanling pigs. J. Nutr. 137:2351-2358.

Gaffin, S.L. and R. Hubbard. 1996. Experimental approaches to therapy and prophylaxis for heat stress and heat-stroke. Wild. Environ. Med. 7:312-334.

Gamou, S., Y. Shimizu, and N. Shimizu. 1990. Adipocytes. In: Pollard, J.W., Walker, J.M. (Eds.), Methods in Molecular Biology: Vol. 5, Animal Culture. The Humana Press, Clifton, NJ, pp. 197–207.

Garriga, C., R.R. Hunter, C. Amat, J.M. Planas, M.A. Mitchell, and M. Moreto. 2006. Heat stress increased apical glucose transport in the chicken jejunum. Am. J. Physiol. Regul. Integr. Comp. Physiol. 290:R195-R201.

Gathiram, P., M.T. Wells, D. Raidoo, J.G. Brock-Utne, and S.L. Gaffin. 1988. Portal and systemic lipopolysaccharide concentrations in heat-stressed primates. Circ. Shock. 25:223-230.

Geraert P.A., J.C. Padilha, and S. Guillaumin. 1996. Metabolic and endocrine changes induced by chronic heat exposure in broiler chickens: growth performance, body composition and energy retention. Br. J. Nutr. 75:195-204.

Glazer, J.L. 2009. Management of heatstroke and heat exhaustion. Am. Fam. Physic. 71:2133-214.

Godfray, H.C.J., I.R. Crute, L. Haddad, D. Lawrence, J.F. Muir, N. Nisbett, J. Pretty, S. Robinson, C. Toulmin, and R. Witeley. 2010. The future of the global food system. Phil. Trans. R. Soc. 365:2769-2777.

Goodyear, L.J. and B.B. Kahn. 1998. Exercise, glucose transport and insulin sensitivity. Annu. Rev. Med. 49:235-261.

Gordon LG, G. Kottra, and E. Frömter. 1989. Electrical impedance analysis of leaky epithelia: theory, techniques, and leak artifact problems. Methods Enzymol.171:642-663.

Gourdine, J.L., J.P. Bidanel, J. Noblet, and D. Renaudeau. 2006. Effects of season and breed on the feeding behavior of multiparous lactating sows in a tropical humid climate. J. Anim. Sci. 84:469-480.

Granada, J.F., G.L. Kaluza, R.L. Wilensky, B.C.Biedermann, R.S. Schwartz, and E. Falk. 2009. Porcine models of coronary atherosclerosis and vulnerable plaque for imaging and interventional research. Euro. Intervent. 5:140-148.

Guilloteau, P., R. Zabielski, H.M. Hammon, and C.C. Metges. 2010. Nutritional programming of gastrointestinal tract development. Is the pig a good model for man? Nutr. Res. Rev. 23:4-22.

Johnson, L.R. 2006. Physiology of the gastrointestinal tract. Elsevier Academic Press, Burlington, MA. Vol 2. 4th ed. 1559-1594.

Hadley, M.E. 2000. Endocrinology: Fifth Edition. Prentice-Hall Inc., Upper Saddle River, NJ. p.255-263.

Hales, J.R. and M.E. Webster. 1967. Respiratory function during thermal tachypnoea in sheep. J. Physiol. 190:241-260.

Hall, G.M., J.N. Lucke, R. Lovell, and D. Lister, 1980. Porcine malignant hyperthermia. VII: Hepatic metabolism. Br. J. Anaesth. 52:11-17.

Hall, D.M., K.R. Baumgardner, T.D. Overley, and C.V. Gisolfi. 1999. Splanchnic tissues undergo hypoxic stress during whole body hyperthermia. Am. J. Physiol. Gastrointest. Liver Physiol. 276:G1195-G1203.

Hall, D.M., G.R. Buettner, L.W. Oberley, L. Xu, R.D. Matthes, and C.V. Gisolfi. 2001. Mechanisms of circulatory and intestinal barrier dysfunction during whole body hyperthermia. Am. J. Physiol. Heart Circ. Physiol. 280:H509-H521.

Hardie, D.G. and D. Carling. 1997. The AMP-activated protein kinase. Fuel gauge of the mammalian cell? Eur. J. Biochem. 246:259-273.

Hardie, D.G. 2004. The AMP-activated protein kinase pathway – new players upstream and downstream. J. Cell Sci. 117:5479-5487.

Hardie, D.G. 2008. AMPK: a key regulator of energy balance in the single cell and the whole organism. Int. J. Obes. London. 32:S7-12

Hayhoe K, D. Cayan, C.B. Field, P.C. Frumhoff, E.P. Maurer, N.L. Miller, S.C. Moser, S.H. Schneider, K.N. Cahill, E.E. Cleland, L. Dale, R. Drapek, R.M. Hanemann, L.S. Kalkstein, J. Lenihan, C.K. Lunch R.P. Neilson, S.C. Sheridan, and J.H. Verville. 2004. Emissions pathways, climate change, and impacts on California. Proc. Natl. Acad. Sci. USA. 101:12422-12427.

Heath, M.E. 1983. The effects of rearing-temperature on body composition in young pigs. Comp. Biochem. Phsiol. A Comp. Physiol. 76:363-366.

Heppner, K.M., K.M. Habegger, J. Day, P.T. Pfluger, D. Perez-Tilve, B. Ward, V. Gelfanov, S.C. Woods, R. DiMarchi, and M. Tschop. 2010. Glucagon regulation of energy metabolism. Physiol. Behav. 100:545-548.

Holowatz L.A., C. Thompson-Torgerson, and W. L. Kenney. 2010. Aging and the control of human skin blood flow. Front. Biosci.15:718-39.

Hossain, Z. and T. Hirata. 2008. Molecular mechanism of intestinal permeability: interaction at tight junctions. Mol. Biosyst. 4:1181-1185.

Huff-Lonergan, E., T. Mitsuhashi, D.D. Beekman, F.C. Parrish Jr., D.G. Olson, and R.M. Robson. 1996. Proteolysis of specific muscle structural proteins by mu-calpain at low pH and temperature is similar to degradation in postmortem bovine muscle. J. Anim. Sci. 74:993-1008.

Hurley, B.F. and E.M Haymes. 1982. The effects of rest and exercise in the cold on substrate mobilization and utilization. Aviat. Environ. Med. 53:1193-1197.

Huynh, T.T.T., A.J.A. Aarnink, M.W.A. Verstegen, W.J.J. Gerrits, M.J.W. Heetkamp, B. Kemp, and T.T. Canh. 2005. Effects of increasing temperatures on physiological changes in pigs at different relative humidities. J. Anim. Sci. 83:1385-1396.

Iannello, S., P. Milazzo, and F. Belfiore. 2007. Animal and human tissue Na,K-ATPase in normal and insulin-resistant states: regulation, behavior and interpretative hypothesis on NEFA effects. Obes. Rev. 8:231-251.

Ikari, A., M. Nakano, Y. Suketa, H. Harada, and K. Takagi. 2005. Reorganization of ZO-1 by sodium-dependent glucose transporter activation after heat stress in LLC-PK1 cells. J. Cell Physiol. 203:471-478.

Ingle DL, D.E. Bauman, R.W. Mellenberger, and D.E. Johnson. 1973. Lipogenesis in the ruminant: effect of fasting and refeeding on fatty acid synthesis and enzymatic activity of sheep adipose tissue. J. Nutr. 103:1479-1488.

Iqbal, J. and M. Mahmood Hussain. 2009. Intestinal lipid absorption. Am. J. Physiol. Endocrinol. Metab. 296:E1183-E1194.

Ithoh, F., Y. Obara, H. Fuse, M.T. Rose, I. Osaka, and H. Takahashi. 1998. Effects of heat exposure on plasma insulin, glucagon and metabolites in response to nutrient injection in heifers. Comp. Biochem. Physiol. 119:157-164.

Jentjens, R.L., A.J. Wagenmakers, and A.E. Jeukendrup. 2002. Heat stress increases muscle glycogen use but reduces the oxidation of ingested carbohydrates during exercise. J. Appl. Physiol. 92:1562-1572.

Johnson, L.R., K.E. Barret, F.K. Ghishan, J.L. Merchant. H.M. Said, and J.D Wood. 2006. Physiology of the gastrointestinal tract. 4th ed. Elsev. Acad. Press, Burlington, MA.

Kamiya, M., Y. Kamiya, M. Tanaka, T. Oki, Y. Nishiba, and S. Shioya. 2006. Effects of high ambient temperature and restricted feed intake on urinary and plasma 3-methylhistidine in lactating Holstein cows. Anim. Sci. J. 77:201-207.

Kappel, M., A. Gyhrs, H. Galbo, and B.K. Pedersen. 1997. The response on glucoregulatory hormones of in vivo whole body hyperthermia. Int. J. Hyperthermia. 13:413-421.

Katsuhiko, D., T. Ohno, and A. Kuroshima. 1982. Role of endocrine pancreas in temperature acclimation. Life Sci. 30:2253-2259.

Katsumata, M., H. Yano, N. Ishida, and A. Miyazaki. 1990. Influence of a high ambient temperature and administration of clenbuterol on body composition in rats. J. Nutr. Sci. Vitaminol. 36:569-578.

Kelker, H.C., J.D. Oppenheim, D.S. Stone-Wolff, D. Henriksen-DeStefano, B.B. Aggarwal, H.C. Stevenson, and J. Vilcek. 1985. Characterization of human tumor necrosis factor produced by peripheral blood monocytes and its separation from lymphotoxin. Int. J. Cancer. 36: 69–73.

Kemp, B.E., K.I. Mitchelhill, D. Stapleton, B.J. Mitchell, Z.P. Chen, and L.A. Witters. 1999. Dealing with energy demand: the AMP-activated protein kinase. Trends Biochem. Sci. 24:22-25.

Kemp, B.E., D. Stapleton, D.J. Campbell, Z.P. Chen, S. Murthy, M. Walter, A. Gupta, J.J. Adams, F. Katsis, B. van Denderen, I.G. Jennings, T. Iseli, B.J. Mitchell and L.A. Witters. 2003. AMP-activated protein kinase, super metabolic regulator. Biochem. Soc. Trans. 31:162-168.

Kielian, T.L. and F. Blecha. 1995. CD14 and other recognition molecules for lipopolysaccharide: a review. Immunopharmacol. 29:187-205.

Kim, B.G., M.D. Lindemann, and G.L. Cromwell. 2009. The effects of dietary chromium (III) picolinate on growth performance, blood measurements, and respiratory rate in pigs kept in high and low ambient temperature. J. Anim. Sci. 87:1695-1704.

Kleiber, M. 1961. *The fire of life: an introduction to animal energetic*. John Wiley and Sons, Inc., New York.

Kletzien, R.F., P.K.W. Harris, and L.A. Foellmi. 1994. Glucose-6-phosphate dehydrogenase: a “housekeeping” enzyme subject to tissue-specific regulation by hormones, nutrients, and oxidant stress. FASEB J. 8:174-181.

Kosatsky, T. 2005. The 2003 European heat waves. Euro. Surveill. 10:148-149.

Kouba, M., D. Hermier, and J. Le Dividich. 2001. Influence of a high ambient temperature on lipid metabolism in the growing pig. J. Anim. Sci. 79:81-87.

Kovats R.S. and K.L Ebi. 2006. Heatwaves and public health in Europe. Euro. J. Pub. Health. 16: 592–599.

Kregel, K.C. and P.T. Wall. 1988. Peripheral vascular responses to hyperthermia in the rat. J. Appl. Physiol. 64:2582-2588.

Lambert, G.P. 2009. Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. J. Anim. Sci. 87:E101-E108.

Lambert, G.P. 2008. Intestinal barrier dysfunction, endotoxemia, and gastrointestinal symptoms: the 'canary in the coal mine' during exercise heat-stress. Med. Sport Sci. 53:61-73.

Lambert, G.P. 2004. Role of gastrointestinal permeability in exertional heatstroke. Exerc. Sport Sci. Rev. 32:185-190.

Lambert, G.P., C.V. Gisolfi, D.J. Berg, P.L. Moseley, L.W. Oberley, and K.C. Kregel. 2002. Selected contribution: Hyperthermia-induced intestinal permeability and the role of oxidative and nitrosative stress. J. Appl. Physiol. 92:1750-1761.

Le Bellego, J. van Milgen, and J. Noblet. 2002. Effect of high temperature and low-protein diets on the performance of growing-finishing pigs. J. Anim. Sci. 80:691-701.

Leon. L.R. 2007. Heat stroke and cytokines. In: Progress in Brain Research vol. 162. Elsevier pp 485.

Lim, C.L., G. Wilson, L. Brown, J.S. Coombes, and L.T. Mackinnon. 2007. Pre-existing inflammatory state compromises heat tolerance in rats exposed to heat stress. Am. J. Physiol. Regul. Integr. Comp. Physiol. 292:R186-R194.

Lima, A.A., L.F. Brito, H.B. Ribeiro, M.C. Martins, A.P. Lustosa, E.M. Rocha, N.L. Lima, C.M. Monte, and R.L. Guerrant. 2005. Intestinal barrier function and weight gain in malnourished children taking glutamine supplemented enteral formula. J. Pediatr. Gastroenterol. Nutr. 40:28-35.

Liu, F., J. Yin, M. Du, P. Yan, J. Xu, X. Zhu, and J. Yu. 2009. Heat-stress-induced damage to porcine small intestinal epithelium associated with down regulation of epithelial growth factor signaling. J. Anim. Sci. 87:1941-1949.

Lopez, J., G.W. Jesse, B.A. Becker, and M.R. Ellersieck. 1991. Effects of temperature on the performance of finishing swine: 1. Effects of a hot, diurnal temperature on average daily gain, feed intake, and feed efficiency. J Anim. Sci. 69:1843-1849.

LoVecchio, F., A.F. Pizon, C. Berret, and A. Balls. 2007. Outcomes after environmental hyperthermia. Am. J. Emergency Med. 25:442-444.

Luber, G. and M. McGeehin. 2008. Climate change and extreme heat events. Am. J. Prev. Med. 35:429-435.

Mansbach II., C.M. and F. Gorelick. 2007. Development and physiological regulation of intestinal lipid absorption. II. Dietary lipid absorption, complex lipid synthesis, and the intracellular packaging and secretion of chylomicrons. Am. J. Physiol. Gastrointest. Liver Physiol. 293:G645-G650.

Mailloux, R.J. and M.E. Harper. 2010. Glucose regulates enzymatic sources of mitochondrial NADPH in skeletal muscle cells; a novel role for glucose-6-phosphate dehydrogenase. FASEB J. 24:2495-2506.

Marder, J., U. Eylath, E. Moskovitz, and R. Sharir. 1990. The effect of heat exposure on blood chemistry of the hyperthermic rabbit. Comp. Biochem. Physiol. 97:245-247.

Marple, D.N., D.J. Jones, C.W. Alliston, and J.C. Forrest. 1974. Physiological and endocrinological changes in response to terminal heat stress in swine. J. Anim. Sci. 39:79-82.

Mastrangelo G., S. Hajat, E. Fadda, A. Buja, U.Fedeli, and P. Spolaore. 2006. Contrasting patterns of hospital admissions and mortality during heat waves: Are deaths from circulatory disease a real excess or an artifact? Med. Hypoth. 66:1025–1028.

McBride, B.W. and J.M. Kelly. 1990. Energy cost of absorption and metabolism in the ruminant gastrointestinal tract and liver: a review. J. Anim. Sci. 68:2997-3010.

McGlone, J.J., W.F. Stansburt, L.F. Tribble, and J.L. Morrow. 1988. Photoperiod and heat stress influence on lactating sow performance and photoperiod effects on nursery pig performance. J. Anim. Sci. 66:1915-1919.

McMillin, J.M. 1990. Blood Glucose. In: Clinical Methods: The history, physical, and laboratory examinations. 3rd ed. Butterworths. Boson, MA.

Melody, J.L., S.M. Lonergan, L.J. Rowe, T.W. Huiatt, M.S. Mayes, and E. Huff-Lonergan. 2004. Early postmortem biochemical factors influence tenderness and water-holding capacity of three porcine muscles. J. Anim. Sci. 82:1195-1205.

Miller, E.R. and D.E. Ullrey. 1987. The pig as a model for human nutrition. Annu. Rev. Nutr. 7:361-382.

Mitev, S., S. Dinevska-Kovkarovska, and B. Miova. 2005. Effect of the acclimation to high environmental temperature on the activity of hepatic glycogen phosphorylase (a + b and a), liver glycogen content and blood glucose level in rat. J. Therm. Biol. 30:563-568.

Mitchell, M.A. and A.J. Carlisle. 1992. The effects of chronic exposure to elevated environmental temperature on intestinal morphology and nutrient absorption in the domestic fowl (Gallus domesticus). Comp. Biochem. Physiol. A. 101:137-142.

Miyoshi, J. and Y. Takai. 2008. Structural and functional associations of apical junctions with cytoskeleton. Biochim. Biophys. Acta. 1778:670-691.

Monteleone, J.A. and D.M. Keefe. 1969. Transient hyperglycemia and aketotic hyperosmolar acidosis with heatstroke. Pediatrics. 44:737-741.

Morrison, D.C. and J.L. Ryan. 1987. Endotoxins and disease mechanisms. Ann. Rev. Med. 38:417-432.

Morth, J.P., B.P. Pedersen, M.J. Buch-Pedersen, J.P. Andersen, B. Vilsen, M.G. Palmgren, and P. Nissen. 2010. A structural overview of the plasma membrane Na^+,K^+-ATPase and H+-ATPase ion pumps. Nat. Rev. Molec. Cell Biol. 12:60-70. Review.

Mount, L.E. 1979. *Adaptation to thermal environment: Man and his productive animals*. Edward Arnold Limited, Thomson Litho Ltd, East Kilbride, Scotland.

Mu, J., J.T. Brozinick, O. Valladares, M. Bucan, and M.J. Birnbaum. 2001. A role for AMP-activated protein kinase in contraction-and hypoxia-regulated glucose transport in skeletal muscle. Mol. Cell 7:1085-1094.

Murray, D. and G.E. Wild. 1980. Effect of fasting on Na-K-ATPase activity in rat small intestinal mucosa. Can. J. Physiol. Pharmacol. 58:643-649.

Nafikov, R.A. and D.C. Beitz. 2007. Carbohydrate and lipid metabolism in farm animals. J. Nutr. 137:702-705.

Najemnikova, E., C.D. Rodgers, and M. Locke. 2007. Altered heat stress response following streptozotocin-induced diabetes. Cell Stress Chap.12:342-352.

