Transitioning to heat stress changes lipid metabolism in Japanese quail (*Coturnix corturnix japonica*)

By

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Table of Contents

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Abbreviations

2-AG	2-arachidonoylglycerol
17α-OHP	17α-hydroxyprogesterone
20α-OHP	20α-hydroxyprogesterone
ACSL5	fatty acid-CoA ligase 5
ADG	average daily gain
ADP	adenosine diphosphate
AFI	average feed intake
ALA	α-linolenic acid
Allo	allo-pregnanolone
ANOVA	analysis of variance
Apo A-IV	apolipoprotein A-IV
ARA	arachidonic acid
ARE	antioxidant/electrophile response element
ASCBG2	fatty acid-CoA ligase bubblegum 2
ATP	adenosine triphosphate
BCA	bicinchoninic acid
BE	base excess
BW	body weight
CAT	catalase
ChREBP	carbohydrate response element binding protein
CO_2	carbon dioxide
DGLA	dihomo-γ-linolenic acid
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
DHEA	dehydroepiandrosterone
DTT	dithiothreitol
DDA	data-dependent acquisition
DIA	data-independent acquisition
DHEA-S	dehydroepiandrosterone sulfate
DHP	dihydroprogesterone
EPA	eicosapentaenoic acid
EDA	11, 14-eicosadienoic acid
FCR	feed conversion ratio
FC	fold change
FABP	fatty acid binding protein
GHG	greenhouse gases
GSH	glutathione
GPx	glutathione peroxidase
GABA	γ-amino butyric acid
HS	heat stress
HSS	heat stress siblings
HSF	heat shock factors
HSP	heat shock proteins
HCO_3	bicarbonate
HPA	hypothalamus-pituitary-adrenal

iCa	ionized calcium
JQ	Japanese quail
LA	linoleic acid
LR-MCF	Protein LoBind Eppendorf tube
LCMS	liquid chromatography mass spectrometry
MUFA	monounsaturated fatty acids
MDA	malondialdehyde
MAGL	monoglyceride lipase
MID1IP1	mid1-interacting protein 1
NEFA	non-esterified fatty acids
Nrf2/Keap1	NF-E2-related factor 2/Kelch-like ECH associated protein 1
NF-κB/IκB	nuclear factor κ-light-chain enhancer of activated B cells/inhibitory κB protein
PUFA	polyunsaturated fatty acids
PA	palmitic acid
PCV	pack cell volume
PO_2	oxygen partial pressure
PCO_2	carbon dioxide partial pressure
PPAR-γ	Peroxisome proliferator-activated receptor gamma
ROS	reactive oxygen species
RH	relative humidity
SOD	superoxide dismutase
SFA	saturated fatty acid
SREBP-1	sterol regulatory element-binding protein 1
sO_2	oxygen saturation
SE	standard error
SRS	stress response system
TAG	triacylglyceride
TBARS	thiobarbituric acid reactive substances
TCA	tricarboxylic acid
TCO_2	total carbon dioxide
TN	thermoneutral
TNS	thermoneutral siblings
TNZ	thermoneutral zone
TMP	1,1,3,3-tetramethoxypropane
TPA	total protein abundance

Overall Abstract

The average surface temperature is predicted to rise 0.5ºC to 6ºC by the year 2100 and will affect domesticated animals. When Japanese quail (*Coturnix coturnix japonica*) are subjected to heat stress (temperatures ≥ 30˚C), a variety of changes may occur including, but not limited to, their ability to regulate blood gases, blood electrolytes, tissue oxidation, and lipids, a class of nutrients that are integral to their health. Most importantly are fatty acids which are a major component of lipids and have been shown to be significantly affected by heat stress in poultry. The extent that heat stress can alter these important aspects of health depends on the length of exposure, severity of heat, and demands of the animal. In addition, poultry chosen for high performance, such as low FCR, may have negative metabolic consequences in early heat stress. By investigating the transition from a thermoneutral zone to heat stress, procedures can be implemented to mitigate heat stress at an earlier stage. To test the hypothesis that multigenerational high performance (FCR) can mitigate the responses to heat stress, FCR of 4 treatment groups were repeated for 10 generations: (1) thermoneutral (TN, 22.2˚C), (2) thermoneutral siblings (TNS, 22.2˚C), (3) heat stress (HS, 31.1˚C), and (4) heat stress siblings (HSS, 31.1˚C). TN and HS were random bred in their respective temperatures. TNS and HSS were siblings reared at the two temperatures. HSS quail were used to determine low FCR at 31.1˚C and their corresponding TNS were mated to create the next generation's TNS and HSS. Body weights (BW), blood gases, blood electrolytes, and sera steroid hormones were measured during the first 4 hours (acute) and after 3 weeks (chronic) of temperature exposure in generation 10. Fatty acid compositions, lipid oxidation, and antioxidant activity of feed, yolk, and adult and embryo brain, liver and kidneys, thigh for adults were investigated. Overall protein abundance in livers of actively laying female Japanese quail was quantified. It was hypothesized that TNS would have the lowest amount of lipid oxidation, HSS would have the highest SOD and CAT activity, HS and HSS would have less PUFA in all organs

due to degradation, HSS would upregulate proteins involved in β-oxidation and the TCA cycle to meet increased energy demands, and HSS would have the lowest levels of glucocorticoids. Significant differences were determined at $P \leq 0.05$. Results of Experiment 1 (Chapter 2) revealed that acute and chronic heat stress at 31.1˚C does not have a clear effect on blood electrolytes, acid-base regulation, and oxygen transport. For Experiment 2 (Chapter 3), of all adult organs analyzed, livers experienced the most variations in concentrations of fatty acids when compared by treatment and sex. Males had significantly more stearic acid in the brain, kidney, and thigh and significantly more PA in all tissues than females. TN brains had significantly less PUFA than that in both TNS and HSS. TN or TNS had significantly more long chain PUFA such as DPA n-6, DPA n-3, DHA, and ARA and significantly less SFA such as PA or stearic acid than that in HS or HSS across all tissues. As revealed in Experiment 3 (Chapter 4), HSS yolk had higher levels of LA and ARA than TN and embryo brain DHA, total PUFA, and stearic acid increased over the duration of incubation as expected for proper brain development. TNS embryo brain had more LA, ALA, DPA n-3, and total n-3 than TN and significantly more stearic acid and SFA than HSS. HS embryo kidneys and gonads had more total SFA than TNS and HSS. HS embryo livers had more stearic acid, SFA:PUFA, and total SFA than TN and TNS. TN and TNS embryo livers had more PUFA than HSS embryo livers. Results of Experiment 4 (Chapter 5) indicated that heat stress did not influence lipid oxidation in any of the tissues; however, brain had the most oxidation, followed by liver> kidney>thigh. There was no significant treatment effect on SOD and CAT activities, but kidneys had significantly more CAT activity than brain, liver, and thigh. Brain and thigh had similar CAT activity. Revealed in Experiment 5 (Chapter 6), HSS had 118 significantly down-regulated proteins and 56 significantly upregulated proteins and HS had 75 significantly down-regulated proteins and 2 significantly upregulated proteins when compared to TN. Differences in protein

abundance among all other comparisons were minimal (≤5) or insignificant. TN and/or TNS had significantly less antioxidants (SOD Cu/Zn and CAT) and lipoprotein transport from liver to egg than those in a heat stress temperature. TN had significantly more proteins involved in adipogenesis, lipogenesis, and fatty acid oxidation than HSS. The lower abundance of β-oxidation enzymes for HS and HSS could indicate that heat stressed quail decreased energy production to prevent further oxidative damage. For Experiment 6 (Chapter 7), chronic males, particularly from TN, had significantly higher levels of glucocorticoids, progestogens, androgens than many of the other treatments and of the 29 sera steroid hormones analyzed, 13 were significantly higher in chronic than acute. Thus, selection for low FCR in heat stress at 31.1˚C did not incur an overall fitness advantage when considering these parameters. However, selection for low FCR in heat stress could possibly reduce oxidation of PUFA or increase retention of PUFA in the brain. Heat stressed quail, regardless of selective breeding, had significantly less health-promoting fatty acids such as DHA and DPA. More research should be conducted across many parameters at temperatures below 31.1˚C to pinpoint how soon quail health is affected and possible procedures that mitigate these effects.

CHAPTER 1
Introduction

1. Climate change

Global average surface temperature is expected to rise 0.5ºC to 6ºC by the year 2100. There are also predictions of increased frequency, intensity, and duration of heat waves, coupled with increasing severe drought events (Talbot, et al., 2018). With these events becoming a more pressing matter in the next few years, it is important to address the physiological changes that are occurring in production animals, particularly poultry. Poultry products are a major provision for food and livelihood in many parts of the world, particularly India as it is the 3rd largest egg producer and 5th largest poultry producer in the world (Pawar, et al., 2016). Important poultry producers such as those in India are expected to experience climate change, leading to a decrease in food availability for both poultry and humans (Verma, 2021). Additionally, the 2020 combined value of poultry production in the United States of America was USD$35.5 billion which could be at risk with climate change (USDA National Agricultural Statistics Service, 2021). In 2006, the USA had a major heat wave that resulted in 700,000 poultry deaths in California (Renaudeau, et al., 2012). Issues like these are predicted to arise as heat wave events and average global surface temperatures increase.

A contributing factor to climate change is the increase in greenhouse gases (GHG), such as carbon dioxide, methane, nitric oxide, and others, in the troposphere. With the increase in GHG, there is a decrease in efficiency of terrestrial radiation to space. The terrestrial radiation is then absorbed by the atmosphere and reemitted at higher altitudes and lower temperatures causing the warming of the lower atmosphere and surfaces (Sejian, et al., 2015). Some GHG linger in the atmosphere longer and short-term changes in GHG concentrations have had and will continue to have long-term effects. GHG have also increased by 75% since 1970 (Sejian, et al., 2015).

Additionally, the global human population is predicted to rise from 7.9 billion to 9.6 billion by the year 2050 (Rojas-Downing, et al., 2017). This will lead to an increase of more than 70% of current agricultural products by 2050, even though 1 in 8 people went hungry between the years 2011 and 2013 (Rojas-Downing, et al., 2017). When global temperatures increase 1.5 to 2.5˚C, 20-30% of plant and animal species will be at risk of extinction (Sejian, et al., 2015). The increase of global surface temperature is inevitable with the amount of GHG that is being produced. The only way the earth can cool itself is by emission of infrared radiation into space, but due to GHG, infrared radiation is being trapped in the atmosphere causing a natural greenhouse effect (Sejian, et al., 2015).