National Aeronautics and Space Administration Goddard Institute for Space Studies (GISS). 2010. GISS Surface Temperature Analysis (GISTEMP). http://www.giss.nasa.gov/research/news/20100121/

National Oceanic and Atmospheric Administration's (NOAA) National Weather Service. 2000. NWS southern region headquarters heat-wave links. www.weather.gov

National Research Defense Council (NRDC), 2007. Global Warming. www.nrdc.org

Nienaber, J.A., G.L. Hahn, and J.T. Yen. 1987. Thermal environment effects on growing-finishing swine, Part II - Carcass composition and organ weights. Transactions of the ASAE 30:1776-1779.

Nienaber, J.A., G.L. Hahn, and R.A. Eigenberg. 1999. Quantifying livestock responses for heat stress management: a review. Int. J. Biometeorol. 42:183–188.

Nienaber, J.A. and G.L. Hahn. 2007. Livestock production system management responses to thermal challenges. Int. J. Biometeorol. 52:149-157.

Niu, C.S., M.T. Lin, I.M. Liu, and J.T. Cheng. 2003. Role of striatal glutamate in heatstroke-induced damage in streptozotocin-induced diabetic rats. Neurosci. Lett. 348:77-80.

Nixdorf-Miller, A., D.M. Hunsaker, and J.C. Hunsaker. 2006. Hypothermia and hyperthermia medicolegal investigation of morbidity and mortality from exposure to environmental temperature extremes. Arch. Pathol. Lab. Med. 130:1297-1304.

O'Brien, M.D., R.P. Rhoads, S.R. Sanders, G.C. Duff, and L.H. Baumgard. 2010. Metabolic adaptations to heat stress in growing cattle. Domest. Anim. Endocrinol. 38:86-94.

O'Neil M.S., R. Carter, J.K. Kish, C.J. Gronlund, J.L. White-Newsome, X. Manarolla, A. Zanobetti, and J.D. Schwartz. 2009. Preventing heat-related morbidity and mortality: New approaches in a changing climate. Maturitas. 64: 98–103.

Oseni, S., I. Misztal, S. Tsuruta, and R. Rekaya. 2003. Seasonality of days open in US Holsteins. J. Dairy Sci. 86:3718-3725.

Ostro, B.D., L.A. Roth, R.S. Green, and R. Basu. 2009. Estimating the mortality effect of the July 2006 California heat wave. Environ. Res. 109:614-619.

Ozturk, O. and S. Gumuslu. 2004. Age-related changes of antioxidant enzyme activities, glutathione status and lipid peroxidation in rat erythrocytes after heat stress. Life Sci. 75:1551-1565.

Patience, J.F., J.F. Umboh, R.K. Chaplin, and C.M. Nyachoti. 2005. Nutritional and physiological responses of growing pigs exposed to a diurnal pattern of heat stress. Livestock Prod. Sci. 96: 205-214.

Patz, J.A., D. Campbell-Lendrum, T. Holloway, and J.A. Foley. 2005. Impact of regional climate change on human health. Nature. 438:310-317.

Prosser, C., K. Stelwagen, R. Cummins, P. Guerin, N. Gill, and C. Milne. 2004. Reduction in heat-induced gastrointestinal hyperpermeability in rats by bovine colostrum and goat milk powders. J. Appl. Physiol. 96:650-654.

Prunier, A., M. Messias de Braganca, and J.L. Dividich. 1997. Influence of high ambient temperature on performance of reproductive sows. Liv. Prod. Sci. 52:123-133.

Quinteiro-Filho, W.M., A. Ribeiro, V. Ferraz-de-Paula, M.L. Pinheiro, M. Sakai, L.R.M. Sa, A.J.P. Ferreira, and J. Palermo-Neto. 2010. Heat stress impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens. 89:1905-1914.

Rahimi, G. 2005. Effect of heat shock at early growth phase on glucose and calcium regulating axis in broiler chickens. Int. J. Poult. Sci. 4:790-794.

Randle, P. J. 1998. Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. Diabetes Metab. Rev. 14:263-283.

Rasmussen, B.B., C.R. Hancock, and W.W. Winder. 1998. Postexercise recovery of skeletal muscle malonyl-CoA, acetyl-CoA carboxylase and AMP-activated protein kinase. J. Appl. Physiol. 85:1629-1634.

Renaudeau, D., E. Huc, and J. Noblet. 2007. Acclimation to high ambient temperature in Large White and Carribean Creole growing pigs. J Anim. Sci. 85:779-790.

Renaudeau, D., N. Quininou, and J. Noblet. 2001. Effects of exposure to high ambient temperature and dietary protein level of performance of multiparous lactating sows. J. Anim. Sci. 79:1240-1249.

Rhoads, M.L., R.P. Rhoads, S.R. Sanders, S.H. Carroll, W.J. Weber, B.A. Crooker, R.J. Collier, M.J. Van Baale, and L.H. Baumgard. 2007. Effects of heat stress on production, lipid metabolism and somatotropin variables in lactating cows. J. Dairy Sci. 90:230. Abs.

Rhoads, M.L., R.P. Rhoads, M.J. Van Baale, R.J. Collier, S.R. Sanders, W.J. Weber, B.A. Crooker, and L H. Baumgard. 2009a. Effects of heat stress and plane of nutrition on lactating Holstein cows: I. production, metabolism, and aspects of circulating somatotropin. J. Dairy Sci. 92:1986-1997.

Rhoads, R.P., S.R. Sanders, L. Cole, M.V. Skrzypek, T.H. Elsasser, G.C. Duff, R.J. Collier, and L.H. Baumgard. 2009b. Effects of heat stress on glucose homeostasis and metabolic response to an endotoxin challenge in Holstein steers. J. Anim. Sci. 87:E-Suppl 2:78

Rhoads, M.L., J.W. Kim, R.J. Collier, B.A. Crooker, Y.R. Boisclair, L.H. Baumgard, and R.P. Rhoads. 2010. Effects of heat stress and nutrition on lactating Holstein cows: II. Aspects of hepatic growth hormone responsiveness. J. Dairy Sci. 93:170-179.

Ronchi, B., U. Bernabucci, N. Lacetera, A.V. Supplizi, and A. Nardone. 1999. Distinct and common effects of heat stress and restricted feeding on metabolic status of Holstein heifers. Zoot. Nutr. Anim. 25:11-20.

Roth, J., C. Rummel, S.W. Barth , R. Gerstberger, and T.Hubschle. 2009. Molecular aspects of fever and hyperthermia. Immunol. Allergy Clin. North Am. 29:229-245.

Rowell, L.B., G.L. Brengelmann, J.R. Blackmon, R.D. Twiss, and F. Kusumi. 1968. Splanchnic blood flow and metabolism in heat-stressed man. J. Appl. Physiol. 24:475-484.

Safranski, T.J., D.L. Harris, J.A. Nienaber, G.L. Hahn, and R.L. Korthals.1997. Heat stress and limit feeding pigs: effects on growth and composition. Proceedings of the Fifth International Livestock Environment Symposium. Bloomington, Minn., May 29–31, ASAE, St. Joseph, Mich., pp 772–780

Sanders, S.R. 2010. *Effects of heat stress on energetic metabolism in rats (*Unpublished PhD dissertation). The University of Arizona, Tucson, AZ.

Sano, H., K. Takahashi, K. Ambo, and T. Tsuda. 1983. Turnover and oxidation rates of blood glucose and heat production in sheep exposed to heat. J. Dairy Sci. 66:856-861.

Sasse, J. and S.R. Gallagher. 2009. Staining proteins in gels.Curr. Protoc. Mol. Biol. 85:1-27.

Sandikci, M., U. Eren, A.G. Onol, and S. Kum. 2004. The effect of heat stress and the use of *Saccharomyces cervisiae* or (and) bacitracin zinc against heat stress on the intestinal mucosa in quails. Revue. Med. Vet. 155:552-556.

Sasse, J. and S.R. Gallagher. 2008. Detection of proteins on blot transfer membranes. Curr. Protoc. Immunol. 8:10B.

Sato, Y., T. Murakami, H. Funatsuki, S. Matsuba, H. Saruyama, and M. Tanida. 2001. Heat-shock-mediated APX gene expression and protection against chilling injury in rice seedlings. J. Exp. Bot. 52:145-151.

Schar, C. and G. Jendritzky. 2004. Hot news from summer 2003. Nature. 432:559-560.

Scheepers, A., H.G. Joost, and A. Schurmann. 2004. The glucose transporter families SGLT and GLUT: Molecular basis of normal and aberrant function. J. Parenter. Enteral. Nutr. 28:364-371.

Schmidt, P. and E.M. Widdowson. 1967. The effect of a low-protein diet and a cold environment on calorie intake and body composition in the rat. Br. J. Nutr. 21:457-465.

Schneider, P.L., D.K. Beede, and C.J. Wilcox. 1988. Nycterohemeral patterns of acid-base status, mineral concentrations and digestive function of lactating cows in natural or chamber heat stress environments. J. Anim. Sci. 66:112-125.

Semenza, J.C., J.E. McCullough, W.D. Flanders, M.A. McGeehin, and J.R. Lumpkin. 1999. Excess hospital admissions during the July 1995 heat wave in Chicago. Am. J. Prev. Medl. 16:269-277.

Sengupta, A. and R.K. Sharma. 1993. Acute heat stress in growing rats: effect on small intestinal morphometry and in vivo absorption. J. Therm. Biol. 18:145-151.

Shehab-El-Deen, M.A.M., J. L. Leroy, M. S. Fadel, S.Y. Saleh, D. Maes, and A. Van Soon. 2010. Biochemical changes in the follicular fluid of the dominant follicle of high producing dairy cows exposed to heat stress early post-partum. Anim. Repro. Sci. 117:189-200.

Shendell, D.G., M.S. Alexander, L. Lorentzon, F.A. McCarty. 2010. Knowledge and awareness of heat-related morbidity among adult recreational endurance athletes. Int. J. Biometeorol. 54:441–448.

Shirazi-Beechey, S.P., A.W. Moran, D. Bravo, and M. Al-Rammahi. 2011. Intestinal glucose sensing and regulation of glucose absorption: implications for swine nutrition. J. Anim. Sci. Epub ahead of print.

Shirazi-Beechey, S.P. 1995. Molecular biology of intestinal glucose transport. Nutr. Res. Rev. 8:27-4.

Shuman, S.H. 1972. Pattern of urban heat-wave deaths and implications for prevention: data from New York and St. Louis during July, 1966. Env. Res. 5:59-75.

Shwartz, G., M.L. Rhoads, M.J. VanBaale, R.P. Rhoads, and L.H Baumgard. 2009. Effects of a supplemental yeast culture on heat-stressed lactating Holstein cows. J. Dairy Sci. 92:935-942.

Sikora A. and E. Grzesiuk. 2007. Heat shock response in gastrointestinal tract. J. Physiol. Pharmacol. 58:43-62

Silanikove, N. 2000. Effects of heat stress on the welfare of extensively managed domestic ruminants. Livest. Prod. Sci. 67:1-18.

Silk, D.B.A., G.K. Grimble, and R.G. Ress. 1985. Protein digestion and amino acid and peptide absorption. Proc. Nutr. Soc. 44:63-72.

Silva, H.R., P.E. Phelan, and J.S. Golden. 2010. Modeling effects of urban heat island mitigation strategies on heat-related morbidity: a case study for Phoenix, Arizona, USA. Int. J. Biometerol. 54:13-22.

Simon, J.F. 1953. Effects of hyperpyrexia on the human blood count, blood chemistry and urine. J. Lab. Clin. Med. 21:400-402.

Singleton, K.D. and P.E. Wischmeyer. 2006. Oral glutamine enhances heat shock protein expression and improves survival following hyperthermia. Shock. 25:296-299.

Song, R., D.N. foster, and G.C. Shurson. 2011. Effects of feeding diets containing bacitracin methylene disalicylate (BMD) to heat-stressed finishing pigs. J. Anim. Sci. Epub before print.

Spencer, R.P. 1969. Intestinal absorption of amino acids: Current concepts. Am. J. Clin. Nutr. 22:292-299.

Spencer, J.D., A.M. Gaines, E.P. Berg, and G.L. Allee. 2005. Diet modifications to improve finishing pig growth performance and pork quality attributes during periods of heat stress. J. Anim. Sci. 83:243-254.

Spurlock, M.E. and N.K. Gabler. 2008. The development of porcine models of obesity and the metabolic syndrome. J. Nutr. 138: 397-402.

Srikandakumar, A., E.H. Johnson, and O. Mahgoub. 2003. Effect of heat stress on respiratory rate, rectal temperature and blood chemistry in Omani and Australian Merino sheep. Small Rumin. Res. 49:193–198.

Steinberg, G.R. and B.E. Kemp. 2009. AMPK in health and disease. Physiol. Rev. 89:1025-1078.

St-Pierre N.R., B. Cobanov, and G. Schnitkey. 2003. Economic Losses from Heat Stress by US Livestock Industries. J. Dairy Sci. 86:E52–E77.

Sussman, C.R. and J.L. Renfro. 1997. Heat shock-induced protection and enhancement of Na+-glucose cotransport by LLC-PK1 monolayers. Am. J. Physiol. 273:F530-F537.

Suzuki Y., A.C. Yeung, and F. Ikeno. 2011. The representative porcine Model for human cardiovascular disease. J. Biomed. Biotechnol. Epub ahead of print.

Tamminga, S. and J.W. Schrama. 1998. Environmental effects on nutrient and energy metabolism in ruminants. Arch. Anim. Nutr. 51:225-235.

Tomita, M., R. Ohkubo, and M. Hayashi. 2004. Lipopolysaccharide transport system across colonic epithelial cells in normal and infective rat. Drug Metab. Pharmacokinet. 19:33-40.

Torlinkska, T., R. Banach, J. Paluszak, and A. Gryczka-Dziadecka. 1987. Hyperthermia effect on lipolytic processes in rat blood and adipose tissue. Acta. Physiol. Pol. 38:361-366.

Turner, J.R. 2006. Molecular basis of epithelial barrier regulation: from basic mechanisms to clinical application. Am. J. Pathol. 169:1901-1909.

Ueda, I. and T. Wada. 1970. Determination of inorganic phosphate by the Molybdovanadate method in the presence of ATP and some interfering organic bases. Anal. Biochem. 37:169-174.

United States Environmental Protection Agency. 2009. www.epa.gov

Viollet, B., B. Guigas, J. Leclerc, S. Hebrard, L. Lantier, R. Mounier, F. Andreelli, and M. Foretz. 2009. AMP-activated protein kinase in the regulation of hepatic energy metabolism: from physiology to therapeutic perspectives. Acta. Physiol. 196:81-98.

Waldron, M.R., A.E. Kulick, A.W. Bell, and T.R. Overton. 2006. Acute experiment mastitis is not casual toward the development of energy-related metabolic disorders in early postpartum dairy cows. J. Dairy Sci. 89:596-610.

Wamelink, M.M.C., E.A. Struys, and C. Jakobs. 2008. The biochemistry, metabolism, and inherited defects of the pentose phosphate pathway: A review. J. Inherit. Metab. Dis. 31:703-717.

Wang, L., B. Xue, K. Wang, S. Li, and Z. Li. 2010. Effect of heat stress on endotoxin flux across mesenteric-drained and portal-drained viscera of dairy goat. J. Anim. Physiol. Anim. Nutr. (Berl). Nov 22. Epub ahead of print.

Welsh, F.K.S., S.M. Farmery, K. MacLennan, M.B. Sheridan, G.R. Barclay, P.J. Guillou, and J.V. Reynolds. 1998. Gut barrier function in malnourished patients. Gut. 42:396-401.

Wheelock, J.B., R.P Rhoads, M.J. Vanbaale, S.R. Sanders, and L.H. Baumgard. 2010. Effects of heat stress on energetic metabolism in lactating Holstein cows. J. Dairy Sci. 93:644-655.

Yalcin, S., V. Bruggeman, J. Buyse, E. Decuypere, M. Cabuk, and P.B. Siegel. 2009. Acclimation to heat during incubation: Blood hormones and metabolites in broilers exposed to daily high temperatures. Poult. Sci. 88:2006-2013.

Yan, Y., Y. Zhao, H. Wang, and M. Fan. 2006. Pathophysiological factors underlying heatstroke. Med. Hypotheses. 67:609-617.

Yang, P.C., S.H. He, and P.Y. Zheng. 2007. Investigation into the signal transduction pathway via which heat stress impairs intestinal epithelial barrier function. J. Gastroenterol. Heptaol. 22:1823-1831.

Young, A.J., M.N. Sawka, L. Levine, B.S. Cadarette, and K.B. Pandolf. 1985. Skeletal muscle metabolism during exercise is influenced by heat acclimation. J. Appl. Physiol. 59:1929-1935.

Young, V.R. 1976. An overview of proteins synthesis, degradation, and the regulation of protein content in skeletal muscle. Environ. Qual. Saf. Suppl. 5:20-42.

Yunianto, V.D., K. Hayashi, S. Kaneda, A. Ohtsuka, and Y. Tomita. 1997. Effect of environmental temperature on muscle protein turnover and heat production in tube-fed broiler chickens. Br. J. Nutr. 77:897-909.

Yu, J., P. Yin, F. Liu, G. Cheng, K. Guo, A. Lu, X. Zhu, W. Luan, and J. Xu. 2010. Effect of heat stress on the porcine small intestine: a morphological and gene expression study. Comp. Biochem. Physiol. 156:119-128.

Zhao, S. J. Wang, X. Song, X. Zhang, C. Ge, and S. Gao. 2010. Impact of dietary protein on lipid metabolism-related gene expression in porcine adipose tissue. Nutr. Metab. 7:6-18.

Zhou, G., R. Myers, Y. Li, Y. Chen, X. Shen, J. Fenyk-Melody, M. Wu, J. Ventre, T. Doebber, N. Fugii, N. Musi, M.F. Hirshman, L.J. Goodyear, and D.E. Moller. 2001. Role of AMP-activated protein kinase in mechanism of metformin action. J. Clin. Invest. 108:1167-1174.