2. Heat stress

For the current study, stress is defined as conditions where environmental demands exceed the natural regular capacity of an animal (Campderrich, et al., 2019). The stressor is the stimulus that induces the state of stress and in the current study, the stressor is heat (Campderrich, et al., 2019). Heat stress (HS) is of particular interest to livestock and poultry producers because of its deleterious effects on reproduction and health of the animals. When animals are subjected to temperatures outside of their thermoneutral zone (TNZ), there is an increase of oxygen consumption; therefore, others have used this metric to determine when an animal is expending more energy to maintain appropriate body temperature. The TNZ of Japanese quail (JQ; *Coturnix coturnix japonica*) decreases as they age, eventually reaching <16-21˚C by sexually maturity (Vohra, 1971). It is important to note that although animals may be outside of their TNZ, it does not necessarily mean they are in heat stress. Some factors that may affect the severity of the response to heat stress are overall conditions, duration, severity, bioavailability of phenolic compounds, breed, and age (Hosseini-Vashan and Raei-Moghadam, 2019).

Poultry species respond to heat stress by increasing panting, water consumption, and resting. They also decrease physical activity, insulation, and feed intake (Mahmoud, et al., 2015). There are also physiological changes that occur within the animal's body that affect various aspects of poultry production such as performance, fatty acid composition, lipid oxidation, and antioxidant utilization. The physiological changes that occur within an animal's body during heat stress include changes in electrolyte balance, pH, digestibility and metabolism of nutrients, and reproductive functions (Akbarian, et al., 2016).

Countries, like India and Nigeria, located in tropical areas, have open-sided housing systems to raise broilers and JQ (Kumari and Nath, 2018; Egbuniwe, et al., 2021). In Nigeria, temperatures inside the aviary ranged from 30.2˚C to 32.2˚C; thus, exceeding the TNZ of JQ (Egbuniwe, et al., 2021). Negative effects of broilers in chronic heat stress include 16.4% decrease in feed intake, 32.6% decrease in body weight gain, but a 25.6% increase in feed conversion ratio (Kumari and Nath, 2018). Layers in heat stress can have 36.4% decreased egg production, 31.6% decreased feed conversion ratio, with increased egg breakages (Kumari and Nath, 2018). Alternatively, feed intake has been reported to decrease 1.2% for every increase in 1˚C between 22 to 21˚C (Kumari and Nath, 2018).

There are varying types of heat stress: acute, chronic, and cyclic. Acute heat stress is a short and rapid rice in ambient temperature. Chronic heat stress is high ambient temperature over days to weeks and this allows for acclimation to the environment. Cyclic heat stress is a limited period of heat stress followed by a period of thermoneutral temperature (Akbarian, et al., 2016). Depending on the type of heat stress exposure, the animal may have different physiological changes.

3. Stress-related phenotypic inheritance

There is indirect and direct exposure of offspring to prenatal stress. Indirect exposure of stress is considered transgenerational because there is transmission of adverse effects from the parent (F0) generation to offspring (F1) generation through epigenetic modifications (Crews, et al., 2012). Direct exposure to stress is considered multigenerational because the stressor is applied to both F0 and F1. In both circumstances there have been observations of increased susceptibility to disease and altered metabolism (Laviola and Macrì, 2013). Due to phenotypic plasticity, the idea that phenotypes are a result of the genes interacting with the environment, it is important to investigate the potentials of communication across generations. For example, if a family is selected for their high performance in a stressful environment, does that confer a fitness advantage in other ways and does that fitness advantage get passed on to their offspring? Oxidative stress can act as a regulator during development to determine phenotypic responses to the environment through redox cell signaling in regulatory processes and epigenetic effects (Laviola and Macrì, 2013).

In chicken and quail, epigenetic effects can occur from the first stage of incubation (Paiva et al., 2018). Epigenetic programming may occur after the embryonic blastocyst stage which may be heavily influenced by storage and incubation conditions (Paiva et al., 2018). Storage and incubation conditions can affect egg quality and subsequently, metabolism (Laviola and Macrì 2013, Paiva et al., 2018). Others showed that conditioning to heat stress at an early age can be protective and confer a fitness advantage in adulthood (Günal, 2012; Kang and Shim, 2021).

The theory for developmental origins of health and disease postulates that exposure to adverse environments during the fetal period and early life may program vulnerability to disease later in life (Ambeskovic, et al., 2019). Alternatively, the early life programming or fetal programming can allow for animals to have a predictive, adaptive response which predisposes the

individual to potential post-natal environment (Laviola and Macrì, 2013). For viviparous animals, adverse environments during the fetal period are predominantly reliant on environmental experiences of the dam or sire. However, eggs of oviparous animals may experience adverse environments that are unrelated to parental experiences.

Studies have shown that prenatal exposure to stress alters brain development and could influence adult human behavior (Ambeskovic, et al., 2019). This alteration in fetal development is well documented in viviparous animals; however, it is not as well defined in oviparous animals such as quail and chicken (Paiva, et al., 2018). Evidence from previous studies also show that prenatal exposure to increased steroid hormones and toxins in the F0 generation changes the behavior and disease incidence of their F2 generation (Anway, et al., 2006; Iqbal, et al., 2012; Ambeskovic, et al., 2019). It has been shown that recurrent prenatal stress exposure over four generations produces offspring with significantly higher anxiety-like behavior than those that were exposed to prenatal stress in only one generation (Ambeskovic, et al., 2019). There was also higher circulation of corticosterone in multigenerational stressed F4 female rats as opposed to males (Ambeskovic, et al., 2019). These investigators noted that anxiety-like behaviors increase incrementally over generations within a lineage where females experience stress during gestation (2019).

Conditioning birds to higher temperatures at an early age may increase heat resistance at a later age (Kumari and Nath, 2018). Adaptation to environmental changes can occur on the genetic level. Considering 18-30% of the genome is involved in environment-specific response genes such as heat shock proteins, osmotic stress protectants, and protein degradation enzymes, animals can adjust to be successful in different environments (Sejian, et al., 2015).

The number of days it takes for a chicken to reach slaughter weight has decreased by half from 1956 to 2020 (van der Wagt, et al., 2020). This significant decrease has placed great importance on the embryonic phase due to its higher proportion of the total lifespan of the chicken. Egg lipids account for 90% of the total energy produced by the embryo or hatchling and is the primary source of energy for the 2nd half of incubation and early post-hatch (van der Wagt, et al., 2020). Therefore, the composition of lipids is important for embryonic development and growth. However, the yolk is only usable at a higher rate after day 10 of incubation due to the full development of the yolk sac membrane and chorioallantois.

3.1 Heat stress and performance

In a study by Paiva et al., meat-type quail subjected to repeated short exposures to cold during late embryogenesis can significantly affect incidences of ascites and improves growth rate (Paiva et al., 2018). This is an indication that the embryos possibly adapted their thermoregulation and cardiovascular systems to lower environmental temperatures (Paiva et al., 2018). In general, when implementing a genetic selection program to improve production traits, there is also an increase in metabolic heat production and therefore susceptibility to heat stress (Renaudeau, et al., 2012). A solution to this issue is to select for high production in heat stressed environments. Animals typically decrease feed intake during times of stress; thus, presenting a challenge for selecting for high producing animals in hot climates. Typically, birds that are selected for high feed conversion ratio, rapid growth, and high egg production rates are also very susceptible to oxidative stress due to demand for rapid metabolic rates (Surai, Kochish, et al., 2019).

In rodents, chronic stress causes a decrease in offspring and maternal growth, maternal care, and an increase in maternal aggression (Laviola and Macrì, 2013). In certain circumstances, the everyday stress resilience hypothesis may be the solution to the aforementioned behaviors.

This hypothesis suggests that resilience can accumulate through regulation of everyday stressors, leading to successful coping (Laviola and Macrì, 2013). For examples, low levels of reactive oxygen species (ROS) have been reported to play a role in redox signaling for stress adaptation, homeostasis, and health management (Surai, et al., 2019).

Heat stress can decrease hemoglobin and pack cell volume due to an increase in water circulation for evaporative cooling; therefore, increasing blood volume (Sejian, et al., 2015). Evaporative cooling in quail is achieved by panting. When panting occurs at a rapid rate, there is excess CO_2 loss, leading to a reduction in the partial pressure of CO_2 (de Moraes, et al., 2019). There is also a rise in blood pH, leading to respiratory alkalosis. The concentration of bicarbonate ions increases, which increases blood pH and leads to respiratory alkalosis. To neutralize the rise in blood pH, birds will excrete bicarbonate ions and retain H^+ from the kidneys (Wasti, et al., 2020).

In addition to changes in hemoglobin and pack cell volume, there is a concomitant change in blood acid-base equilibrium when poultry are adapting to heat stress. Such changes include the ability to regulate electrolytes such as Na, K, and Ca (Gezen, et al., 2005). The electrolytes are important to maintaining osmotic pressure in cells and for maintenance of molecular traffic in and out of cells (Park and Kim, 2017). Heat stressed broiler chicken at 35˚C for 8 hours, decreased feed intake, bicarbonate, potassium, and CO_2 (Beckford, et al. 2020). The heat stressed ducks also decreased blood electrolytes such as Na, K, and Cl (Park and Kim, 2017).

3.2 Heat stress and fatty acid composition

Fatty acids are of particular interest for a heat stress study because they are ubiquitous molecules that serve many functions in the body. Such functions include metabolic fuel, structure, and signaling molecules (Ben-Hamo, et al., 2013). The composition of fatty acids is important to

support the energetic needs of the bird. Birds will make seasonal adjustments in the composition of structural fatty acids, especially increasing unsaturated fatty acids in the fall and winter (Ben-Hamo, et al., 2013). During fasting situations such as heat stress, birds will rely exclusively on lipid metabolism for energy with a preferential metabolism of short-chained unsaturated fatty acids from body tissue fat. This will change the fatty acid composition of stored fat towards a higher concentration of saturated fatty acids (Ben-Hamo, et al., 2013). Saturated fatty acids have a greater tendency to be deposited in the body and unsaturated fatty acids are more readily oxidized for energy (Ben-Hamo, et al., 2011). Results from a study of fasted heat stressed quail showed that there was a lower turnover rate of fatty acids in phospholipids as compared to triacylglycerides (Ben-Hamo, et al. 2013). The study also revealed that all tissues had decreased proportions of palmitic acid (C16:0) (Ben-Hamo, et al., 2013).

Fatty acid composition can affect heat production. In a study on JQ that were fed either an unsaturated fatty acid diet or a saturated fatty acid diet, researchers noted that those fed saturated fatty acids had lower feed intake, high concentrations of oleic acid in their liver, lower levels of arachidonic acid, and lower body temperatures (Ben-Hamo, et al., 2011). Fasting quail fed the saturated fatty acid diet also had lower body temperatures than those that were fasting and fed the unsaturated fatty acid diet (Ben-Hamo, et al., 2011). With high levels of oleic acid in quail tissue, there is a decrease in thermogenesis (Ben-Hamo, et al., 2011).

Unlike other species, where adipose tissue is the predominant tissue for lipid synthesis, birds use their livers for this purpose (Noble and Cocchi, 1990). Male Ross broiler chickens were raised from 1 to 42 days of age in either a thermoneutral temperature (21˚C) or a cyclic heat stress temperature (37˚C). Researchers determined no significant differences in cholesterol, triacylglyceride, low density lipoproteins, and high-density lipoprotein levels in the blood

(Hosseini-Vashan and Raei-Moghadam, 2019). However, they determined lower indication of lipid oxidation by use of the thiobarbituric reactive substances assay (TBARS) and higher abdominal fat weight and liver weight in birds raised in the thermoneutral temperature (Hosseini-Vashan and Raei-Moghadam, 2019). The higher abdominal fat weight in the broilers raised in thermoneutral temperatures indicated that the heat stressed birds were expending more energy and possibly lipid stores to decrease heat increment and body temperature (Hosseini-Vashan and Raei-Moghadam, 2019).

It is essential to study early life programming in poultry species because they are reaching slaughter weight at physiologically younger ages, which means bird welfare, health, and productivity need to be optimized early in life (Cherian, 2011). Essential fatty acids for chick development are linoleic (C18:2 n-6) and α- linolenic (C18:3 n-3). These fatty acids are transferred from the dam to the yolk which supply lipids for energy, PUFA-rich phospholipid membranes, and eicosanoids (Cherian, 2011). The yolk is about 30-31% lipids and 80% of these yolk lipids are absorbed by the developing chick embryo during incubation (Cherian, 2011). Yolk essential fatty acids are the main factor known to influence lipid and fatty acid composition of the chick during embryogenesis and early post-hatch. In particular, the concentrations of n-6 and n-3 essential fatty acids in the yolk can determine metabolic and immune tissue function during development and may persist post-hatch (Cherian, 2011). The concentrations of n-3 and n-6 in the maternal diet affects fertilized eggs. This was demonstrated in a study were chicks hatched from dams that ate high n-3 diets retained higher DHA (C22:6 n-3) in their liver, heart, spleen, and bursa compared to chicks from low n-3 eggs (Cherian, 2011). Lowering the n-6:n-3 ratio decreased the inflammation response.

3.3 Heat stress, lipid oxidation, and antioxidants

Through the theory of evolution of animals from algae and from anaerobic life to aerobic life, many have hypothesized that oxidative damage still occurs because mechanisms for completely neutralizing damage from excess oxygen was not inherited along with the ability to use oxygen as an energy source (Laviola and Macrì, 2013). The evidence is present in various molecular structures such as polyunsaturated fatty acids and certain amino acids (cysteine, methionine, histidine, phenylalanine, tyrosine, and tryptophan) (Berrill, et al., 2011). Both classes of molecules are essential to life and are highly susceptible to oxidation. The oxidation of polyunsaturated fatty acids can change membrane composition, structure, fluidity, and membrane-bound enzymatic activity (Surai, et al., 2016).

Under normal conditions, 1-5% of oxygen intake is converted to free radicals because the conversion to water or energy is not perfect within complexes I and II of the electron transport chain (Surai, et al., 2016; Akbarian, et al., 2016). When an animal is in a normal state, it will typically have enough antioxidants to trap and neutralize free radicals. However, during abnormal events such as heat stress, there will be increased oxidation reactions and an inadequate supply of antioxidants (Hosseini-Vashan and Raei-Moghadam, 2019). Heat stress or increase in environmental temperature increases the concentration of free radicals and therefore oxidation in the plasma, liver, and heart (Hosseini-Vashan and Raei-Moghadam, 2019). The antioxidant strategy is an attempt to decrease free radical production by decreasing oxygen availability. Decreasing oxygen availability includes decreasing activity of enzymes responsible for ROS such as NADPH oxidase and xanthine oxidase, maintaining iron and copper bound to proteins to prevent new radical formation, sustaining mitochondria integrity, scavenging for free radicals with the use of molecules like vitamins E, C, and glutathione (GSH), apoptosis, and autophagy (Sahin, et al.,

2013; Chauhan, et al., 2020; Madkour, et al., 2021). However, others found that heat stress in avian muscle cells increases NADPH oxidase (Kikusato, et al. 2015).

GSH is the most abundant non-protein thiol in avian cells. It controls the redox balance and signaling, regulates transcription factors and gene expression, and controls mitochondrial ROS generation (Surai, et al., 2019). In a study with 5-week-old JQ that were reared at 34˚C for 8 hours per day for 12 weeks, results showed that there was an 84.8% increase in hepatic malondialdehyde, a 25.8% decrease in hepatic SOD, a 52.3% decrease in CAT, and a 45.5% decrease in glutathione peroxidase (Surai, et al., 2019).

Previous research has tested varying amounts of antioxidants such as propolis in male Ross 708 broilers during heat stress (32˚C for 9 hours from day 15 to 42 of age) and concluded that inclusion of propolis significantly increases mobility and significantly decreases panting (Mahmoud, et al., 2015). Thus, these investigators demonstrated that antioxidants play a large role in modulating the effects of heat stress in poultry species.

3.4 Heat stress and proteomics

Previous research has also revealed that birds will increase (1) mobilization of energy to maintain homeostasis and (2) proteins such as sterol regulatory element-binding protein 1 (SREBP-1) and carbohydrate response element binding protein (ChREBP) may play significant roles in regulating fat deposition in broiler carcasses during heat stress (De Antonio, et al., 2017). Male Cobb 500 broilers that were subjected to heat stress at 31.8˚C from 7 to 42 days of age had higher liver gene expression for SREBP-1 at day 42 (De Antonio, et al., 2017). Results of others have also indicated that triglyceride and cholesterol levels in the liver of heat stressed JQ were increased, further supporting the idea that heat stress changes lipid metabolism (Pu, et al., 2020).

It is important to have heat shock factors (HSF) because there is an increase in misfolded proteins in the cytoplasm during adaption to climate change. In avian cells, there are only 2 heat shock factors expressed: HSF1 and HSF3. HSF1 is rapidly activated by mild heat stress and mediates transcriptional activity only in the brain. Once it is activated, HSF1 trimerizes with other HSF1 molecules and is transported into the nucleus. In the nucleus, HSF1 initiates transcription of HSP (Sejian, et al., 2015). HSF3 is the master regulator of heat stress genes and is only activated in blood cells after heat stress (Surai, Kochish, et al., 2019). HSF2 has not been found in avian cells, but in other species, it is activated after prolonged stress and is involved in normal cellular processes, embryonic development, and cellular differentiation (Sejian, et al., 2015).

Heat shock proteins (HSPs) are important molecules associated with heat tolerance. Typically, there are two phases of heat stress in which HSPs play a role. Phase 1 occurs about 1 hour after heat stress and where pathways that involve adenosine receptors, mitochondrial potassium ATP-dependent channels, and various kinases are activated. Phase 2 is more delayed and has a longer-lasting response. During phase 2, heat shock genes and HSPs are active and cause protein assembly and disassembly (Kumari and Nath, 2018). There is a notably higher concentration of HSP70 after 2, 3, and 5 hours of heat stress with expression varying by tissue (Kumari and Nath, 2018). HSP70 protects intestinal mucosa from heat stress injury by increasing antioxidant capacity and inhibiting lipid oxidation in broilers. HSP70 activation is tissue, age, and time dependent in avian species (Surai, et al., 2019).

NF-E2-related factor 2/Kelch-like ECH associated protein 1 (Nrf2/Keap1) and nuclear factor κ-light-chain enhancer of activated B cells/inhibitory κB protein (NF-κB/IκB) systems are major regulators of stress response. Nrf2/Keap1 is one of the fastest responding systems to a change in environment because it can upregulate antioxidants in minutes. It is a redox-sensitive

master regulator of oxidative stress signaling for adaptive stress response (Surai, et al., 2019). Oxidative stress ultimately causes Nrf2 to bind to the antioxidant/electrophile response element (ARE) and upstream promoter regions of genes encoding for antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (Akbarian, et al., 2016). In a study on 180 10-day-old JQ that were heat stressed for 8 hours a day at 34˚C, researchers reported an increase in NF-κB and HSP70 levels and a decrease in Nrf2 levels in hepatic cells (Sahin, et al., 2012).

Other investigators also disclosed that proteins involved in carbohydrate metabolism were differentially expressed in chronic heat stress (Kang and Shim, 2020). Therefore, it is important to further investigate the effects of heat stress on proteins involved in lipid metabolism in the liver.