APPENDIX

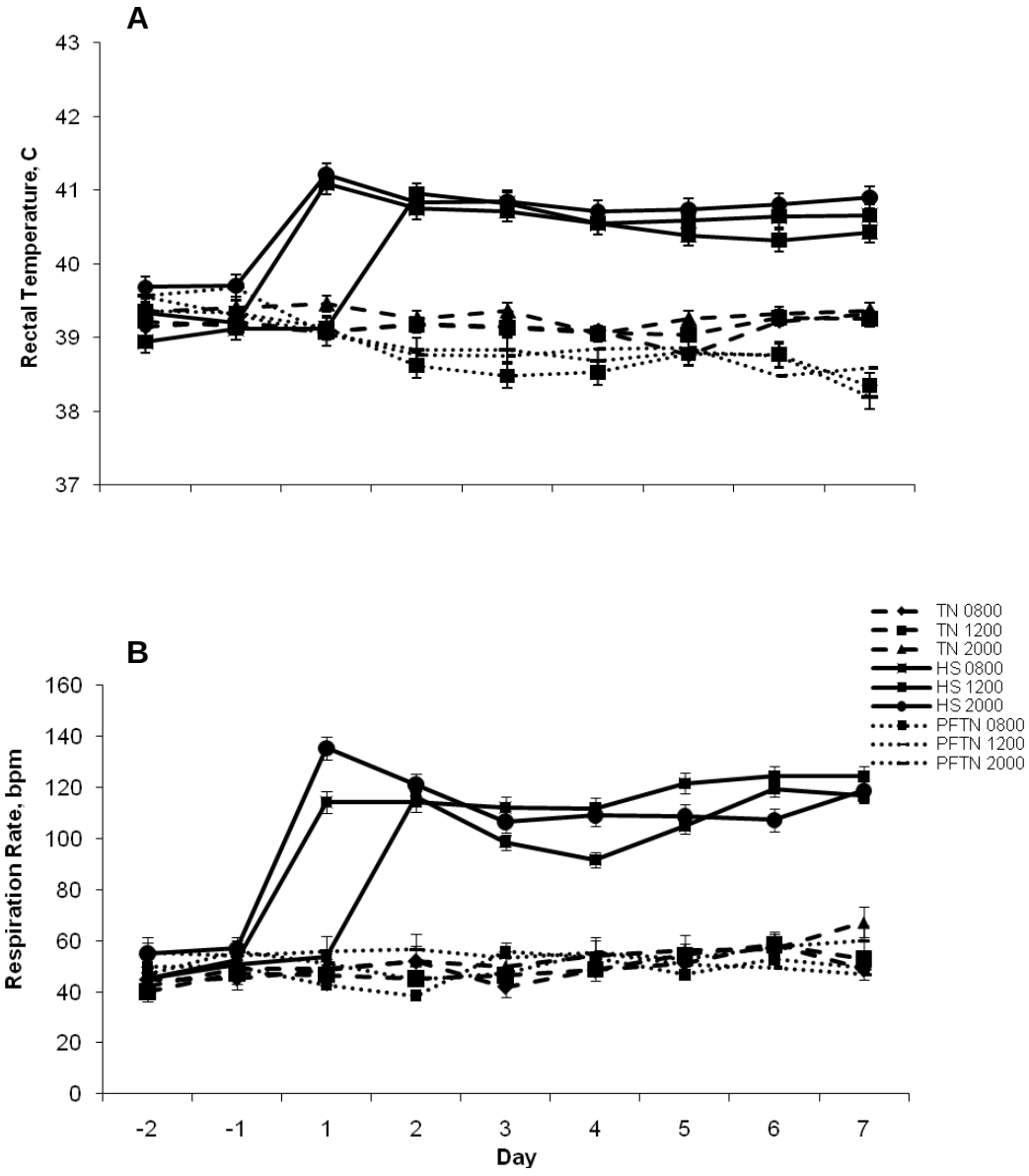

Figure A-1.1: Effects of constant environment [ad-libitum intake in thermal neutral (TN; 20℃) conditions; heat stress (HS; 35℃) or pairfee ding in thermal neutral conditions (PFTN)] on (A) Rectal temperatures and (B) Respiration rates at all timepoints in growing pigs.

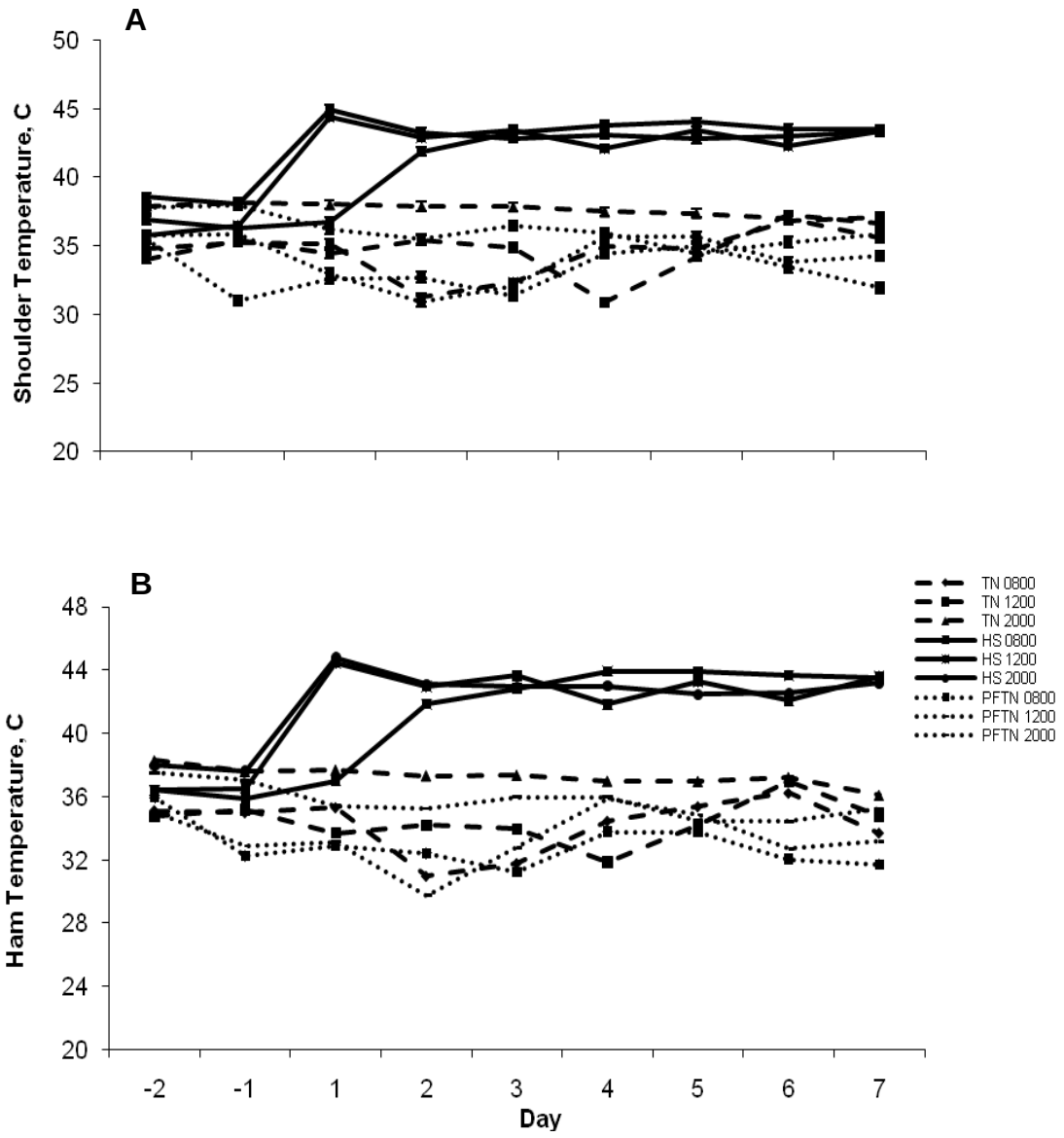

Figure A-1.2: Effects of constant environment [ad-libitum intake in thermal neutral (TN; 20℃) conditions; heat stress (HS; 35℃) or pairfee ding in thermal neutral conditions (PFTN)] on (A) Skin temperature at the shoulder and (B) Skin temperature at the ham at all timepoints in growing pigs.

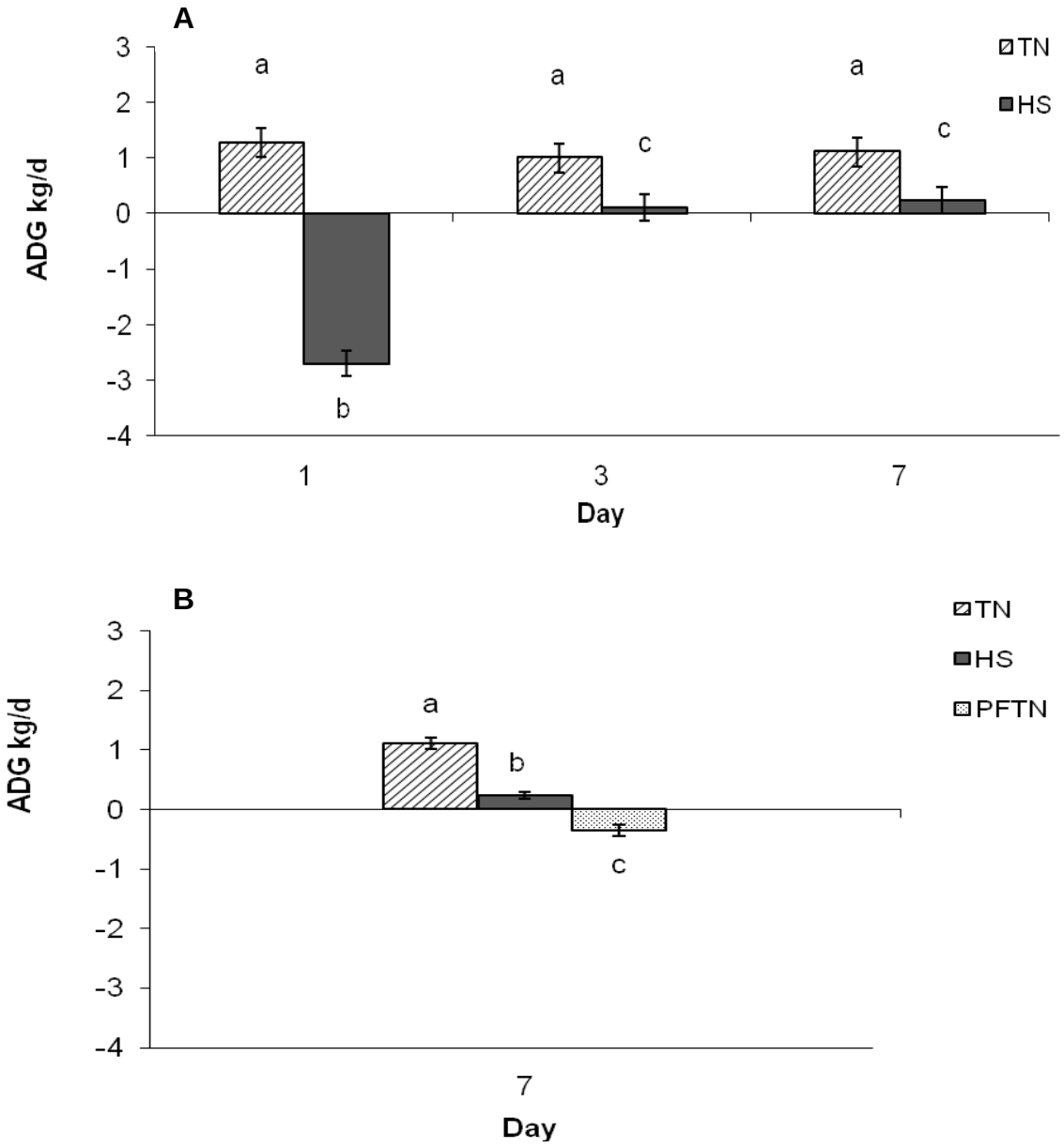

Figure A-1.3: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) and heat stress (HS; 35℃) conditions on the (A) Temporal change in average daily gain and (B) Effects of TN, HS, and pair-feeding in thermal neutral conditions (PFTN) on average daily gain at the end of the experiment. $^{a,b,c}P<0.05$

Table A-1.1 Effects of heat stress (HS) and plane of nutrition on production parameters in growing pigs

	Environment				
Parameter	TN[1]	HS	PFTN[2]	SEM	*P*
ADFI[1], kg/d	2.00[a]	1.06[b]	1.09[b]	0.14	<0.05
ADG[2], kg/d	1.11[a]	0.24[b]	-0.35[c]	0.08	<0.01
Δ BW[3], kg[2]	7.76[a]	1.65[b]	-2.47[c]	0.57	<0.01

[1]Thermal Neutral
[2]Pair-fed Thermal Neutral
[3]Average Daily Feed Intake
[4]Average Daily Gain
[5]Change in Body Weight
[a,b,c]$P < 0.05$

Table A-1.2 Effects of heat stress (HS) and plane of nutrition on plasma energetic variables in growing pigs

	Environment				
Parameter	TN[1]	HS	PFTN[2]	SEM	*P*
NEFA, mmol/L[3]	0.072[a]	0.086[a]	0.184[b]	0.029	0.05
Glucose, mg/dL	116.8	105.8	105.5	6.0	0.37
BUN, mg/dL[4]	10.0	8.0	8.3	1.1	0.37
Insulin, ng/mL	0.18	0.12	0.06	0.03	0.03
Cholesterol, mg/dL	92.7	92.7	94.3	3.9	0.96
Creatinine, mg/dL	1.0[a]	1.51[b]	1.20[a]	0.12	<0.01
Triglycerides, mg/dL	37.5	47.3	45.5	5.2	0.34
ALK Phos, IU/L[5]	203.5[a]	103.3[b]	209.8[a]	15.6	<0.01

[1]Thermal Neutral
[2]Pair-fed Thermal Neutral
[3]Non-esterified Fatty Acids
[4]Blood Urea Nitrogen
[5]Alkaline Phosphatase
[a,b,c]$P < 0.05$

Table A-1.3 Effects of heat stress (HS) and plane of nutrition on energetic variables of the Longissimus Dorsi (LD) and liver in growing pigs

	Environment				
Parameter	TN[1]	HS	PFTN[2]	SEM	*P*
LD HSP 70[3]	1.29[a]	2.27[b]	1.02[c]	0.06	0.02
LD ATPase[4]	135.5	189.2	143.3	38.8	0.54
Liver ATPase	104.3[a]	88.8[a]	68.0[b]	7.7	0.02
LD AMPK, mg/mL[5]	0.257	0.299	0.226	0.037	0.24
Liver AMPK, mg/mL	1.29[a]	1.32[a]	0.99[b]	0.11	0.08

[1]Thermal Neutral
[2]Pair-fed Thermal Neutral
[3]Heat-Shock Protein 70, arbitrary units
[4]Na^+/K^+ ATPase pump activity, µmol Pi/mg protein/h
[5]AMP-Activated Kinase Phosphorylation, mg/mL
[a,b,c]$P < 0.05$

Table A-1.4 Effects of heat stress (HS) and plane of nutrition on adipose tissue lipogenic enzyme activity in growing pigs

	Environment				
Parameter	TN[1]	HS	PFTN[2]	SEM	*P*
FAS activity[3]	19.9[a]	12.9[ab]	7.3[b]	2.8	0.03
G-6-PDH activity[4]	34.9[a]	26.6[b]	35.5[a]	2.5	0.02
G-3-PDH activity[5]	68.6	64.6	64.4	6.6	0.90

[1]Thermal Neutral
[2]Pair-fed Thermal Neutral
[3]Fatty acid synthase, nmol NADPH oxidized/min/mg protein
[4]Glucose-6-phosphate dehydrogenase, nmol NADPH produced/min/mg protein
[5]Glycerol-3-phosphate dehydrogenase, nmol NADH oxidized/min/mg protein
[a,b,c]$P < 0.05$

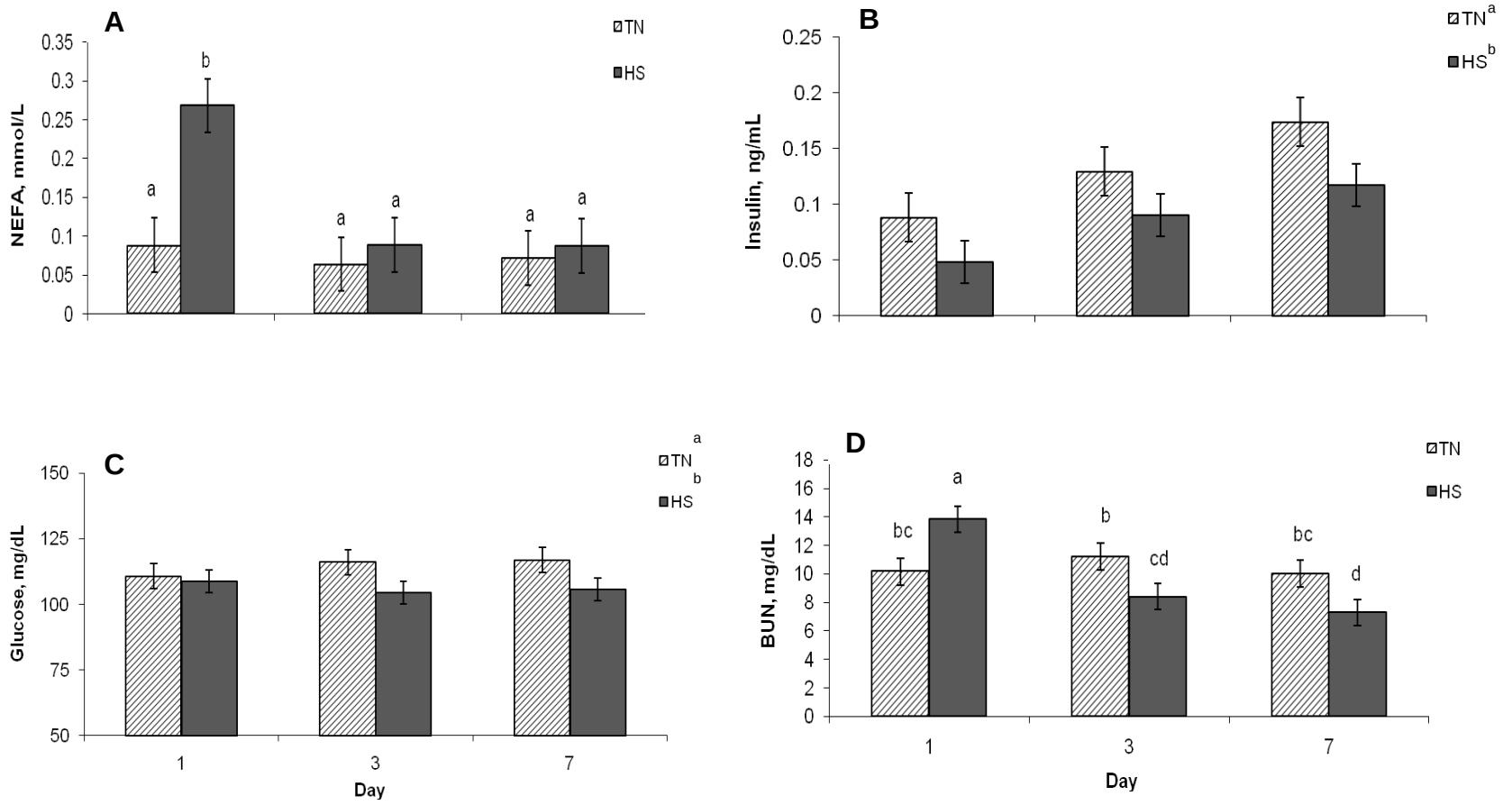

Figure A-1.4: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃), heat stress (HS; 35℃) and pair-feeding in thermal neutral conditions (PFTN) on (A) Plasma non-esterified fatty acids (NEFA), (B) Plasma insulin, (C) Plasma glucose, and (D) Plasma blood urea nitrogen (BUN) throughout the experiment in growing pigs. $^{a,b,c}P<0.05$

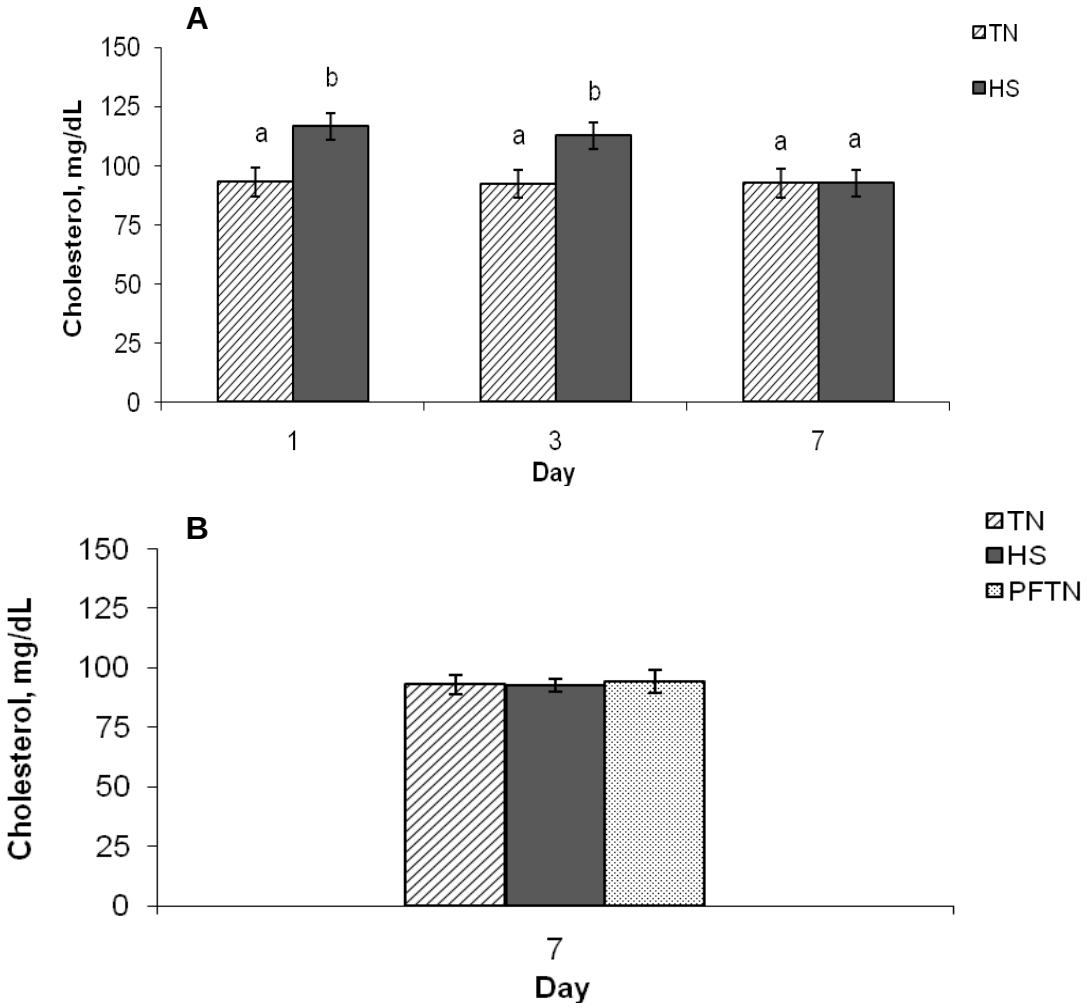

Figure A-1.5: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) and heat stress (HS; 35℃) conditions on the (A) temporal change in plasma cholesterol and (B) effects of TN, HS, and pair-feeding in thermal neutral conditions (PFTN) on change in plasma cholesterol at the end of the experiment. $^{a,b,c}P<0.05$

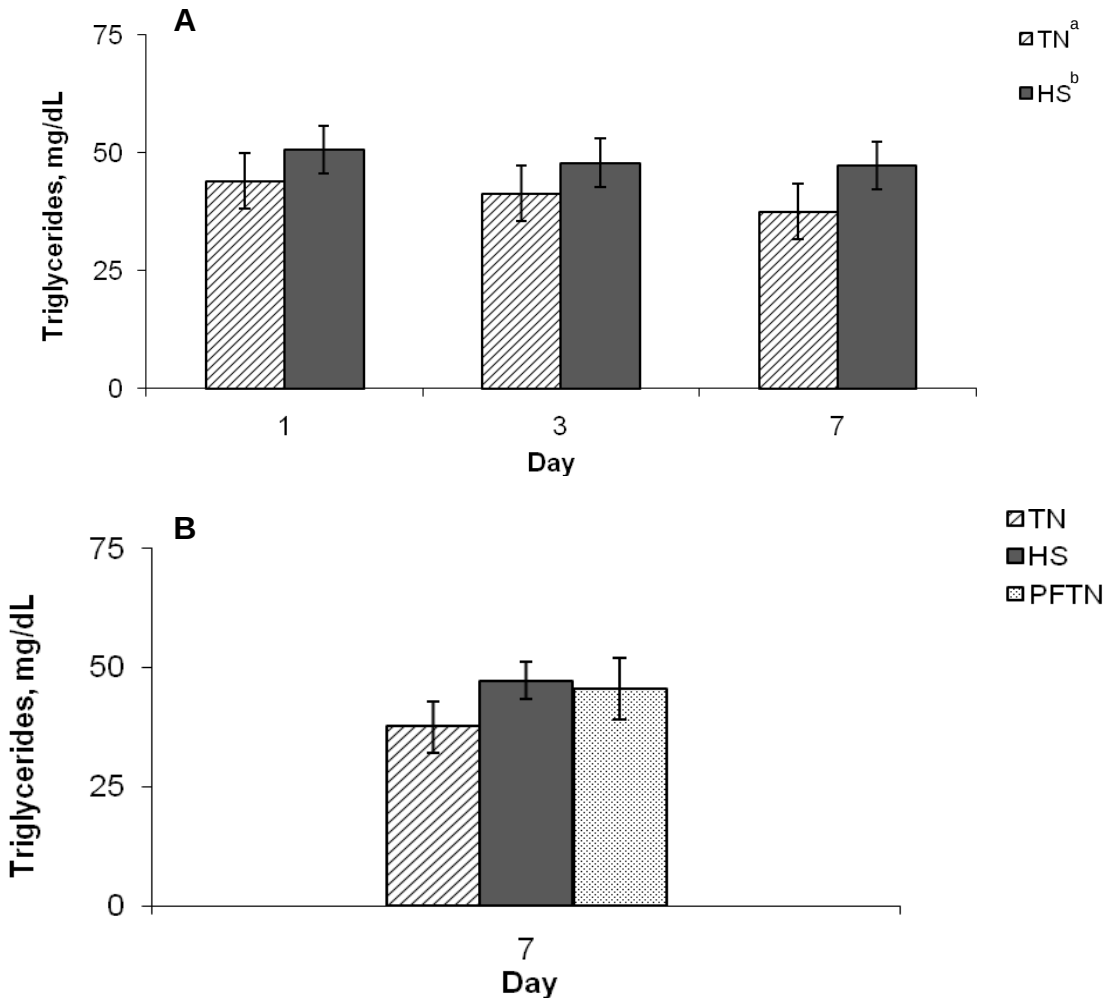

Figure A-1.6: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) and heat stress (HS; 35℃) conditions on the (A) temporal change in plasma triglycerides and (B) effects of TN, HS, and pair-feeding in thermal neutral conditions (PFTN) on change in plasma triglycerides at the end of the experiment. [a,b,c]$P<0.05$

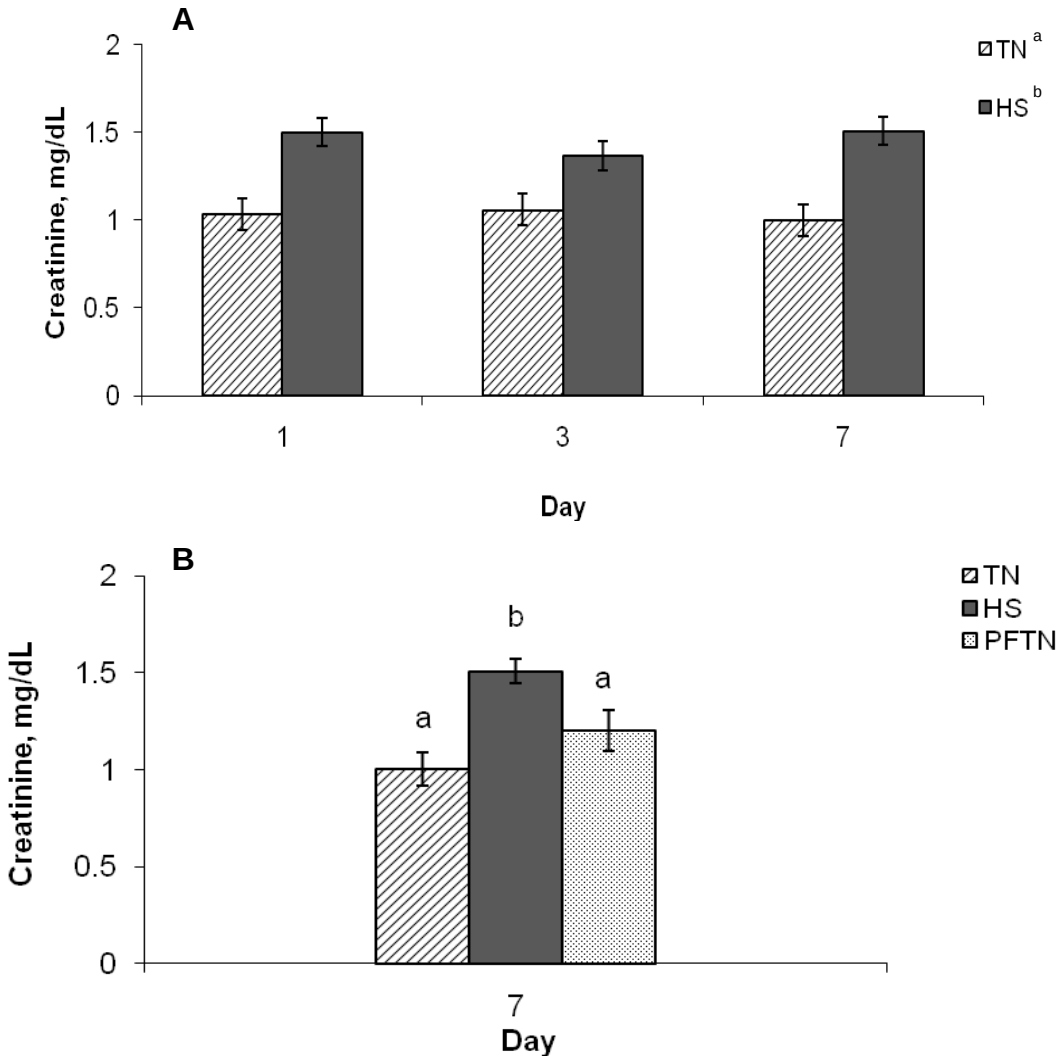

Figure A-1.7: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) and heat stress (HS; 35℃) conditions on the (A) temporal change in plasma creatinine and (B) effects of TN, HS, and pair-feeding in thermal neutral conditions (PFTN) on change in plasma creatinine at the end of the experiment. $^{a,b,c}P<0.05$

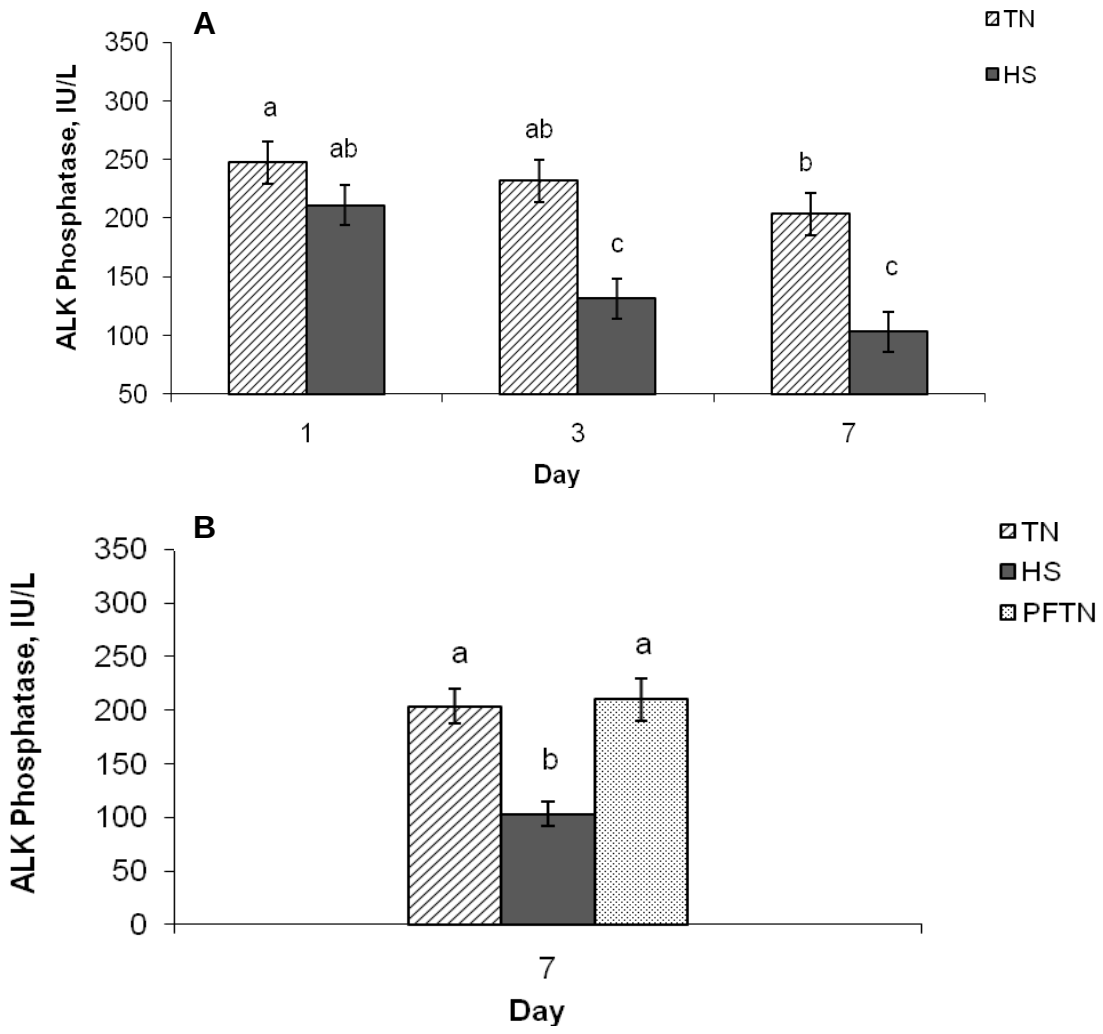

Figure A-1.8: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) and heat stress (HS; 35℃) conditions on the (A) temporal change in plasma alkaline phosphatase (ALK phosphatase) and (B) effects of TN, HS, and pair-feeding in thermal neutral conditions (PFTN) on change in plasma ALK phosphatase at the end of the experiment. $^{a,b,c}P<0.05$

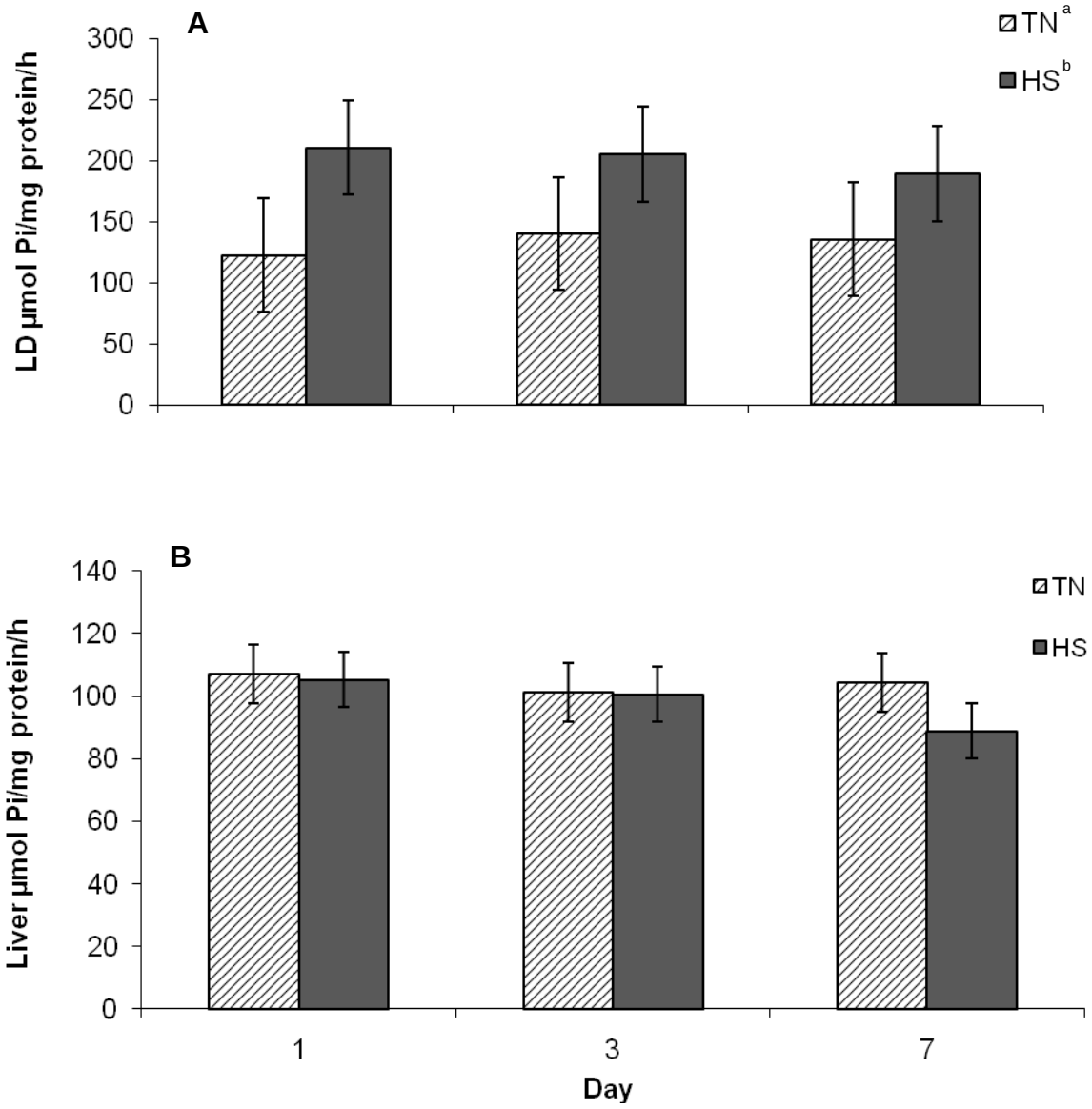

Figure A-1.9: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃), or heat stress (HS; 35℃) on (A) Longissumus Dorsi (LD) Na^+/K^+ ATPase activity and (B) liver Na^+/K^+ ATPase throughout the experiment in growing pigs. $^{a,b,c}P<0.05$