3.5 Heat stress and steroid hormones

All steroid hormones originate from cholesterol. This system of hormones is highly responsive to heat stress and can influence many different functions of the body. They can be categorized into multiple classes depending on their primary function. Glucocorticoids (corticosterone and cortisol) are largely responsible for stress response, immune system regulation, gluconeogenesis, and fat storage (Wingfield, 2005; Honda, et al., 2022). Estrogens (estradiol and estriol) play a pivotal role as sex hormones in female ovaries and male testes. They also impact adipose and cardiovascular systems by interacting with various nuclei in the brain that control food intake and satiety (Barros and Gustafsson, 2011; Lee, et al., 2012). Therefore, indirectly, the influence of estrogens on metabolism may be through the regulation of feed intake (Barros and Gustafsson, 2011). The direct effect of estrogens on carbohydrate metabolism is supported by the presence of estrogen receptors throughout insulin-induced and insulin-independent glucose uptake (Barros and Gustafsson, 2011). Androgens (testosterone, dihydrotestosterone, and

dehydroepiandrosterone) are responsible for the development of the male reproductive system. Others have reported that heat stress downregulates the expression of enzymes essential to testosterone biosynthesis in Leydig cells and causes Leydig cell hyperplasia (Li, et al., 2015). Progestogens (progesterone, 17α-hydroxyprogesterone, and 20α-hydroxyprogesterone) are important as precursors to cortisol, corticosterone, aldosterone, testosterone, and estradiol. Neurosteroids such as allo-pregnanolone is converted to progesterone and has been investigated for its role in modulating stress (Locci and Pinna, 2017; Locci and Pinna, 2019). Dehydroepiandrosterone (DHEA) may also be an important steroid precursor. Adrenal cortex in origin but can also be synthesized by neurons in the brain for paracrine actions.

Heat stress is known to increase plasma corticosterone and cortisol. In prolonged exposure to heat stress, the high levels of corticosterone and cortisol will increase gluconeogenesis and cause increase vasodilation, proteolysis, lipolysis, and decrease insulin sensitivity (Sejian, et al., 2015). In JQ subjected to 34˚C for 4 hours, researchers described an increase in both serum and yolk corticosterone and 17β-estradiol concentrations (Pu, et al., 2020). This transfer of steroid hormones from females to offspring have also been demonstrated in numerous other species such a barn swallows, European starlings, and chicken (Saino, et al., 2005; Janczak, et al., 2006; Love and Williams, 2008).

As with other species, an increase in corticosteroids will decrease reproduction through the dampening of sex steroid hormones such as testosterone, estradiol, and progesterone. Maternal heat stress can also increase reactive oxygen species activity in the oviducts and embryos which decrease the quality of oocyte and follicular development (Sejian, et al., 2015). Additionally, corticosterone has lipogenic effects and causes an increase of fat pad size (Tedford, 1995). There

can be detrimental effects of corticosterone exposure during embryonation; thus, further investigation of steroid hormones and heat stress should be conducted.

4. Importance of JQ as a heat stress model

Livestock is 40% of the world's agriculture-related gross domestic product (Sejian, et al., 2015). JQ are a significant part of the poultry industry. JQ can be an alternative to chicken for egg and meat production. When considering generation interval as the time between F0 hatch to F1 hatch, JQ have an average generation interval of 59 days (42 days to sexual maturity of F0 + 17 days for F1 incubation and hatch) as opposed to the chicken which have an average generation interval of 161 (140 days to sexual maturity of F0 + 21 days for F1 incubation and hatch). This relatively shorter generation interval of JQ is desirable because there can be increased rate of genetic selection for particular traits such as heat tolerance. The thermoneutral zone of quail is 18 to 30˚C with optimal temperatures between 21 to 27˚C (Shanaway, 1994; Alagawany, et al., 2017). JQ are also less susceptible to common chicken diseases which makes them ideal for stress challenges (Rahman, et al., 2016).

Quail are excellent models for heat stress (Huss, et al., 2008; Carvalho, et al. 2020). Information obtained from quail are relevant to many different species. They also have hardy embryos and have been used in studies related to fetal alcohol syndrome (Huss, et al., 2008). In general, birds are ideal models for investigating multigenerational stress response because the fetal environment is outside of the female body. This allows for easy manipulation and study. Additionally, the neuroendocrine system of birds is very similar to humans (Laviola and Macrì, 2013).

Understanding how heat stress changes an animal's metabolism is the basis of developing future mitigation strategies (Sejian, et al., 2015). For examples, it has been noted that heat stress

increases carbohydrates utilization causing insulin to activate and upregulate HSP. This increase in insulin concentration in the blood can lead to antilipolytic and lipogenic activities in the body and cause an increase in lipid retention in heat stressed animals (Sejian, et al., 2015). Heat stress also increases blood flow to the external body rather than internal; thus, leading to a change in gut conformation and a decrease in intestinal barrier function (Sejian, et al., 2015).

5. Current study

Due to the role of steroid hormones in stress response and their subsequent impact on energy metabolism, it is important to investigate overall effects in multigenerational heat stressed JQ. Although there have been many studies on heat stress in high performing poultry, there have been comparatively fewer studies based on birds chosen specifically for high performance in a heat stressed environment (Park and Kim, 2017; Wang, et al., 2018; Beckford, et al., 2020; Livingston, et al., 2022). Therefore, the overarching goal of this study was to determine if phenotypic selection for high performance in a heat stressed environment has a familial link. In other words, is there a fitness advantage when birds are chosen based on their siblings' performance in a heat stressed environment and does that confer a difference in how their lipids are metabolized? A tangential goal of the study was to determine the effects of marginal heat stress on multiple generations of JQ. This information is important for both producers and wildlife conservationists for mitigation of heat stress. It can also build a more comprehensive picture of the transition from the thermoneutral zone to heat stress.

Questions that were sought out to be answered by this project were: (1) Do quail adapt their energy metabolism and fat metabolism after multiple generations of heat stress, (2) Are there also protective changes in fatty acid composition over time, and (3) Are there protective mechanisms,

such as increased circulation of antioxidants, that are triggered in response to increased oxidative molecules?

To test these questions, the following parameters were analyzed after 10 generations of selective breeding for high performance in heat stress (1) concentration of various blood gases, blood electrolytes, and animal performance, (2) fatty acid composition of adult tissues, yolk, and subsequent embryonic tissue, (3) level of lipid oxidation and antioxidant status of adult tissues, yolk, and subsequent embryonic tissue, (4) steroid hormone levels in adult sera, and (5) protein expression in adult female livers. Overall, it was hypothesized that selection for high performance in heat stress will confer a fitness advantage over time; therefore, lessening the burden of heat stress.

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CHAPTER 2
Changes in Japanese quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress

Abstract: The average surface temperature of the Earth is predicted to rise 0.5ºC to 6ºC by the year 2100. When Japanese quail (*Coturnix coturnix japonica*) are subjected to heat stress, their blood acid-base equilibrium and their ability to regulate electrolytes such as Na, K, and Ca may change. This disequilibrium may influence egg-shell quality, enzyme functions, and synthesis of tissue proteins. To determine effects of multi-generation heat stress on Japanese quail, they were subjected to the following treatments (1) control (TN, non-sibling random mating at thermoneutral temperature [22.2˚C]); (2) thermoneutral siblings (22.2˚C, TNS); (3) heat stress (HS, non-sibling random mating at 31.1˚C); and (4) heat stressed siblings (HSS, offspring of TNS with a high feed conversion ratio (FCR) as determined in HSS, 31.1˚C). Body weights (BW), blood gases, and electrolytes of 24 quail per treatment were measured during the first 4 hours (acute) and after 3 weeks (chronic) of heat exposure (31.1˚C) in generation 10. Analyses of data were tested for significance at $P \leq 0.05$. Models included treatments, length of exposure, sex, and their interactions. Results showed that acute and chronic heat stress at 31.1˚C does not have a clear effect on blood electrolytes, acid-base regulation, and oxygen transport. Across treatments, sexes, lengths of exposure, and their interactions, acute HSS males or females were significantly different than chronic TN males in BW, PCO_2, PO_2, sO_2, and Na. Chronic HS males and females did not have significantly different blood electrolytes, acid-base regulation, and oxygen transport than chronic HSS males and females. Thus, selection for low FCR in heat stress at 31.1˚C did not incur a fitness advantage when considering these parameters. Sexually mature males had significantly higher levels of hematocrit and hemoglobin than sexually immature quail and sexually mature females.

1. Introduction

Data from 1880 to 2018 suggest that the average surface temperature of earth will rise 0.5ºC to 6ºC by 2100 (Lindsey and Dahlman, 2019). In general, animals experience heat stress

when internal heat production exceeds the ability of the animal to dissipate heat to the external environment (Talbot et al., 2018). Even with temperature-controlled barns, changes in the earth's surface temperature due to anthropogenic climate change may have effects on various parameters including concentration of blood gases and electrolyte.

Japanese quail (*Coturnix coturnix japonica*) are of particular interest due to their importance as an option for meat and eggs (El-Tarabany, 2016; Nasar, et al., 2016). Tropical areas such as Bangladesh and Egypt have several successful quail farms, indicating that quail are adapted to hot and humid climates (El-Tarabany, 2016; Rahman, et al., 2016). They are also relatively easy to raise as approximately 92.3% of the 52 Bangladeshi quail farmers had no prior experience in quail farming (Rahman, et al., 2016). As well, Japanese quail have short generation intervals and can reach sexual maturity at 5 to 6 weeks of age. Their thermoneutral zone is 18 to 30˚C with optimal temperatures between 21 to 27˚C; whereas chicken have upper critical temperatures of 23.86˚C to 25.46˚C (Shanaway, 1984; Alagawany, et al., 2017; Chang, et al., 2018). Thus, Japanese quail can be raised in higher ambient temperatures without sacrificing performance. It is important to obtain biochemical information about quail raised at or above 30˚C, and to understand how it can be useful for growth of broilers and other birds at higher temperatures.

When quail are subjected to heat stress, their blood acid-base equilibrium may change as well as their ability to regulate electrolytes such as Na, K, and Ca (Gezen, et al., 2005). Typically, as laying hens experience temperatures outside of their thermoneutral zone, they will perform rapid shallow breathing, also known as gular fluttering, because they do not have sweat glands. Gular fluttering is when a bird opens its beak and rapidly moves its neck muscles to allow moisture to evaporate from its lungs, throat, and mouth (Talbot, et al., 2018). This behavior increases air passage in the upper respiratory tract and can cause blood alkalosis and moderate to severe

dehydration (Renaudeau, et al., 2012). This disequilibrium may influence eggshell quality, enzyme functions, and synthesis of tissue proteins (Gezen, et al., 2005). Eggshell quality may decrease due to decreased Ca intake or blood alkalosis (Renaudeau, et al., 2012). Decreasing Ca intake will decrease blood bicarbonate which is essential to eggshell formation.