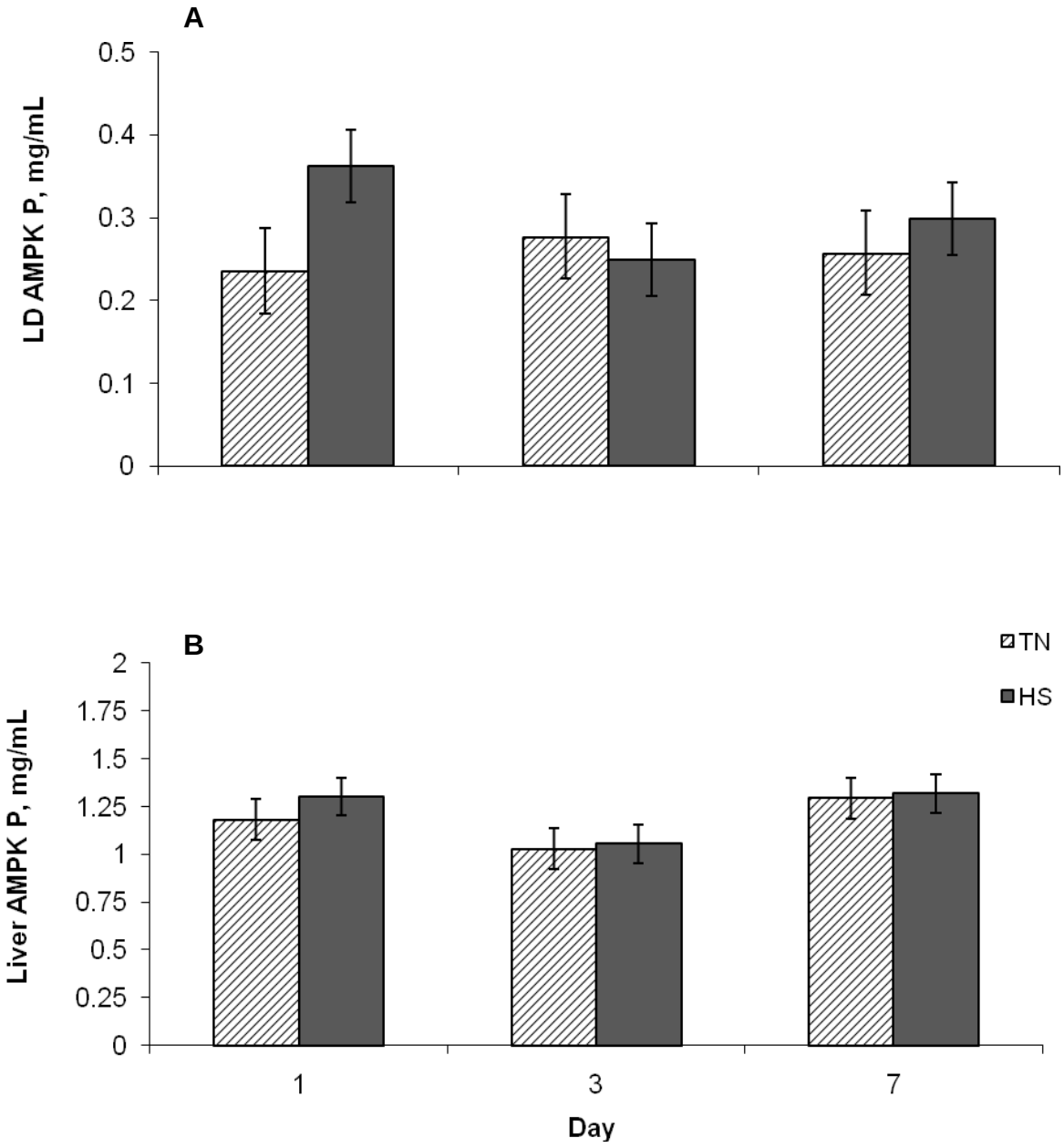

Figure A-2.0: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃), or heat stress (HS; 35℃) on (A) Longissumus Dorsi (LD) AMPK phosphorylation and (B) liver AMPK phosphoryation throughout the experiment in growing pigs. $^{a,b,c}P<0.05$

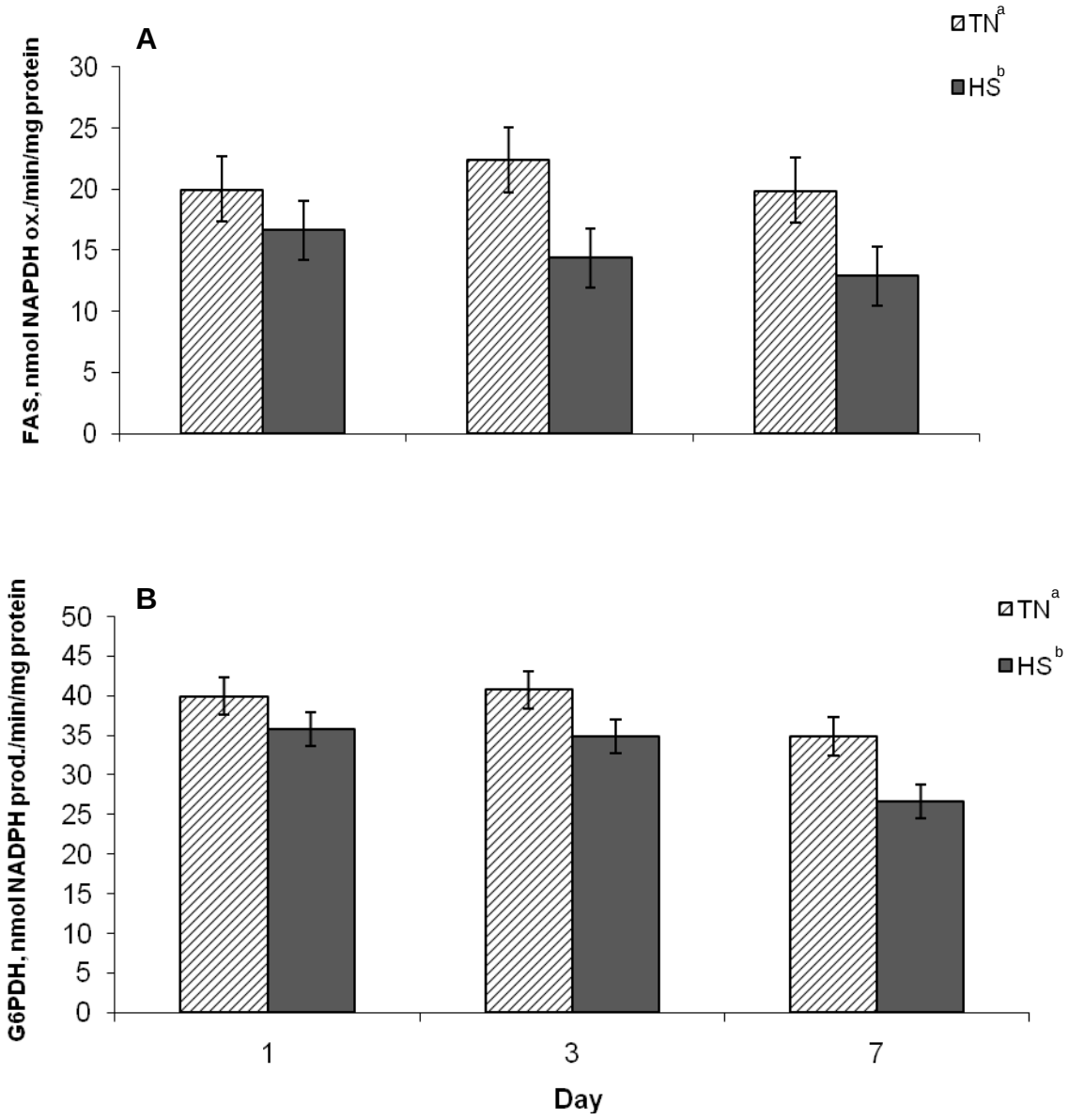

Figure A-2.1: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃), or heat stress (HS; 35℃) on (A) Adipose tissue fa tty acid synthase (FAS) enzyme activity and (B) Adipose tissue glucose-6-phosphate dehydrogenase (G-6-PDH) enzyme activity throughout the experiment in growing pigs. [a,b,c]*P*<0.05

Table A-1.5 Effects of heat stress (HS) on plasma variables in growing pigs

	Day of Experiment									
	1		3		7			*P*		
Parameter	TN[1]	HS	TN	HS	TN	HS	SEM	Trt	Day	T x D[2]
Total Protein, g/dL	6.4	7.0	6.4	6.7	6.3	6.8	0.1	<0.01	0.44	0.60
Albumin, mg/dL	3.32^{a}	3.72^{b}	3.42^{ab}	3.25^{a}	3.50^{ab}	3.46^{a}	0.1	0.56	0.38	0.10
CK, IU/L[3]	8785	4787	9140	8342	7768	6392	2180	0.26	0.66	0.75
GGT, IU/L[4]	47.3^{a}	47.8^{a}	37.0^{b}	53.2^{a}	46.8^{a}	47.8^{a}	3.9	0.07	0.80	0.11
AST, IU/L[5]	53.5	55.2	76.0	79.5	54.0	50.8	11.7	0.95	0.08	0.96
HCO_3, mEq/L[6]	30.5	25.7	31.0	28.0	28.3	26.6	1.4	<0.01	0.35	0.53
Calcium, mg/dL	10.72	10.05	10.82	10.16	10.87	10.31	0.16	<0.01	0.44	0.93
Magnesium, mg/dL	1.60	1.73	1.62	1.76	1.67	1.82	0.07	0.02	0.43	0.99
Potassium, mg/dL	6.6	6.1	6.8	5.9	6.8	6.4	0.4	0.07	0.76	0.79
Sodium, mg/dL	139.7^{b}	142.2^{c}	138.8^{ab}	139.3^{b}	141.0^{bc}	136.5^{a}	1.1	0.60	0.10	<0.01
Chloride, mEq/L	100.5^{a}	105.3^{b}	99.0^{a}	104.0^{b}	101.8^{ab}	100.7^{a}	1.1	<0.01	0.30	<0.01
Phosphorus, mg/dL	8.1^{b}	9.3^{c}	7.5^{ab}	7.3^{a}	8.8^{c}	7.3^{a}	0.3	0.48	<0.01	<0.01

[1]Thermal Neutral
[2]Treatment x Day interaction
[3]Creatine Kinase
[4]Gamma-glutamyl Transferase
[5]Aspartate Amino Transferase
[6]Bicarbonate
$^{a,b,c}P<0.05$

Table A-1.6 Effects heat stress (HS) on plasma variables in growing pigs

	Environment				
Parameter	TN[1]	HS	PFTN[2]	SEM	*P*
Total Protein, g/dL	6.3[a]	6.8[b]	7.4[c]	0.1	<0.01
Albumin, mg/dL	3.50[a]	3.46[a]	4.25[b]	0.15	<0.01
Sodium, mEq/L	141.0[a]	136.6[b]	139.8[a]	0.82	<0.01
Potassium, mEq/L	6.80	6.41	6.73	0.44	0.75
Chloride, mEq/L	101.8	100.7	99.3	0.9	0.26
Calcium, mg/dL	10.87[a]	10.31[b]	10.68[ab]	0.16	0.03
Phosphorus, mg/dL	8.80[a]	7.29[b]	8.43[a]	0.32	<0.01
Magnesium, mg/dL	1.67	1.82	1.75	0.08	0.33
HCO_3^-, mEq/L[1]	28.3	26.6	25.0	1.68	0.47
AST, IU/L[2]	54.0	50.8	76.5	8.85	0.16
CK, IU/L[3]	7768	6392	9257	2011	0.60
GGT, IU/L[4]	46.8	47.8	40	3.6	0.33

[1]Thermal Neutral
[2]Pair-fed Thermal Neutral
[3]Bicarbonate
[4]Aspartate Aminotransferase
[5]Creatine Kinase
[6]Gamma-glutamyl Transferase
[a,b,c]$P < 0.05$

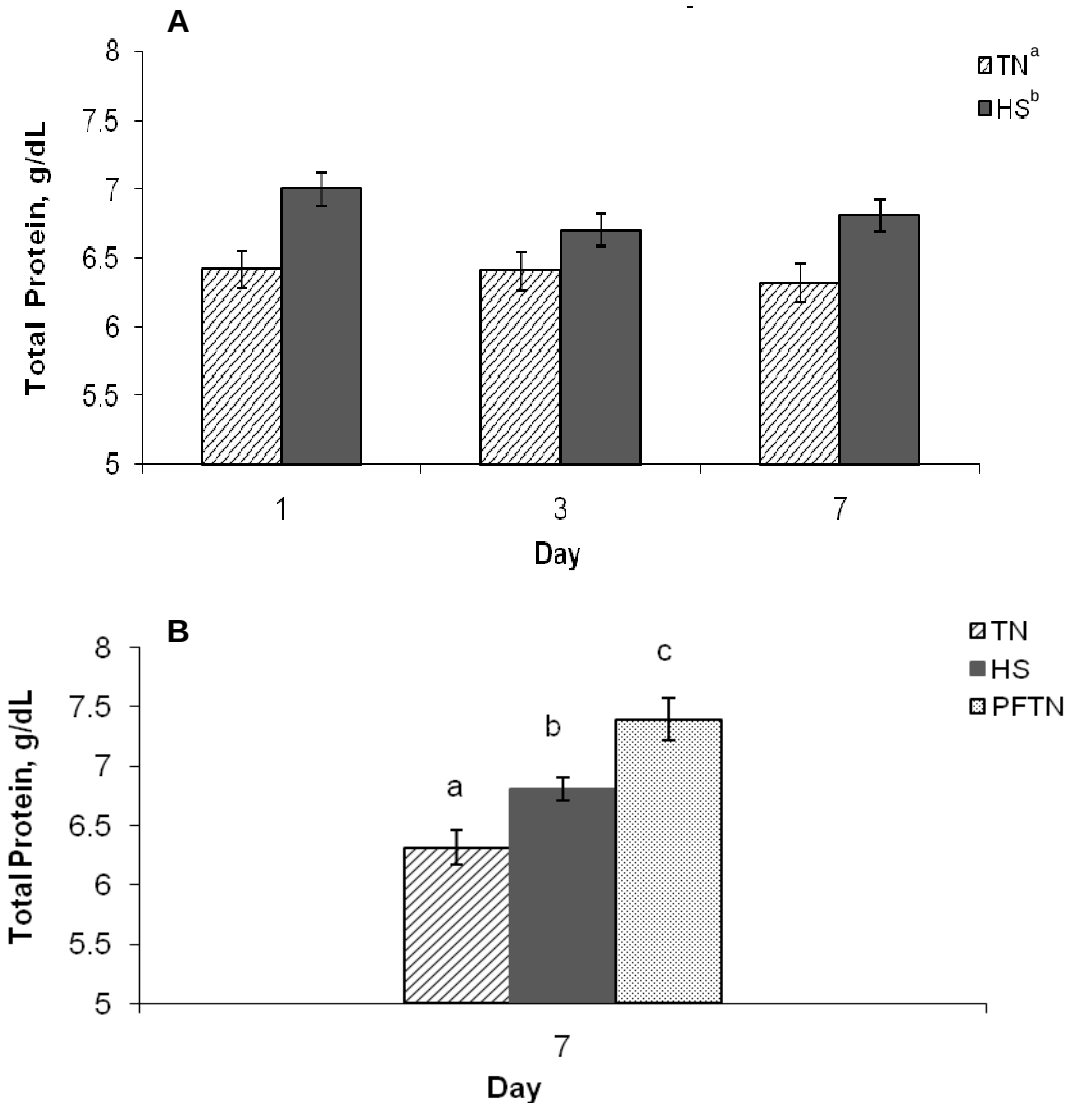

Figure A-2.2: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) and heat stress (HS; 35℃) conditions on the (A) temporal change in plasma total protein and (B) effects of TN, HS, and pair-feeding in thermal neutral conditions (PFTN) on change in plasma total protein at the end of the experiment. [a,b,c]$P<0.05$

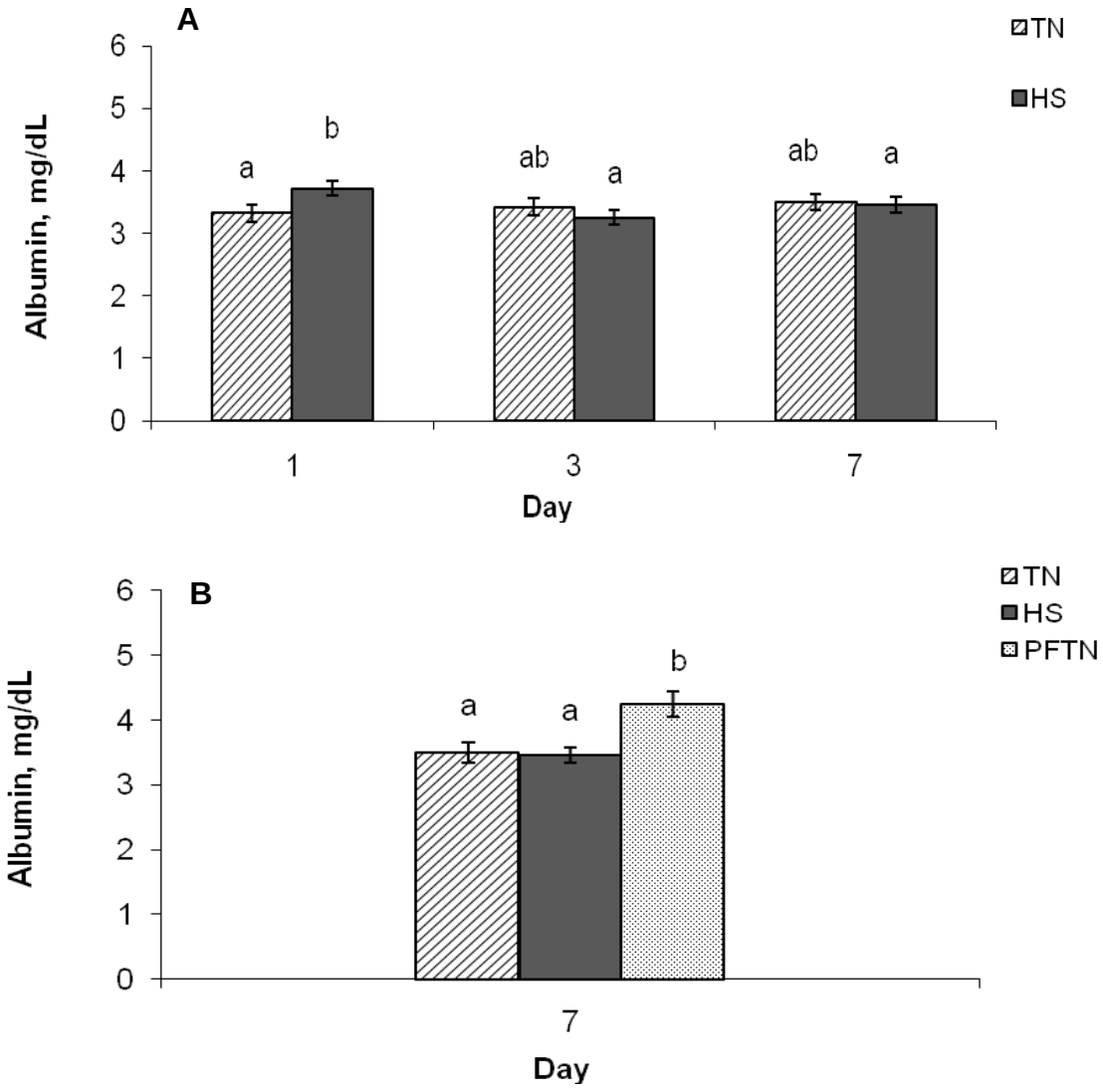

Figure A-2.3: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) and heat stress (HS; 35℃) conditions on the (A) temporal change in plasma albumin and (B) effects of TN, HS, and pair-feeding in thermal neutral conditions (PFTN) on change in plasma albumin at the end of the experiment. $^{a,b,c}P<0.05$

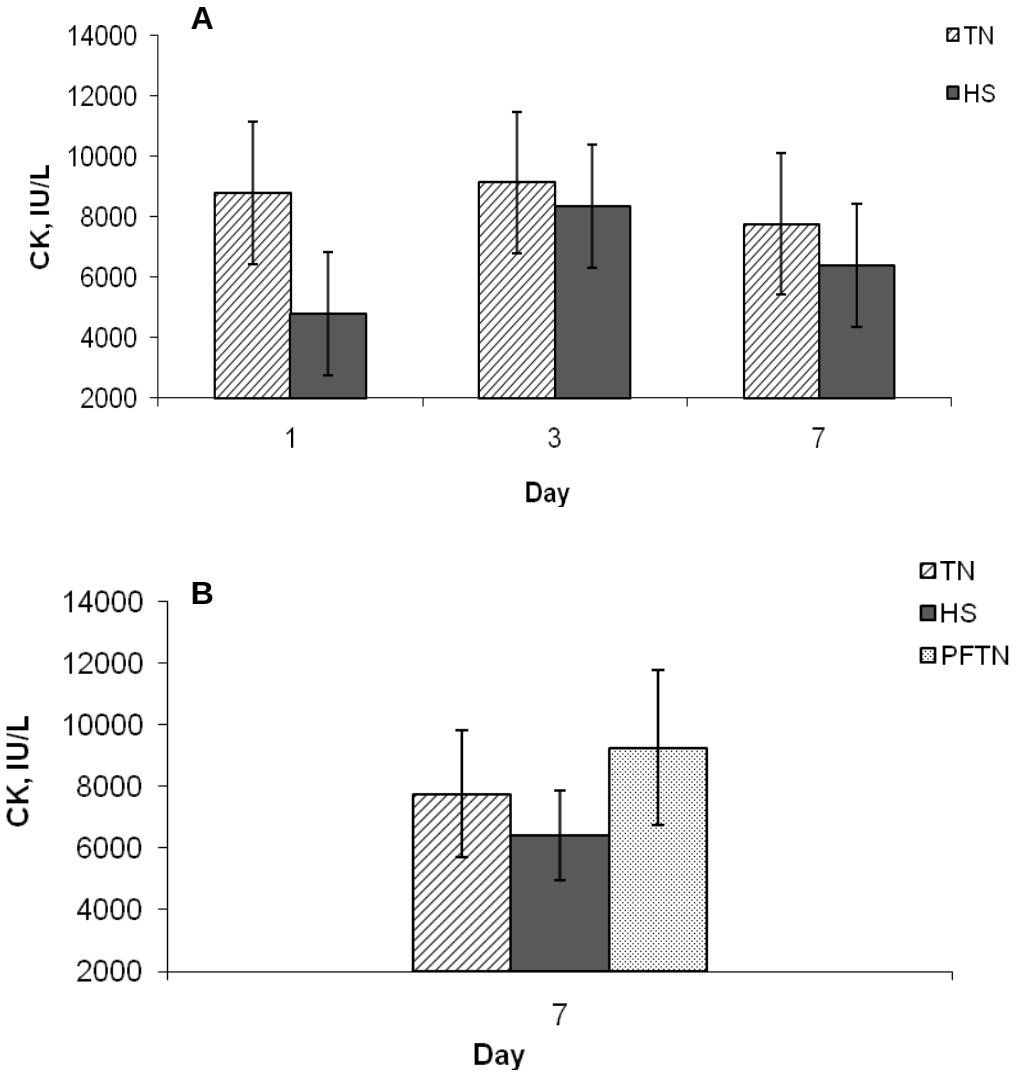

Figure A-2.4: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) and heat stress (HS; 35℃) conditions on the (A) temporal change in plasma creatine kinase (CK) and (B) effects of TN, HS, and pair-feeding in thermal neutral conditions (PFTN) on change in plasma CK at the end of the experiment. $^{a,b,c}P<0.05$

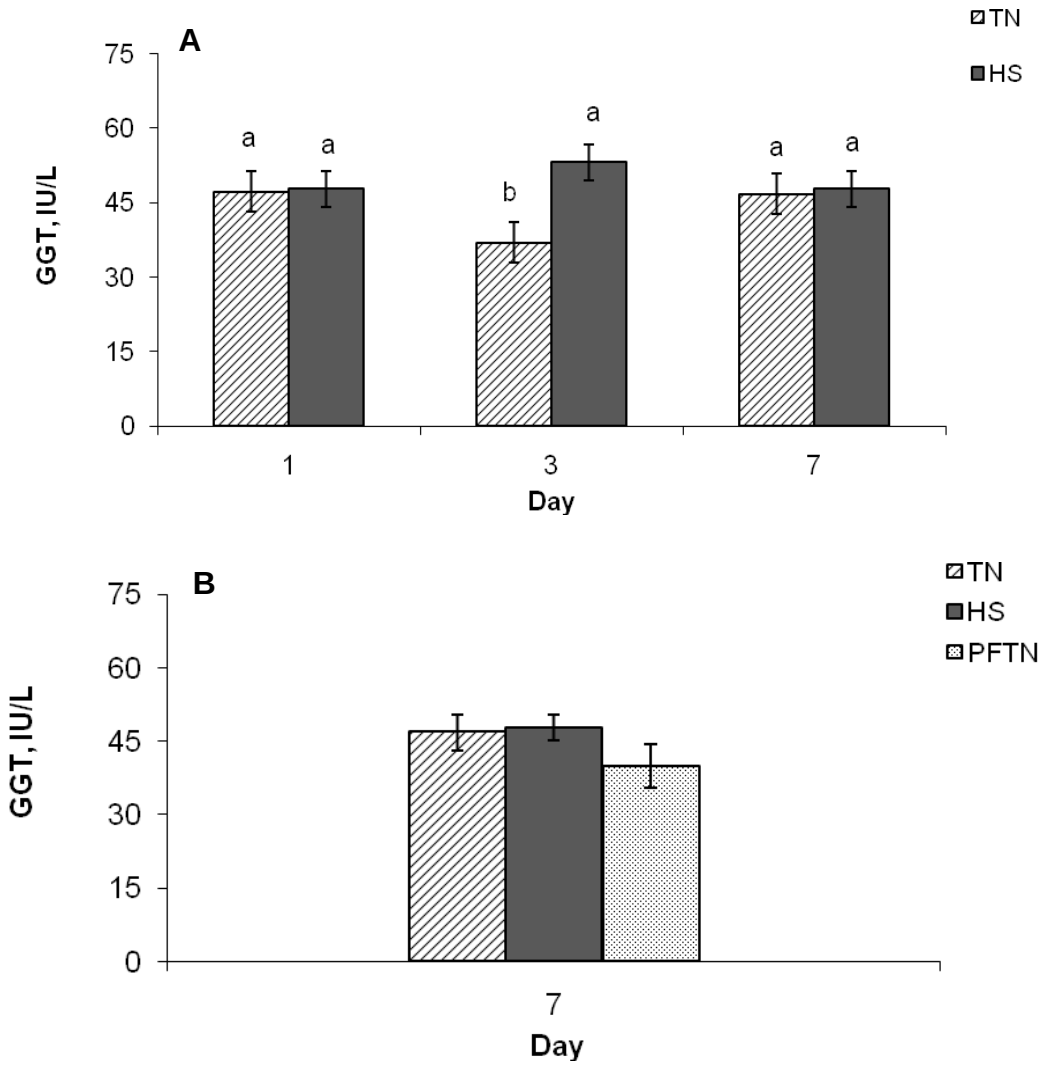

Figure A-2.5: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) and heat stress (HS; 35℃) conditions on the (A) temporal change in plasma gamma-glutamyl transpeptidase (GGT) and (B) effects of TN, HS, and pair-feeding in thermal neutral conditions (PFTN) on change in plasma GGT at the end of the experiment. $^{a,b,c}P<0.05$

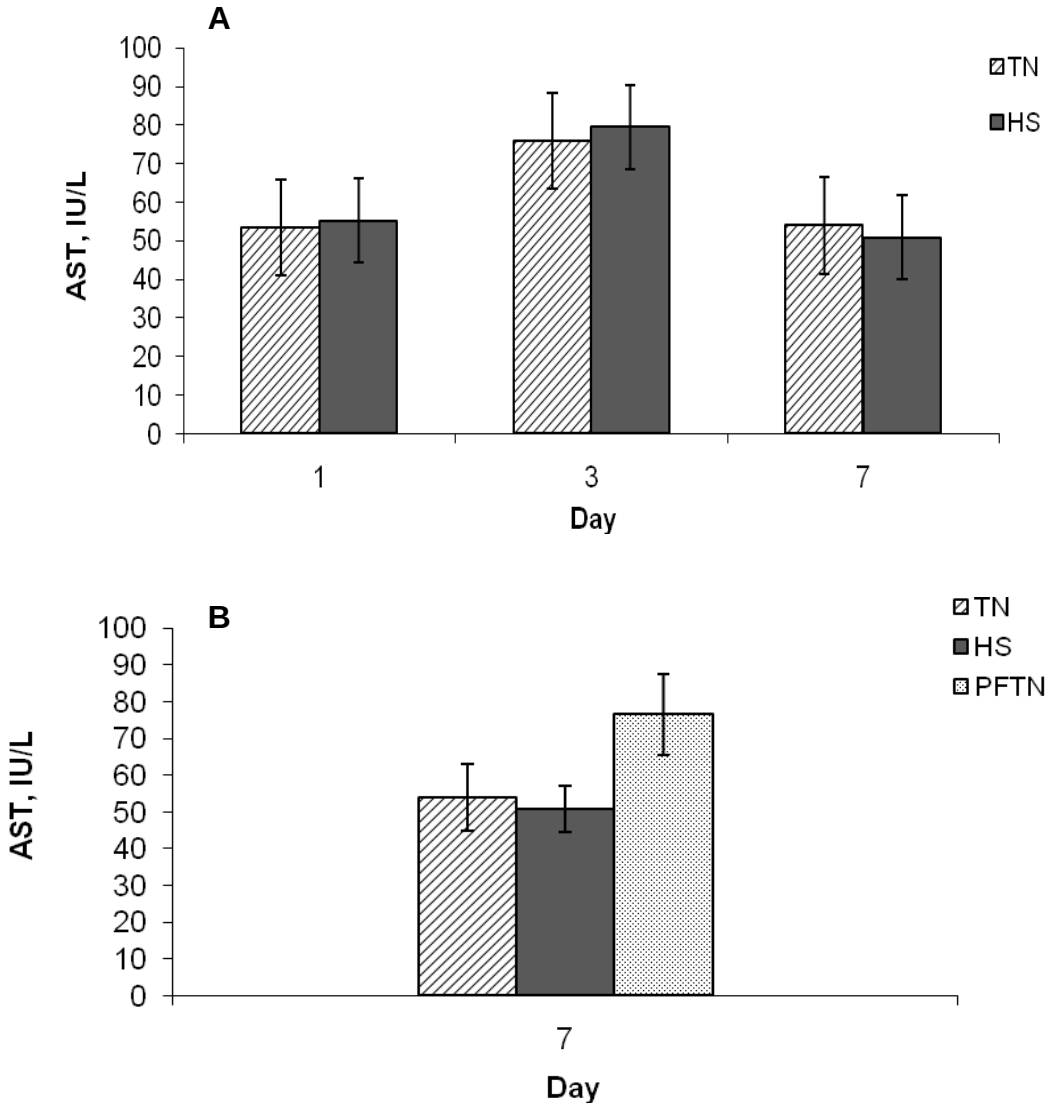

Figure A-2.6: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) and heat stress (HS; 35℃) conditions on the (A) temporal change in plasma aspartate aminotransferase (AST) and (B) effects of TN, HS, and pair-feeding in thermal neutral conditions (PFTN) on change in plasma AST at the end of the experiment. [a,b,c]$P<0.05$

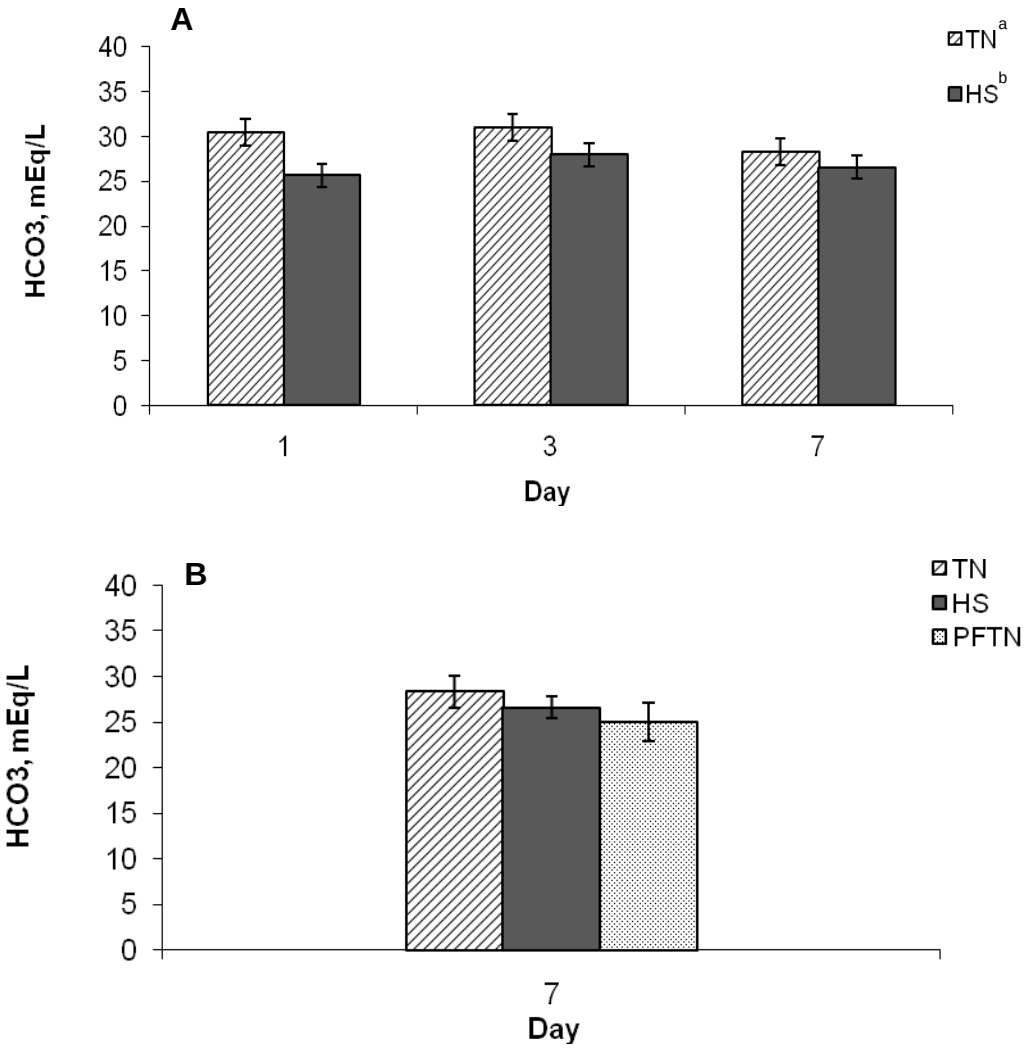

Figure A-2.7: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) and heat stress (HS; 35℃) conditions on the (A) temporal change in plasma bicarbonate (HCO_3^-) and (B) effects of TN, HS, and pair-feeding in thermal neutral conditions (PFTN) on change in plasma HCO_3^- at the end of the experiment.
[a,b,c]$P<0.05$