Other important electrolytes to consider are Na^+ and K^+, which are the main electrolytes fed to maintain acid/base balance for osmotic pressure (de Moraes, et al., 2019). Na^+ accounts for 93% of total cation content in blood plasma (Baloš, et al., 2016). It is used in many physiological processes such as osmotic relationships, muscle cell contractions, adrenal gland function, and carbohydrate absorption and energy turnover (Baloš, et al., 2016). It also plays a critical role in maintaining blood plasma pH and activity of most mitochondrial enzymes. More than one-third of ATP consumed by a resting animal is used for active transport of Na^+ and K^+ (Baloš, et al., 2016). K^+ is used for protein and carbohydrate metabolism, normal heart function, and permeability of cell membranes. When an animal experiences stress, there is an increase in plasma proteins which leads to an increase in adrenaline-mediated renal excretion of K^+ ions into urine (Baloš, et al., 2016). However, once glycogen is re-established, K^+ returns to the liver and if adaptation to stress occurs, K^+ levels are restored.

Stressed birds require higher amounts of vitamins and minerals in their diet because there is a change in metabolism, a decrease in feed intake, and a decrease in vitamin stability (Laganá, et al., 2007). Researchers found that heat stressed broilers have a decrease of about 6.8% in feed intake and a decrease of 8.4% in body weight gain (Laganá, et al., 2007). However, once they were supplemented with vitamins and minerals, their feed conversion ratios were significantly higher than control (Laganá, et al., 2007). This study also found that cyclic heat stress is less detrimental

to birds than chronic heat stress due to the respite in high temperature during part of the day (Laganá, et al., 2007).

Understanding the bird's response to heat stress will be particularly important in less-developed countries as they may have less resources available for upkeep of controlled, indoor poultry housing, thus, requiring birds to use thermoregulatory processes such as gular fluttering or feather fluffing (Rahman, et al., 2016). Producers in more developed countries will also benefit from this information due to current consumer demand for pastured poultry farming (Hwang, et al., 2020).

The objective of the current study was to determine if there was an effect on concentrations of blood gases and electrolytes in whole blood of Japanese quail after 10 generations of cyclic heat stress. It was hypothesized that there will be fewer changes across treatments during acute heat stress due to strong homeostatic regulations on electrolytes and blood gases. In HS and HSS (see treatments below), the blood pH is expected to become more alkaline as CO_2 levels decrease and bicarbonate levels increase. A strong interaction between treatment, sex, and age are expected due to physiological changes that occur with sexual maturation. Sexually mature females need to drastically mobilize electrolytes to meet maintenance and egg production requirements; therefore, it was hypothesized that chronic HS and HSS females will have lower levels of Na, K, and ionized Ca (iCa) in their blood than TN and TNS.

2. Materials and methods

2.1 Experimental design

Animal care and use was approved by the Institutional Animal Care and Use Committee at the University of California Davis (Protocols #19473 and 21370; Davis, CA). All birds were hatched at 32.78˚C with 61% RH and were wing banded to identify familial lineage. After hatch,

they were reared together in brooder cages until 3.5 weeks of age. Sexual dimorphisms were apparent at 3.5 weeks and birds were separated into their respective treatments. The 4 treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS). HS was obtained through repeated generation of mating in 31.1˚C. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers with 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that were determined as having high fitness were mated. High fitness was quantified by a low feed conversion ratio (FCR) in HSS (Equation 1).

Equation 1. $Feed\ conversion\ ratio = \left(\frac{average\ feed\ intake}{average\ daily\ gain}\right)$

The ratios were only compared to other families in HSS and within their respective generation. After determining FCR for 1 week, ½ of males and ½ of females were classified as low FCR and the other ½ were classified as having high FCR. The low FCR birds were paired 1:1 (male: female, n = 80 pairs) to create Generation 0 (F0) TNS and HSS. Maternal wing band numbers were recorded and used to represent the offspring's family. Non-sibling mating was ensured in all treatments; first cousin pairings were acceptable. TN was obtained through multiple generations of mating in 22.2˚C (Figure 1).

The first day of heat exposure occurred 3 days after relocation of birds into their adult cages to allow for acclimation. HS and HSS experienced cyclic heat stress, in which temperatures increased from 22.2˚C to 31.1˚C between 0630h to 1100h (4.5 hours), were maintained at 31.1˚C between 1100h to 1630h (5.5 hours), decreased from 31.1˚C to 22.2˚C between 1630h to 2200h (5.5 hours), and were maintained at 22.2˚C from 2200h to 0630h (8.5 hours). The relative humidity remained constant at 50%. The chamber had at least 15 air exchanges per hour and temperature

was maintained through forced air heating. These treatment groups were repeated for 10 generations.

Birds were fed *ad libitum* feed and water. A starter game bird crumble (Purina® Game Bird Startena®, Purina Animal Nutrition, Arden Hills, MN) was fed from 0 to 6-7 weeks of age. A laying hen pellet (Purina® Layena Pellets, Purina Animal Nutrition, Arden Hills, MN) was fed from 6-7 to 17 weeks of age.

2.2 Performance measurements

To determine infertility, embryo death, and embryo twin/deformities, a total of 160 eggs for TN, HS, and HSS and a total of 152 eggs for TNS were collected when the quail were 69-day-old to 82-day-old, stored at 12.78˚C, incubated together for 15 days, then broken out. If an embryo failed to form, infertility was assumed. Death was counted during 15 days of incubation. Multiple embryo formation or a physically defected quail was considered twins or deformed, respectively. Percentage of each abnormality for eggs in each treatment was determined.

Average feed intake (AFI) was determined by averaging male and female daily feed intake over 7 days. Average daily gain (ADG) was determined as shown in Equation 2.

Equation 2. $Average\ daily\ gain\ = \frac{(BW_{d7} - BW_{d0})}{7\ days}$

BW_{d0}, initial body weight and BW_{d7}, body weight after 7 days.

2.3 Blood collection and analysis

To reduce stress, singularly caged birds were handled no more than 2 times. They were fasted for approximately 1 hour and weighed (BW) before blood was drawn through the right or left jugular vein. Analyses were performed using a VetScan iSTAT-1 handheld blood analyzer (Abbott Laboratories, San Diego, CA) and CG8+ cartridges from treatments (n = 24; 12 males and females) during the first 4 hours (acute) or after 3 weeks (chronic) of heat exposure (31.1˚C).

Fresh, whole blood (100 ml) was applied to the iSTAT CG8+ cartridge immediately after drawing due to rapid coagulation. The CG8+ cartridges detected electrolytes [Na^+ (mmol/L), K^+ (mmol/L), iCa (mg/dL)], glucose (mg/dL), hematology [(% PCV), hemoglobin (g/dL)], and blood gases [pH, PCO_2 (mmHg), PO_2 mmHg), TCO_2 (mmol/L), HCO_3 (mmol/L), base excess (BE, mmol/L), sO_2 (% saturated oxygen)]. Na^+ was measured to monitor electrolyte imbalances. Glucose was measured to determine if there was dysregulation of carbohydrate metabolism. Hematocrit and hemoglobin were measured to determine if there were more red blood cells in circulation for increased oxygen transport. PCO_2 measured the partial pressure of CO_2. PO_2 measured the partial pressure of oxygen. TCO_2 measured total carbon dioxide and was calculated from pH and PCO_2. HCO_3 measured bicarbonate. BE was calculated from HCO_3 and pH.

2.4 Statistical analysis

Analyses of data were performed in R 4.0.0 (R Core Team, 2020; RStudio Team, 2022) to test significance ($P \leq 0.05$). The Shapiro-Wilk's test was used to determine normality of residuals of models and data were considered normal at $W \geq 0.95$ or $P \geq 0.05$. Levene's test and Q-Q plots were used to determine homogeneity of variances and variances were considered equal at $P \geq 0.05$. For all data, analysis of variance (ANOVA) was used to assess significance for linear models which included sex, generation, and treatment as main effects. Tukey's method for comparison was used for analysis of significant pairwise differences of means. Cages were considered replicates as well as experimental units. All data were reported as means ± SE where appropriate.

3. Results

3.1 Performance

There were no significant differences in percentages of infertile eggs, embryo deaths, and deformed/twin embryos across all treatments ($P > 0.05$; Table 1). There were no significant

differences in body weight during acute heat stress when comparing males and females and across treatments (Figure 2). However, there were significant differences in body weight when comparing males and females in chronic heat stress where females in all treatments had higher body weights than males in all treatments (Figure 2). The p-value for the interaction between length of exposure and sex was <0.0001. However, overall, there was no treatment effect on body weight (p=0.12).

Only 100 individual cages were available for the FCR trial. Therefore, n=100 HSS were transferred to individual cages at 2.5 weeks-old while n=100 HS were transferred to the individual cages at 3.5-weeks-old. Thus, only sex effect within treatment was statistically analyzed. HS males had significantly lower AFI (P<0.0001) and ADG (P<0.0001) than HS females; however, FCR (p=0.32) was not significantly different between sexes. HSS males had significantly lower AFI (p=0.030) and ADG (p=0.00069) than HSS females; however, HSS males had a significantly higher FCR (p=0.0013) than HSS females.

3.2 pH and base excess

As shown in Figure 3, the pH of acute male and female quail was not significantly different when compared across sex and treatment (p=0.30). However, the pH of the chronic HS females was significantly greater than that for chronic TNS females (p=0.043). For BE, chronic TNS females was 1.09 mmol/L, significantly less than that for acute HSS males with a BE of 6.55 mmol/L (p=0.0029), acutely HSS females with a BE of 5.55 mmol/L (p=0.044), and chronic HS females with a BE of 6.83 mmol/L (p=0.0008).

3.3 Blood gases

When comparing PCO_2 by length of exposure, sex, and treatment, acute HS males had significantly lower levels (40.3 mmHg) than acute HSS females (47.4 mmHg, p=0.034) and chronic HS males (47.4 mmHg, p=0.036); however, acute HS males were not significantly

different than any other treatments (Table 3). Acute HS females had significantly lower levels of PCO_2 (mmHg) than acute HSS males (p=0.0048), acute HSS females (p=0.0017), chronic HS males (p=0.0018), and chronic HSS males (p=0.015); but there were no significant differences for any of the other treatments (Table 3). Values for acute HSS females were significantly lower than those for chronic TN females (p=0.0274) and chronic TN males (p=0.0079). Chronic TN females had significantly higher values than chronic HS males (p=0.029). The value for acute HSS males was significantly higher than that of chronic TN males (p=0.021). Chronic HS males had significantly higher PCO_2 than chronic TN males (p=0.0085).