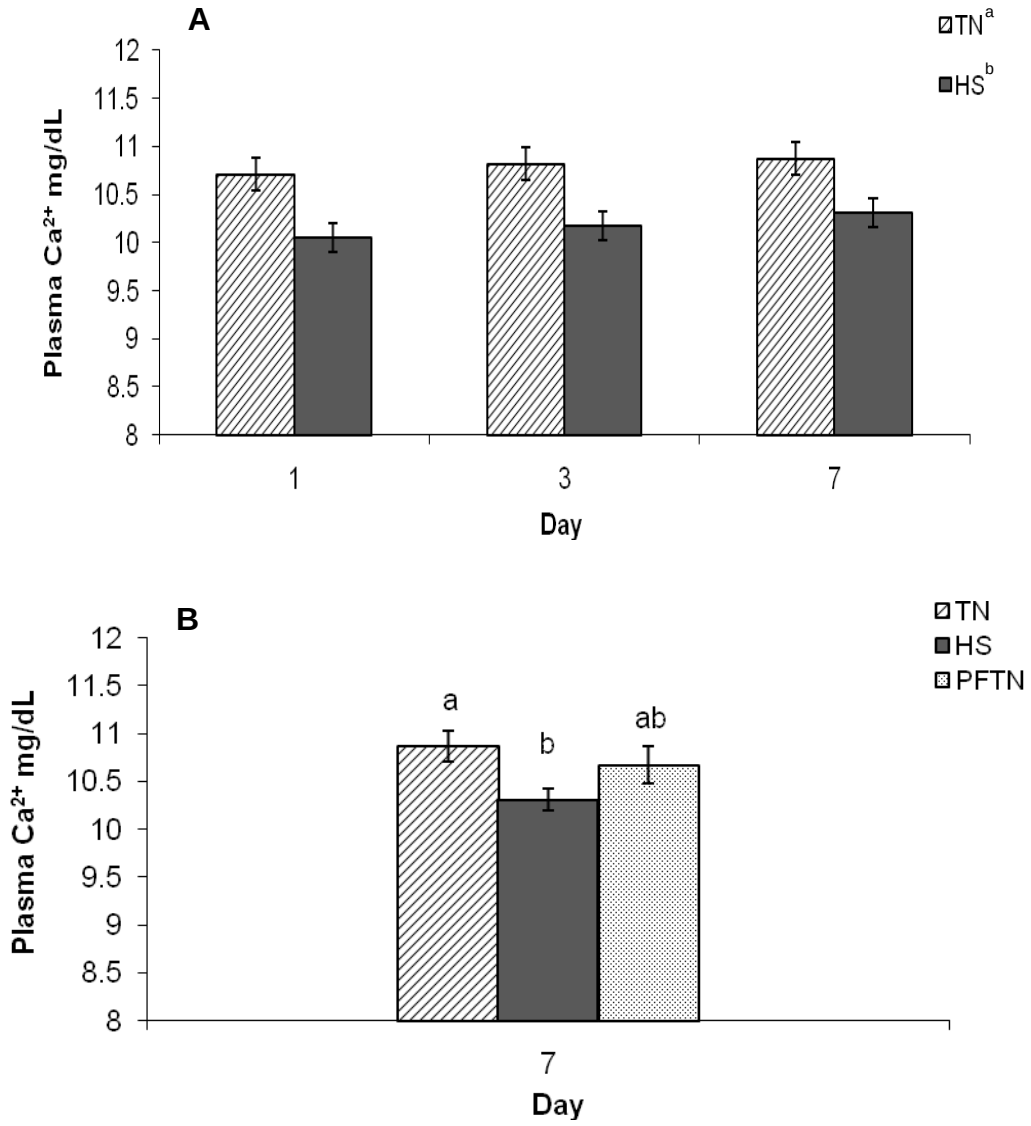

Figure A-2.8: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) and heat stress (HS; 35℃) conditions on the (A) temporal change in plasma calcium and (B) effects of TN, HS, and pair-feeding in thermal neutral conditions (PFTN) on change in plasma calcium at the end of the experiment. [a,b,c]$P<0.05$

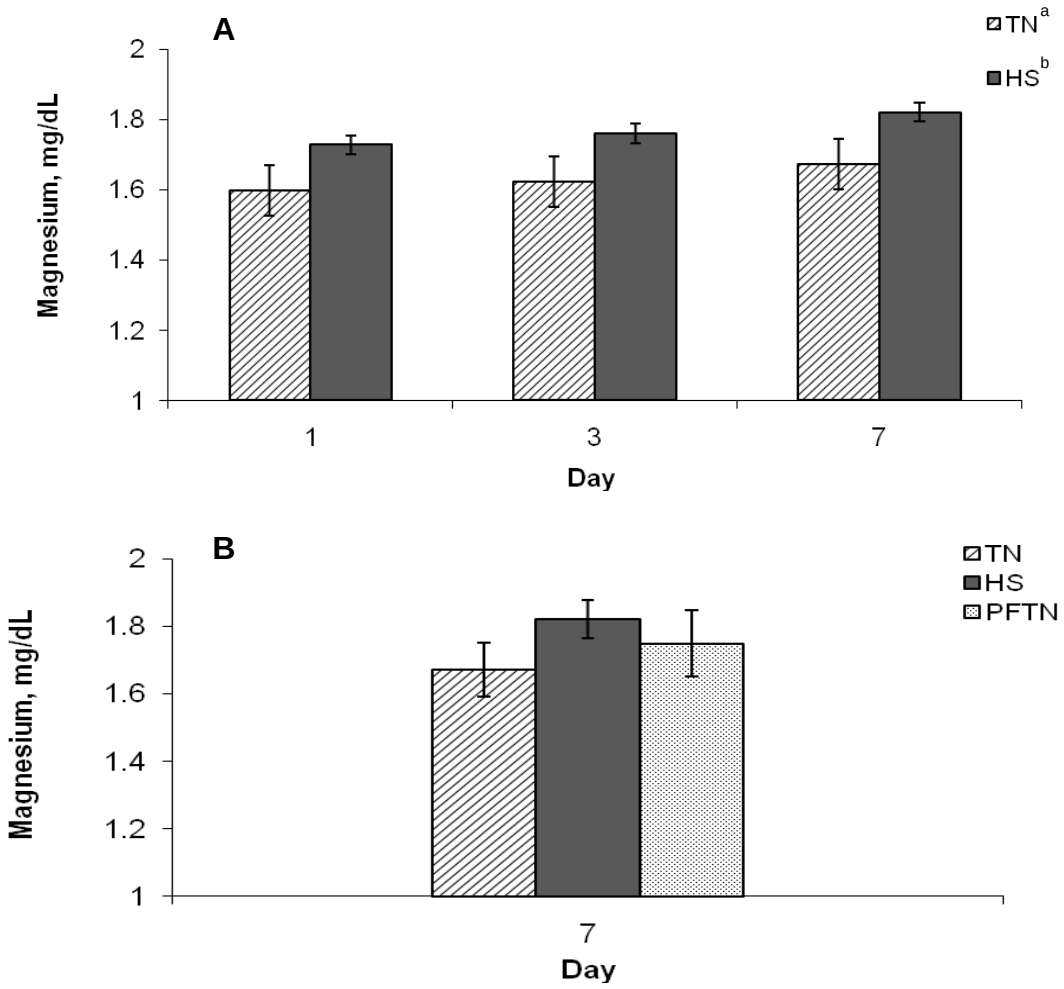

Figure A-2.9: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) and heat stress (HS; 35℃) conditions on the (A) temporal change in plasma magnesium and (B) effects of TN, HS, and pair-feeding in thermal neutral conditions (PFTN) on change in plasma magnesium at the end of the experiment. [a,b,c]$P<0.05$

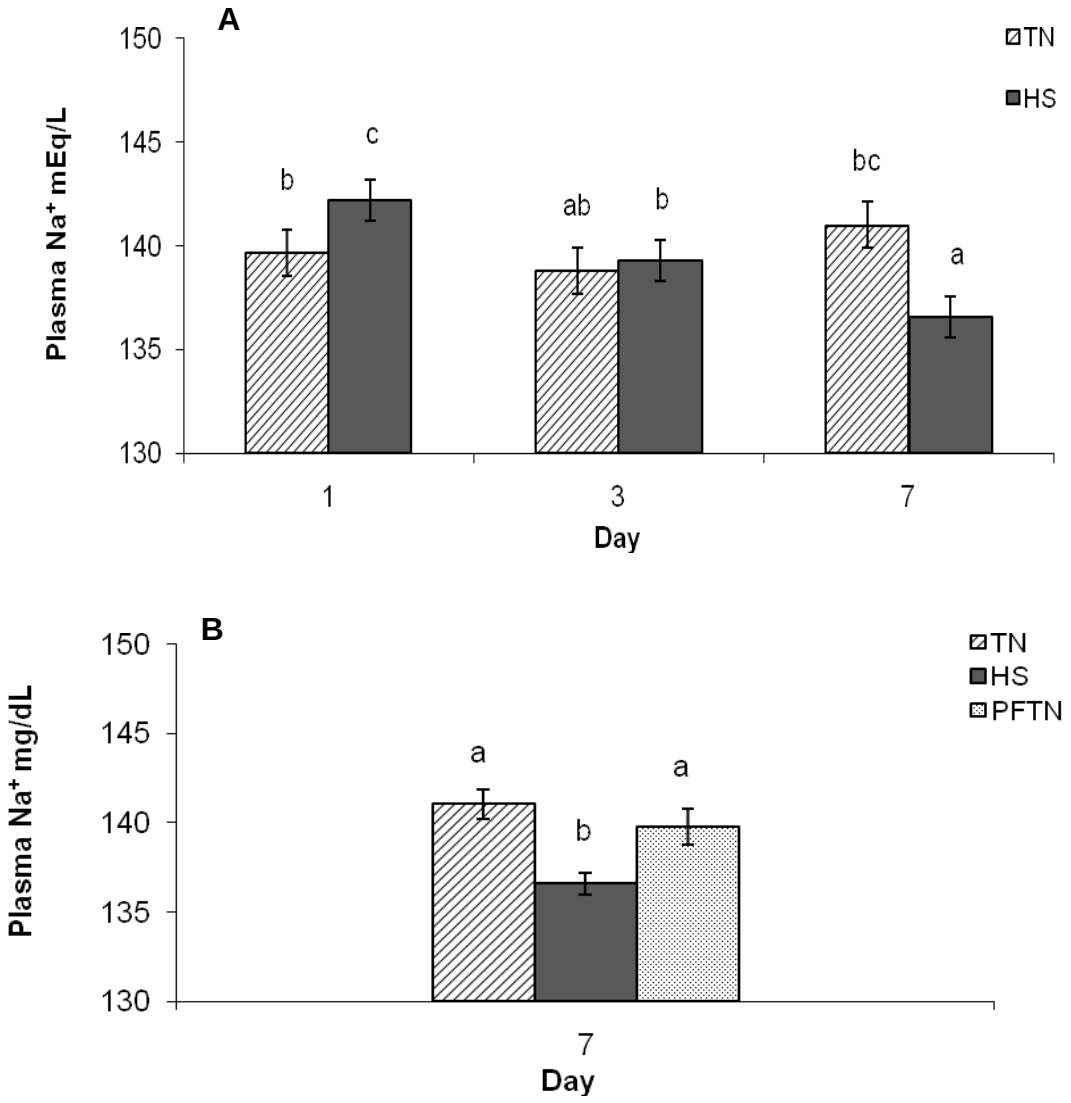

Figure A-3.0: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) and heat stress (HS; 35℃) conditions on the (A) temporal change in plasma sodium and (B) effects of TN, HS, and pair-feeding in thermal neutral conditions (PFTN) on change in plasma sodium at the end of the experiment. $^{a,b,c}P<0.05$

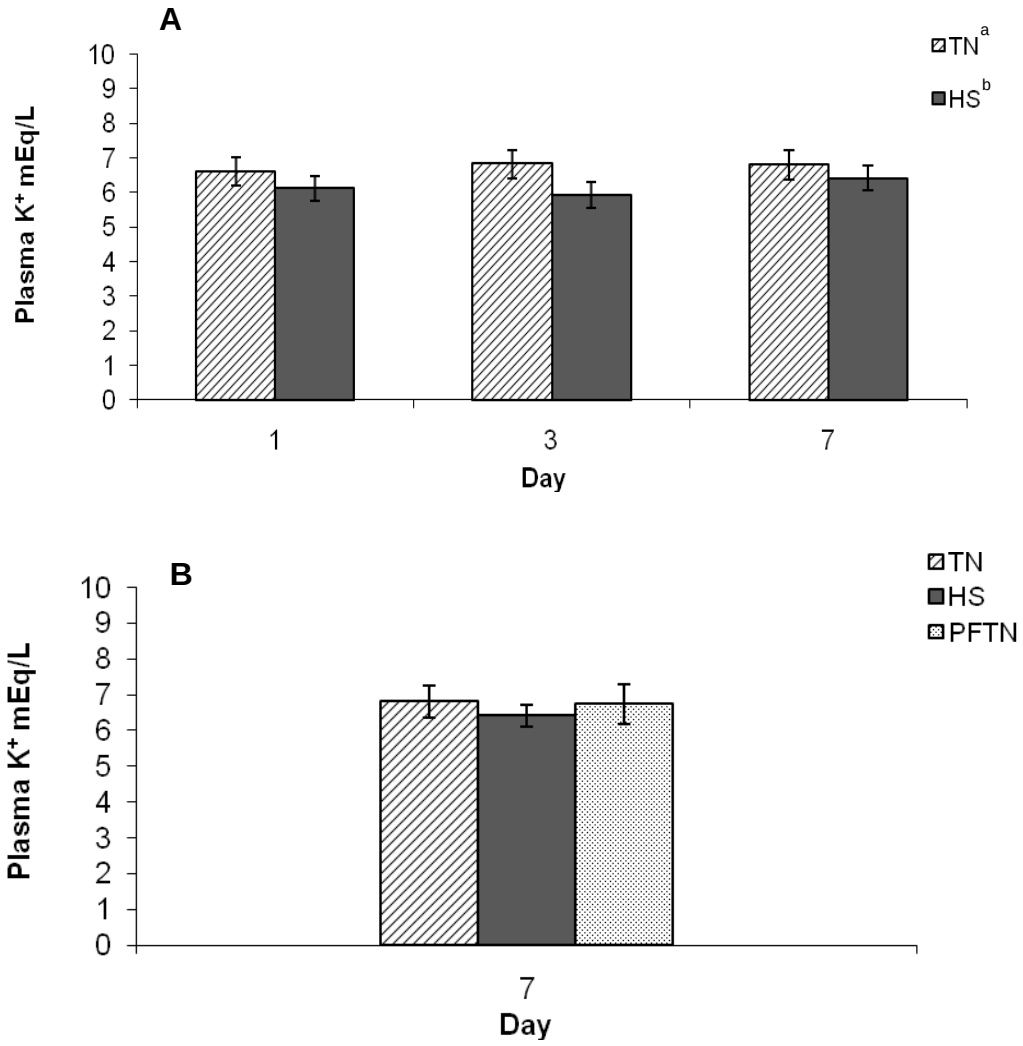

Figure A-3.1: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) and heat stress (HS; 35℃) conditions on the (A) temporal change in plasma potassium and (B) effects of TN, HS, and pair-feeding in thermal neutral conditions (PFTN) on change in plasma potassium at the end of the experiment. $^{a,b,c}P<0.05$

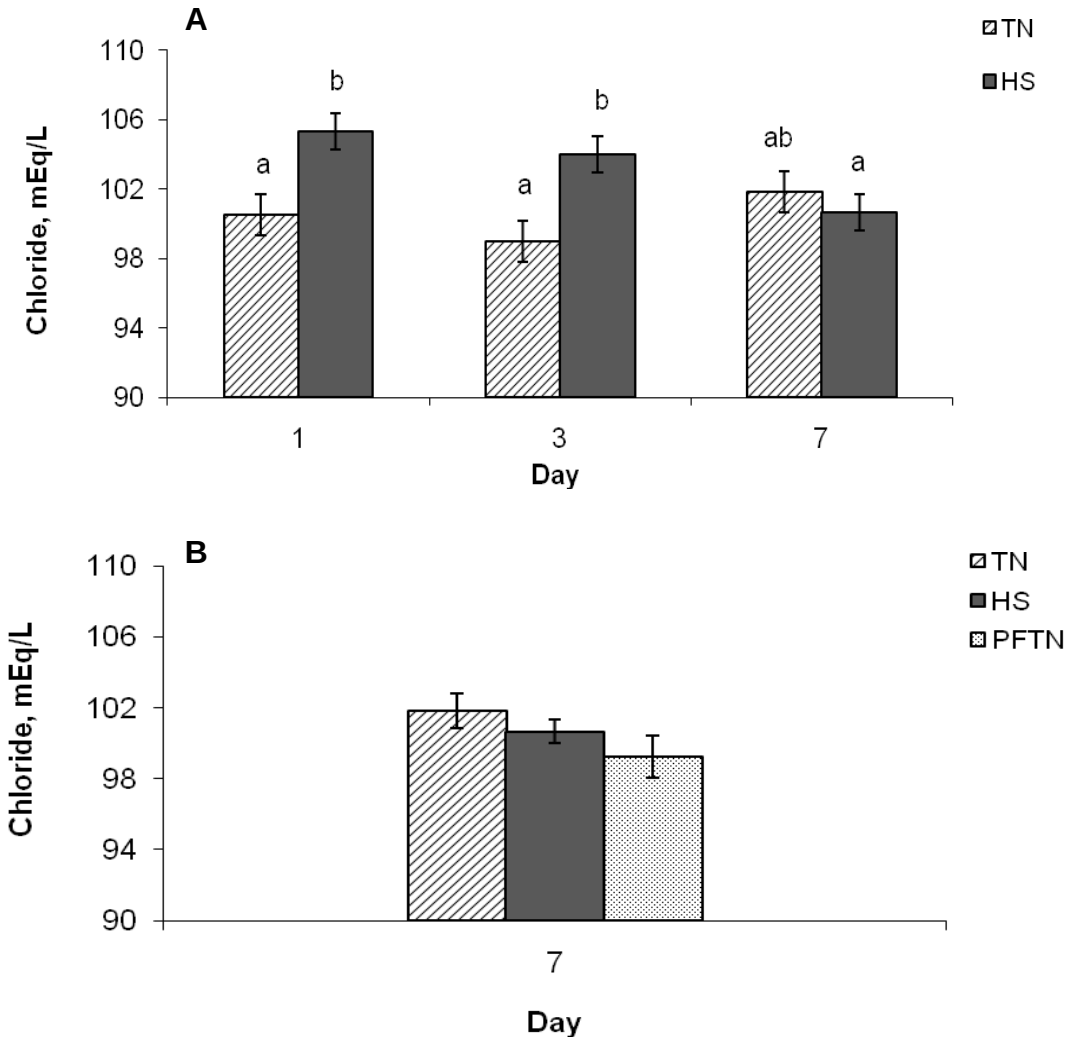

Figure A-3.2: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) and heat stress (HS; 35℃) conditions on the (A) temporal change in plasma chloride and (B) effects of TN, HS, and pair-feeding in thermal neutral conditions (PFTN) on change in plasma chloride at the end of the experiment. $^{a,b,c}P<0.05$