When comparing PO_2 by length of exposure, sex, and treatment, chronic TN males had significantly higher levels than acute HS males (p=0.037), acute HS females (p=0.015), and acute HSS males (p=0.0004; Table 3). While chronic TNS males had significantly higher levels of PO_2 than acute HSS males (p=0.043), the value was not significantly different from that than all other treatments. Chronic TN females had significantly higher levels of PO_2 than acute HSS males (p=0.016).

Values for HCO_3 and TCO_2 are shown in Table 3. For both measurements, chronic TNS females had significantly lower levels than acute HSS males (p=0.0014; p=0.0093 for HCO_3 and TCO_2, respectively), acute HSS females (p=0.02; p=0.0011), and chronic HS females (p=0.0036; p=0.0026). Chronic TNS males also had a significantly lower level of HCO_3 than both acute HSS males (p=0.007; p=0.0091) and chronic HS females (p=0.016; p=0.020); but, the level was not significantly different from that of other treatments.

The percentage of sO_2 was significantly lower for acute HSS males than for acute TN females (p=0.048), chronic TN males (p=0.0009), and chronic TN females (p=0.014; Table 3). However, sO_2 values for acute HSS males were not significantly different from that of all other

treatments. The acute TNS female value was significantly lower than that of chronic TN males (p=0.043); however, it was not significantly different from that of all other treatments.

3.4 Blood electrolytes

Electrolyte levels are shown in Table 4. The data from the current study did not have clear temperature effects on Na^+ levels in blood; however, when comparing acute and chronic quail across treatments and sex, acute quail had significantly less Na^+ than chronic quail (P<0.0001). When comparing for treatment effect only, TN had significantly lower levels of Na^+ than HS (p=0.016) and HSS (p=0.0014). HSS also had significantly lower levels of Na^+ than TNS (p=0.016). The Na^+ levels for acute TN females, acute HSS males, and acute HSS females were significantly lower than chronic TN males (P<0.0001, for all comparisons), chronic TNS males (P<0.0001, p=0.0001, p=0.0001, for acute TN females, acute HSS males, and acute HSS females, respectively), chronic HSS males (p=0.014, p=0.038, p=0.047 as above), chronic TN females (P<0.0001, for all comparisons), and chronic TNS females (p=0.0001, p=0.0005, p=0.0007 as above). Chronic TN males had significantly higher levels of Na^+ in the blood than all acute treatments (P≤0.006), chronic HS males (p=0.028), chronic HS females (p=0.022), and chronic HSS females (p=0.001). Acute HS females and acute TNS females had significantly lower levels of Na^+ than chronic TN females (p=0.0004, p=0.0005, for acute HS females and acute TNS females, respectively), chronic TNS females (p=0.015, p=0.022 as above), and chronic TNS males (p=0.0039, p=0.0057 as above). Chronic HSS females had significantly lower levels of Na^+ than chronic TN females (p=0.040). Chronic TN females had significantly higher levels of Na^+ than acute HS males (p=0.0003), acute TN males (p=0.0025), and acute TNS males (p=0.0042). Chronic TNS males had significantly higher levels of Na^+ than acute TN males (p=0.021), acute TNS males (p=0.026), and acute HS males (p=0.003).

There were no significant differences among treatment, sex, or length of exposure in K^+ levels in the blood; however, when comparing acute and chronic quails across treatments and sex, acute quail had significantly more K^+ than chronic quails (p=0.0024). When only looking at treatment effects, TN had significantly higher levels of iCa than HS (p=0.0065). When only looking at length of exposure, chronic had significantly less iCa than acute (p=0.022). Chronic TN females were significantly higher than chronic HS females (p=0.039) in the amount of iCa, but there were no other significant differences among treatments (Table 4).

3.5 Glucose, hematocrit, and hemoglobin

As seen in Figure 4, acute TN males had significantly higher glucose levels than acute TNS females (p=0.0043), acute TNS males (p=0.029), chronic TNS males (p=0.019), and chronic HSS females (p=0.003). Acute TN females had significantly higher glucose levels than acute TNS females (p=0.015) and chronic HSS females (p=0.011). Acute TNS females had significantly lower glucose levels than chronic TN males (p=0.034). Chronic HSS females had significantly lower glucose levels than acute HSS males (p=0.039), chronic TN males (p=0.024), and chronic TNS females (p=0.041).

For both hematocrit and hemoglobin (Figures 5 and 6), all acute males, acute females, and chronic females were not significantly different from each other. There were no significant differences among treatments in chronic males; however, all chronic males were significantly different than all acute males, all acute females, and all chronic females except chronic HSS females (p=0.024; p=0.027, for hematocrit and hemoglobin, respectively).

4. Discussion

4.1 Performance

Percentage of infertile eggs (0.046% to 0.069%) and embryo deaths (0.038% to 0.089%), observed during egg breakouts was lower than that reported for Japanese quail (Omid, et al., 2018). The fertility for eggs from quails raised in a thermoneutral temperature (22˚C) reported by Omid, et al. (2018) was 88.32% and the total embryonic mortality was 25%. However, the fertility for eggs from quail raised in a heat stressed temperature (34˚C for 8 h/day) reported by these investigators was 79.96% and the total embryonic mortality was 35.71% (Omid, et al., 2018). The lower infertility and mortality rates observed in the current study could be due to the number of eggs sampled (152 to 160) as compared to 20 sampled in the study by Omid, et al. (2018). In the current study, there was no treatment effect on the percentages of abnormalities which may have been due to adaptability of quail to 31.1˚C. Others have tested heat stress in quail at 33˚C or higher and have observed temperature effects on infertility and embryo mortality (Ipek and Dikmen, 2014; Omid, et al., 2018).

Significant differences among sex and age supported our hypothesis; however, there were no treatment differences. BW for 3.5- to 4.5-week-old males and females were 100-106g and 102-105g, respectively, for all treatments (Figure 2). These were lower than the average body weight of about 128.79 to 167.64 g reported for 4- to 5-week-old Japanese quail (Sarica, et al., 2015). Possibly, weight differences were due to genetic lines. Japanese quail used in the study by Sarica, et al. (2015) were from a commercial hatchery in Turkey while the Japanese quail used in the current study were from a line of breeding quail maintained at UC Davis (Woodard, et al., 1973; Sarica, et al., 2015). In the current study, heat stress did not significantly affect body weight;

however, other studies found that heat stress decreases body weight in quail (Del Vesco and Gasparino, 2013; Sarica, et al., 2015; Alagawany, et al. 2017).

The AFI for HS and HSS were similar to reports of others for 4- to 6-week-old quail (Kar, et al., 2017). The ADG for HS was lower than that reported for 5- to 6-week-old Japanese quail (Kar, et al., 2017). ADG for HSS was similar to reports of 3.57g for ADG of 4- to 5-week-old quails (Kar, et al., 2017). The FCR of all sexes within treatments, except HS males, was higher than others have reported for quail at 5.60 (4- to 5-week-old) and 6.37 (5- to 6-week-old) (Kar, et al., 2017). In the present work, male HS FCR was high due to lack of significant weight change from week 4.5 to 5.5. The results from Kar, et al. were from quail raised at 23.89˚C; therefore, perhaps high ambient temperature caused the FCR of HS and HSS to be higher than those raised at a thermoneutral temperature (Kar, et al., 2017). High FCR was also determined for 4-week-old broiler chicken that were housed at 32˚C (Baziz, et al., 1996; Laganá, et al., 2007; Campderrich, et al., 2019). Additionally, feed efficiency or FCR is recommended for selection during heat stress because when selecting for body weight alone, there is reduced heat tolerance (Bowen and Washburn, 1984).

4.2 pH, base excess, and blood gases

Base excess is the amount of acid or base required to return the pH of the blood to 7.4 (Rabi, et al., 2017). Results supported the hypothesis that there would be little changes across treatments. However, the hypothesis that HS and HSS would have more alkaline blood was proven to be false. Results suggested that there was no significant difference among treatments, sex, and length of exposure. The significant differences in pH and base excess occurred between chronic TNS and HS females with TNS females having significantly lower pH and base excess than HS females. This could indicate that generational selection for low FCR in mildly heat stressed

environments can elicit a better buffering system in those that were chosen for heat stress, but never exposed to heat stress.

Overall, the acid-base balance in HSS during acute heat stress had more significant differences than those in the chronic TN treatment. However, there were no other clear differences on the acid-base balance between treatments that were heat stressed and those that were not. Similarly, other studies on heat stressed turkeys have found no significant differences in acid-base balance (Keskin and Durgun, 1997). Additionally, a study on different genetic lines of chicken and their acid-base regulation during heat stress at 35˚C showed that chronic exposure to this temperature did not change pH, PO_2, PCO_2, and HCO_3 (Wang, et al., 2018).

When there is excess loss of CO_2, the PCO_2 will decrease along with a decrease in H_2CO_3 in the blood due to an increase of HCO_3^- release from the kidneys and reduction of H^+ excretion to maintain acid-base equilibrium (de Moraes, et al., 2019). Researchers have found a decrease in blood PCO_2 in heat stressed broilers which is similar to the results seen in the acute HS male and females as compared to chronic TN males; however, it was not significantly different, and it was not seen in acute HSS and chronic HS and HSS male and female quail in the current study (Table 4; Park and Park, 2017).

In chicken, when there was high pH and low PCO_2, there was a decrease in Ca^{2+} in the blood (Sandercock, et al., 2001). Heat stress also decreased the water-holding capacity of proteins due to oxidative damage and decreased meat quality (Sandercock, et al., 2001). However, heat stress caused increased decomposition of glycogen and an increased rate of muscle glycolysis which will change meat quality due to a decrease in pH (Sandercock, et al., 2001). To alleviate issues with changes in pH during heat stress, others have found that adding NH_4Cl and KCl to drinking water can help maintain blood CO_2 and pH levels (Pawar, et al., 2016). Sources also

found that addition of sodium bicarbonate increases bicarbonate levels in the blood (Pawar, et al., 2016).