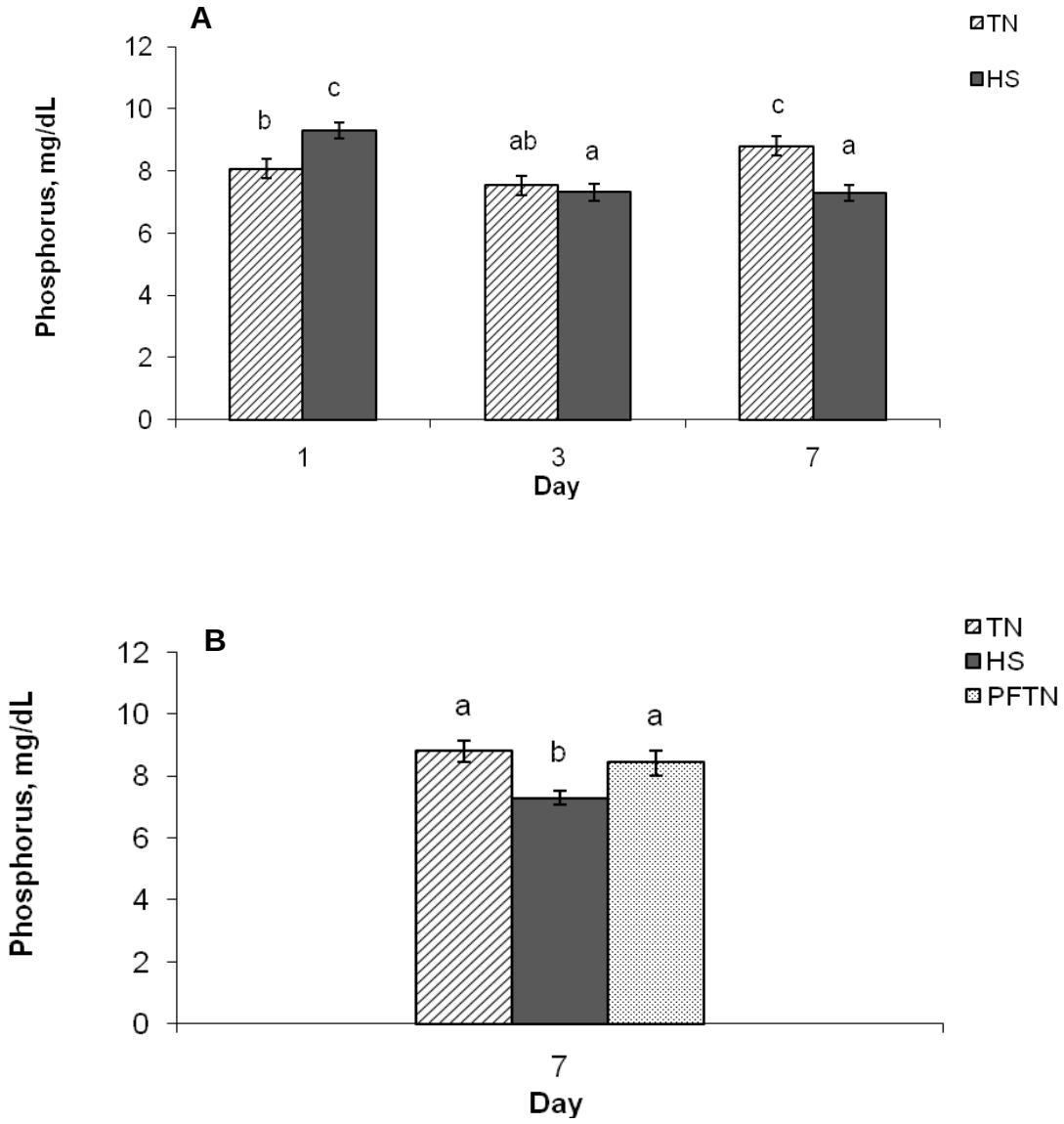

Figure A-3.3: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) and heat stress (HS; 35℃) conditions on the (A) temporal change in plasma phosphorus and (B) effects of TN, HS, and pair-feeding in thermal neutral conditions (PFTN) on change in plasma phosphorus at the end of the experiment. $^{a,b,c}P<0.05$

Table A-1.7 Effects of heat stress (HS) and plane of nutrition on intestinal permeability measures in growing pigs

	Treatment				
Parameter	TN[1]	HS	PFTN[2]	SEM	*P*
Plasma LPS[1]	27.2	36.6	34.1	4.1	0.25
TER[2]	897	648	741	170	0.59
APP[3]	1.1[a]	2.4[b]	1.7[ab]	0.4	0.05

[1]Thermal Neutral
[2]Pair-fed Thermal Neutral
[1]LPS – Lipopolysaccharide, EU (endotoxin units)
[2]TER – Transepithelial electrical resistance, Ω x cm^2
[3]APP – Apparent permeability coefficient for FITC-LPS, µg/mL/min/cm^2
[a,b,c]*P* <0.05

Table A-1.8 Effects of heat stress (HS) and plane of nutrition on intestinal nutrient transport in growing pigs

	Treatment				
Parameter	TN[1]	HS	PFTN[2]	SEM	*P*
Glucose, µA/cm^2	1.67	1.33	2.10	0.31	0.23
Glutamine, µA/cm^2	0.38	0.54	0.37	0.14	0.53
Lysine, µA/cm^2	0.87	1.36	1.54	0.50	0.66
Na^+/K^+ ATPase[4]	142.7[a]	119.7[ab]	77.4[b]	19.4	0.11

[1]Thermal Neutral
[2]Pair-fed Thermal Neutral
[3]Jejunum Na^+/K^+ ATPase, µmol Pi/mg protein/h
[a,b,c]*P* <0.05

Table A-1.9 Effects of heat stress (HS) and plane of nutrition on intestinal morphology in growing pigs

	Treatment				
Parameter	TN[1]	HS	PFTN[2]	SEM	*P*
Villous height, µm	503[a]	385[b]	409[b]	10	<0.01
Villous width, µm	151[a]	178[b]	143[a]	4	<0.01
Crypt depth, µm	292[a]	276[b]	239[c]	3	<0.01

[1]Thermal Neutral
[2]Pair-fed Thermal Neutral
[a,b,c]*P* <0.05

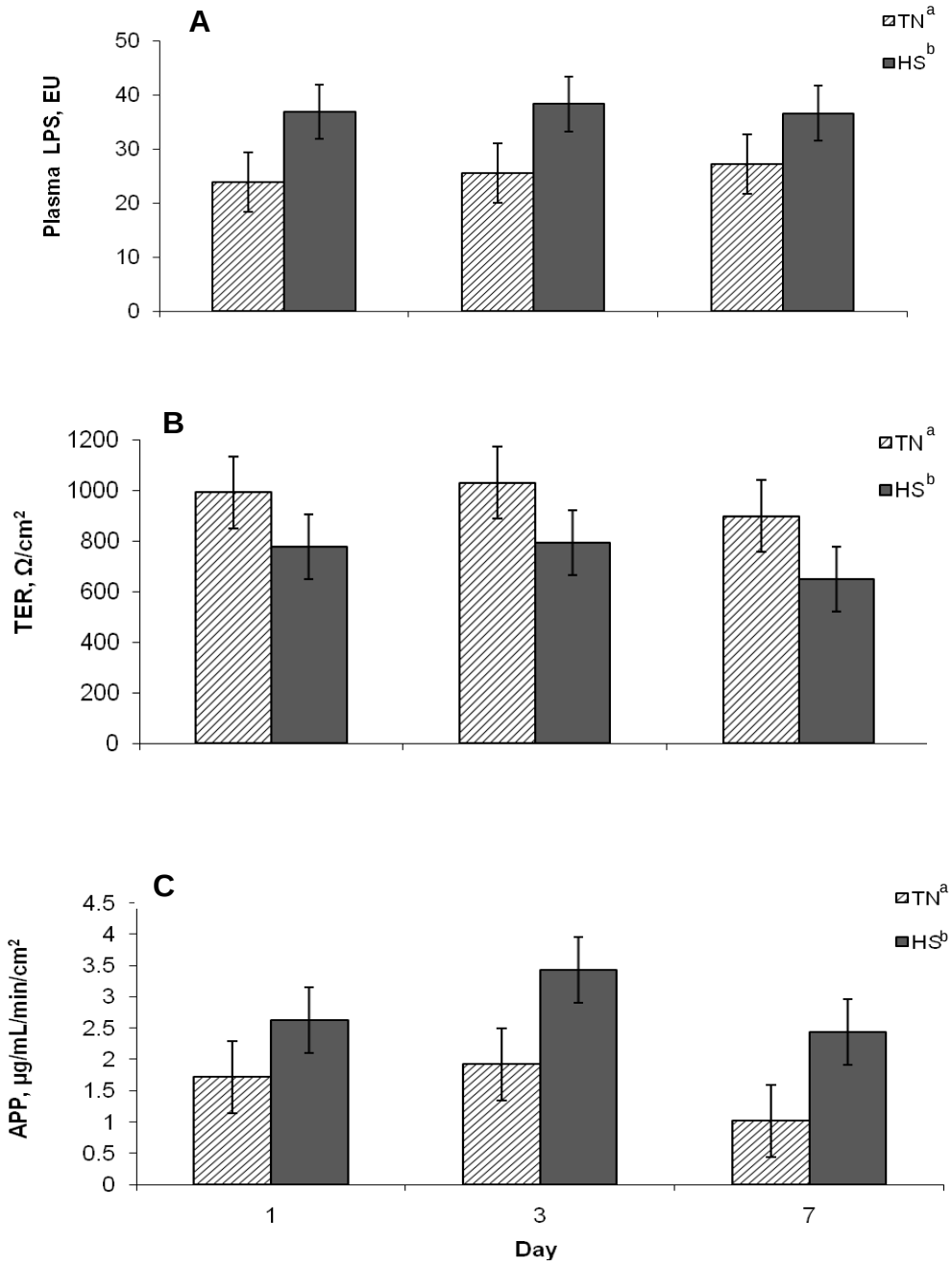

Figure A-3.4: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) or heat stress (HS; 35℃) on (A) Plasma LPS (B) Transepithelial electrical resistance (TER), and (C) Apparent permeability coefficient (APP) throughout the experiment in growing pigs. $^{a,b,c}P<0.05$

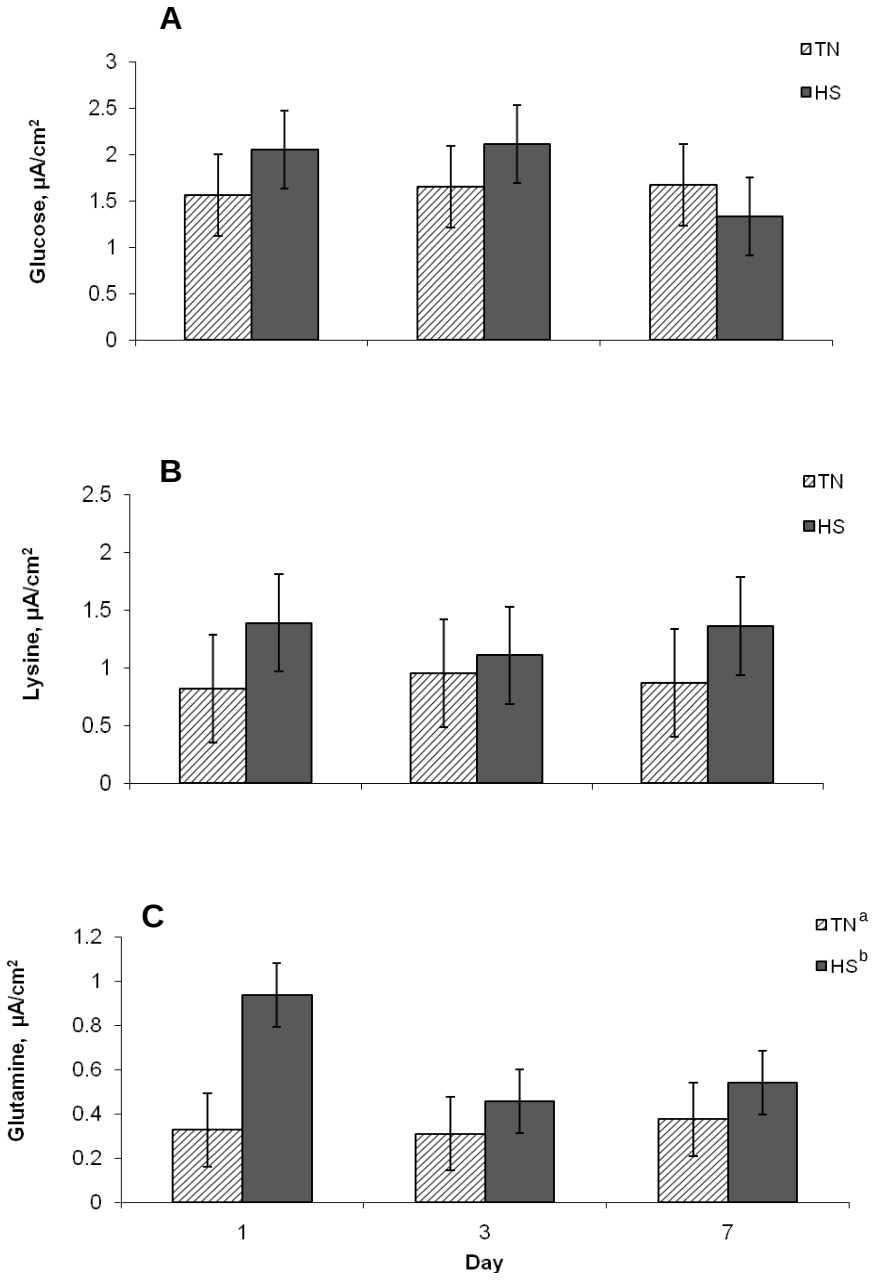

Figure A-3.5: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) or heat stress (HS; 35℃) on (A) Glucose transpor t (B) Lysine transport, and (C) Glutamine transport throughout the experiment in growing pigs. [a,b,c]*P*<0.05

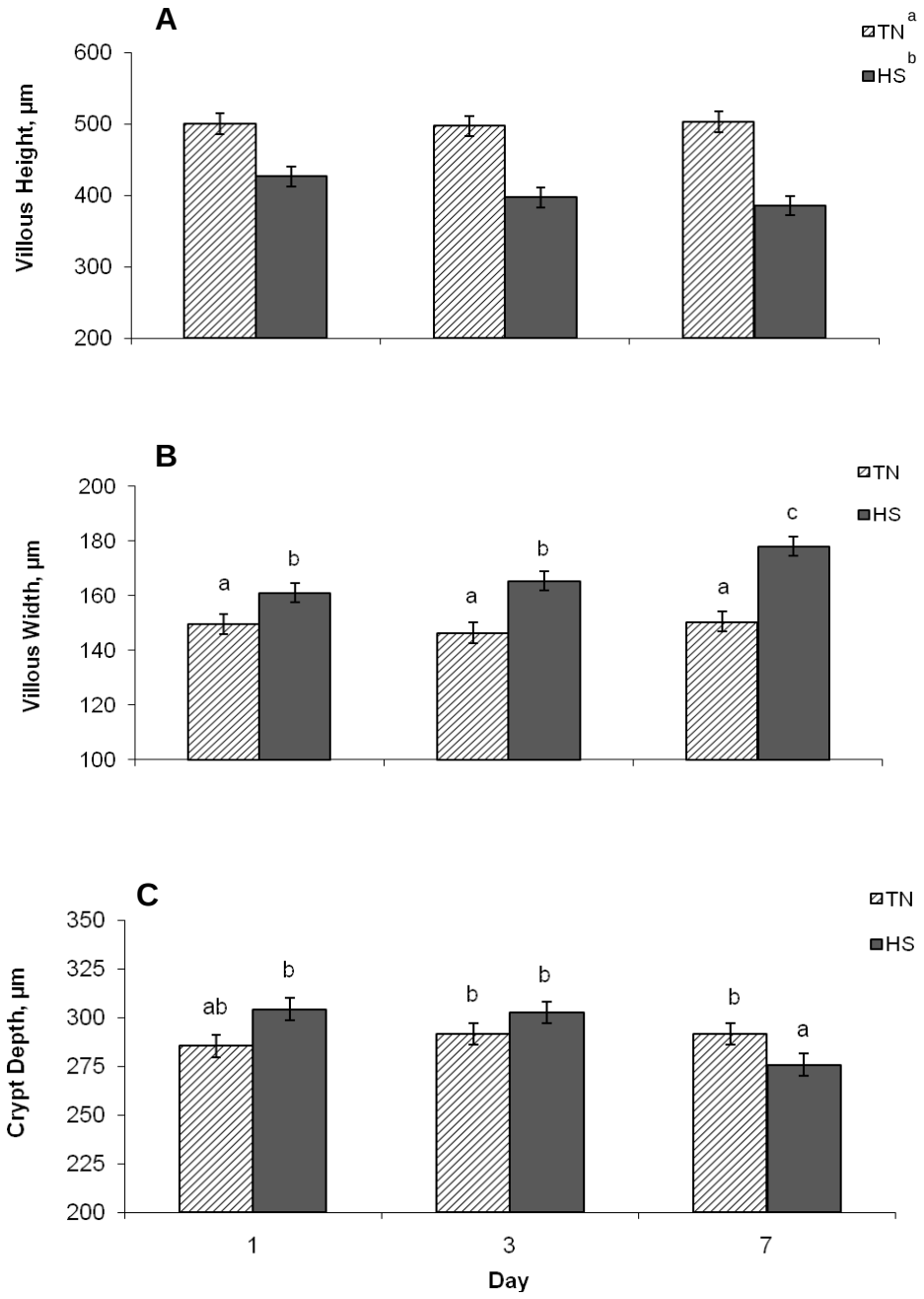

Figure A-3.6: Effects of ad-libitum feed intake in thermal neutral conditions (TN; 20℃) or heat stress (HS; 35℃) on (A) Villus height (B) Villus width, and (C) Crypt depth throughout the experiment in growing pigs. [a,b,c]$P<0.05$