Acute HSS males having significantly lower levels of oxygen saturation (sO_2) in their blood than acute TN females, chronic TN males, and chronic TN females suggested that the initial exposure to heat stress caused more difficulty for breathing in the HSS than HS. However, those in the TN treatment had consistently higher levels of oxygen saturation in their blood.

4.3 Blood electrolytes

When other livestock species experience heat stress, there is usually a deficiency in both Na^+ and K^+ which lead to metabolic alkalosis and acid-base imbalances (Baloš, et al., 2016; Rojas-Downing, et al., 2017). In the current study, the most significant differences occurred between acute HSS and chronic TN. However, because there were no significant differences among all treatments in the acute phase of heat stress, results suggests that heat stress at 31.1˚C did not induce acid-base imbalance. However, when focusing on chronic TN males, chronic HS males and females, and chronic HSS females, there were significant differences. TN males had significantly higher levels of Na^+ than HS males and females and HSS females during the chronic heat stress phase of the study. This suggested that quails subjected to chronic heat stress at 31.1˚C did experience electrolyte imbalances.

If an animal is experiencing stress, there can be an increase in plasma proteins which leads to an increase in renal excretion of K^+ into the urine (Baloš, et al., 2016). However, when the animal has adapted to the stress, glycogen is reestablished and K^+ levels are restored (Baloš, et al., 2016). In this study, K^+ levels did not seem affected by treatment, indicating that the heat stress may not have been severe enough to metabolize protein or glycogen stores.

When birds experience heat stress, there is a decrease in activity of carbonic anhydrase and feed intake. Carbonic anhydrase is essential to make ion carbonate for eggshells and a decrease in feed intake limits free or iCa in the blood. There may also be an increase in blood pH which would also decrease Ca^{2+} blood levels (de Moraes, et al., 2019). This current study revealed significantly lower levels of iCa in chronic HS females when compared to chronic TN females; however, there were no other significant differences among treatments. This finding was likely due to the age of sampling as the birds were not yet reproductively active; thus, they did not require as much mobilization of electrolytes nor were there extreme physiological differences between sexually immature quail. This difference was not driven by pH differences because the pH of chronic TN females and chronic HS females were not significantly different from each other.

Although the current study did not find significantly lower levels of Na^+, K^+, and iCa in all birds subjected to heat stress, other studies have. A study in meat-type ducks that were heat stressed at 34 to 43˚C reported a decrease in Na^+, K^+, and chloride ions in the blood of heat stressed ducks. This decrease may have caused disruptions to Na^+/K^+ ATP pumps which are responsible for 30-60% of the body's energy and is important for maintenance of moisture balance in cells (Park and Park, 2017).

4.4 Glucose, hematocrit, and hemoglobin

Chronic TNS females had the highest levels of glucose. This effect could be due to the higher demand of energy from heat stressed groups and possibly conservative carbohydrate use in TNS during the acute phase and in chronically exposed males.

Typically, dehydration or cardiovascular disorders increase hematocrit levels. When comparing across all treatments, sexes, lengths of exposure, and their interactions, results from the current study showed that all males during chronic exposure had significantly higher levels of

hematocrit and hemoglobin. However, there was a shared significance with chronic HSS females. The higher levels of hematocrit in chronically exposed males may be indicative of effects from testosterone on hematocrit levels (Kobayashi, et al., 2022). Other researchers have found that exogenous testosterone increases red blood cell counts, hemoglobin concentration, and hematocrit levels in Japanese quail (Kobayashi, et al., 2022).

In a study on female Japanese quail that were housed in 38˚C for 8 hours a day, researchers found no difference in red blood cell count, concentration of hemoglobin, pack cell volume %, or white blood cells when compared to those in a thermoneutral temperature (Usman, et al., 2008). This is contrary to others who reported that Japanese quails were sensitive to high environmental temperatures and the metabolic stress response was triggered at temperatures higher than 25˚C (de Moraes, et al., 2019).

Contrary to the findings of the current study, hematocrit of heat stressed broilers were observed to decrease due to the damage of the red blood cells (Park and Park, 2017). In meat-type ducks that were heat stressed at 34-43˚C, researchers found that there was a decrease in red blood cells and hemoglobin which led to iron deficiency (Park and Park, 2017). Therefore, as previously mentioned, the heat stress experienced by the Japanese quail in this study was likely mild enough to not elicit strong physiological changes in their blood.

5. Conclusions

Many have researched the effects of heat stress at 32˚C and above; however, little research has been done on mild heat stress at temperatures between 27 to 31.9˚C. From this current study, the most notable differences seen were:

1. Acute and chronic heat stress at 31.1˚C does not have a clear effect on blood electrolytes, acid-base regulation, and oxygen transport.

2. Across treatments, sexes, lengths of exposure, and their interactions, acute HSS males or females were significantly different than chronic TN males in body weight, PCO_2, PO_2, sO_2, and Na.
3. Chronic HS males and females did not have significantly different blood electrolytes, acid-base regulation, and oxygen transport than chronic HSS males and females. This finding indicated that selection for low FCR in heat stress at 31.1˚C does not incur a fitness advantage when considering these parameters.
4. Sexually mature males had significantly higher levels of hematocrit and hemoglobin than sexually immature quails and sexually mature females.

Future research should focus on blood analysis in Japanese quail selected for low FCR in a thermoneutral temperature (22˚C), mild heat stress temperature (30-31˚C), and high heat stress temperature (≥33˚C). The current findings seemed to indicate that more studies should evaluate effects of higher temperatures on Na. Even though permission to conduct research at temperatures ≥33˚C is often difficult to obtain from institutional animal care and use committees, studies using the higher temperature could inform producers that have animals in potentially heat stressed environments when to expect physiological changes in their quail and how to adapt their feed to meet the animals' needs at different temperatures.

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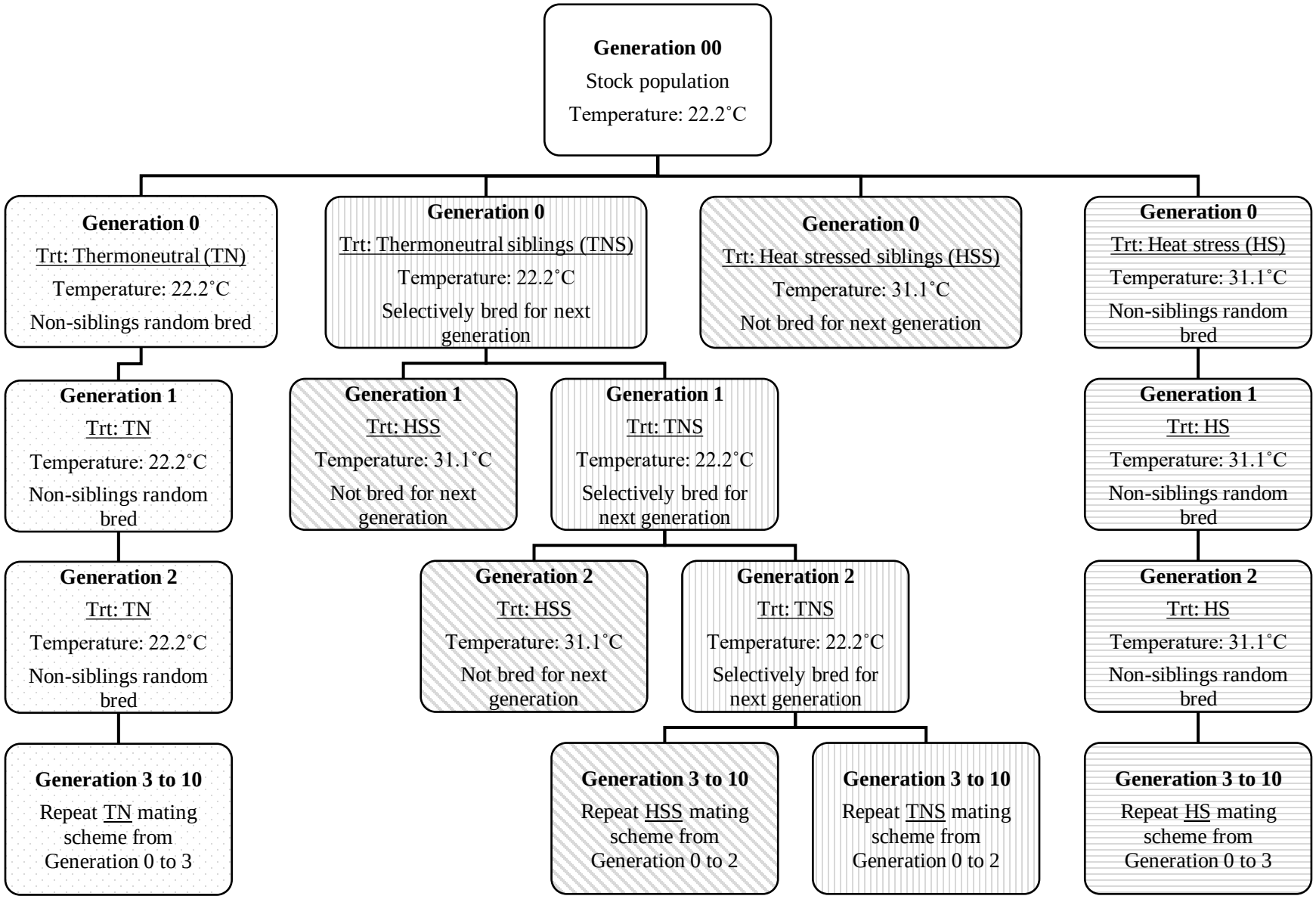

Figure 2.1. Schematic representation of treatments for 10 generations of Japanese quail.

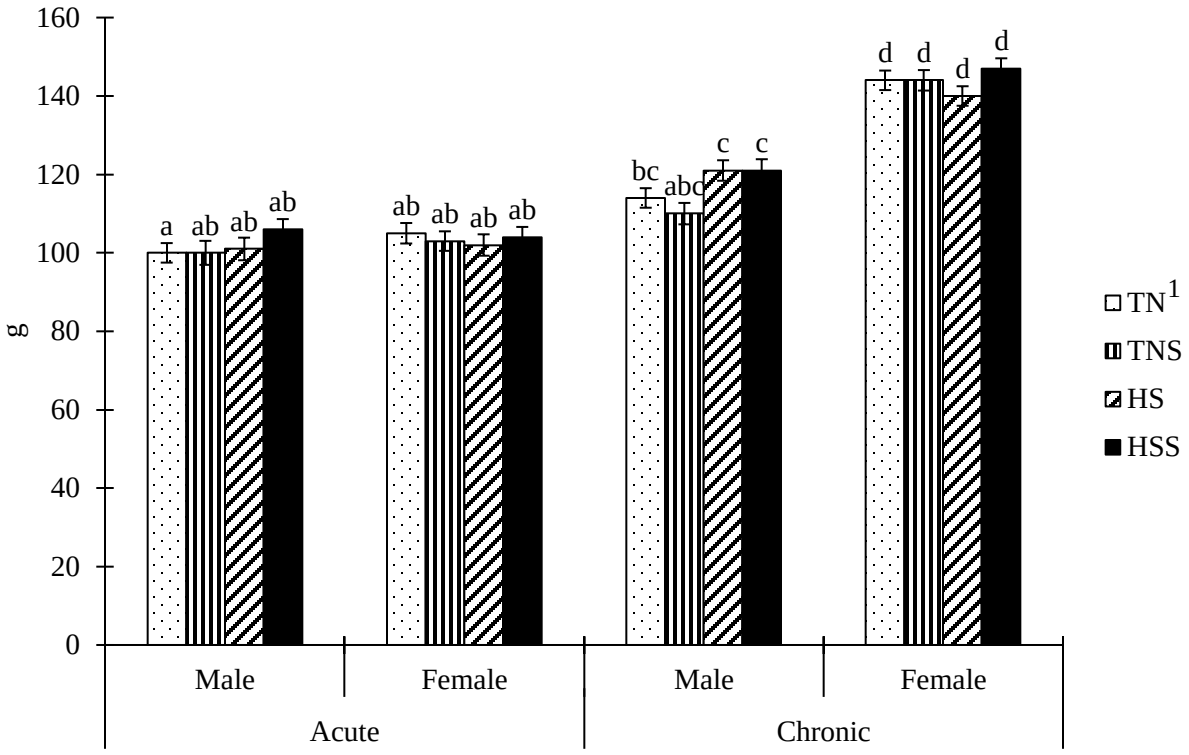

Figure 2.2. Body weight[2] of quail exposed to acute and chronic[3] temperatures.

[1] Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[2] Body weight was compared across treatment, length of exposure[1], sex, and their interactions.

[3] Acute, exposure to respective temperature for 4 hours; chronic, exposure to respective temperatures for 3 weeks.

[a-d] Superscripts indicate significant differences at $P \leq 0.05$.

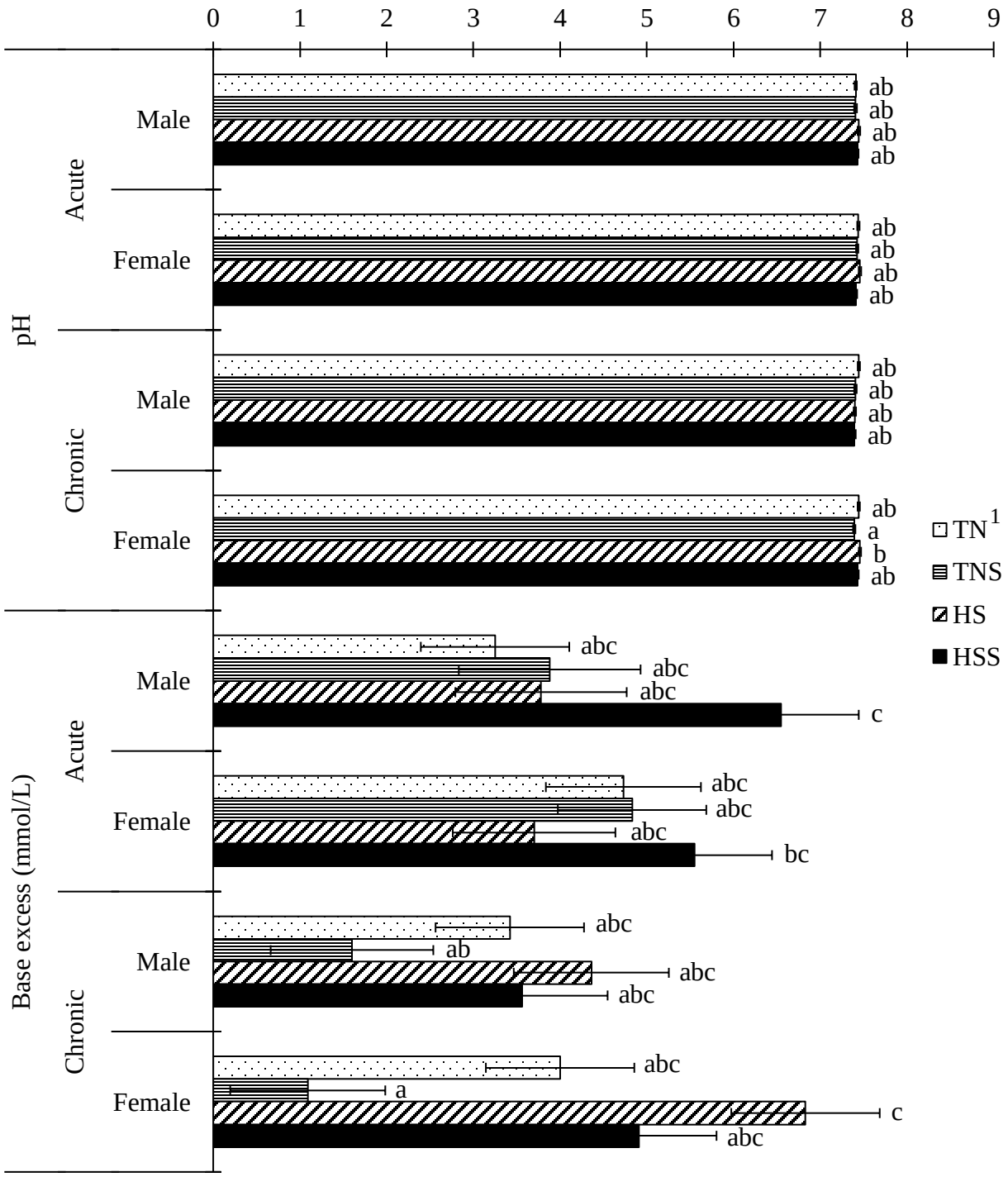

Figure 2.3. Blood pH and base excess[2] of quail exposed to acute and chronic[3] temperatures.
[1] Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[2] Blood pH (scale as 0 to 9) and base excess (mmol/L) were compared across treatment, length of exposure[2], sex, and their interactions.
[3] Acute, exposure to respective temperature for 4 hours; chronic, exposure to respective temperatures for 3 weeks.
[a-c] Superscripts indicate significant differences at $P \leq 0.05$.

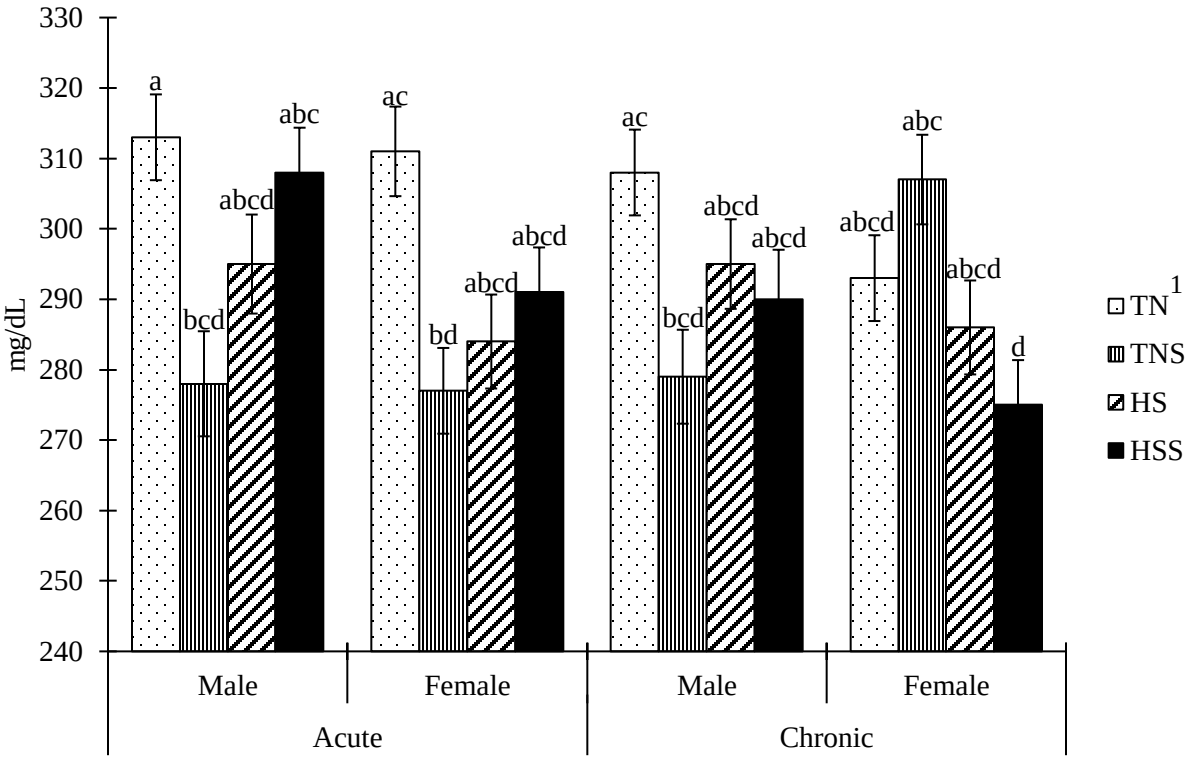

Figure 2.4. Blood glucose[2] of quail exposed to acute and chronic[3] temperatures.

[1] Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[2] Blood glucose was compared across treatment, length of exposure[2], sex, and their interactions.

[3] Acute, exposure to respective temperature for 4 hours; chronic, exposure to respective temperatures for 3 weeks.

[a-d] Superscripts indicate significant differences at $P \leq 0.05$.

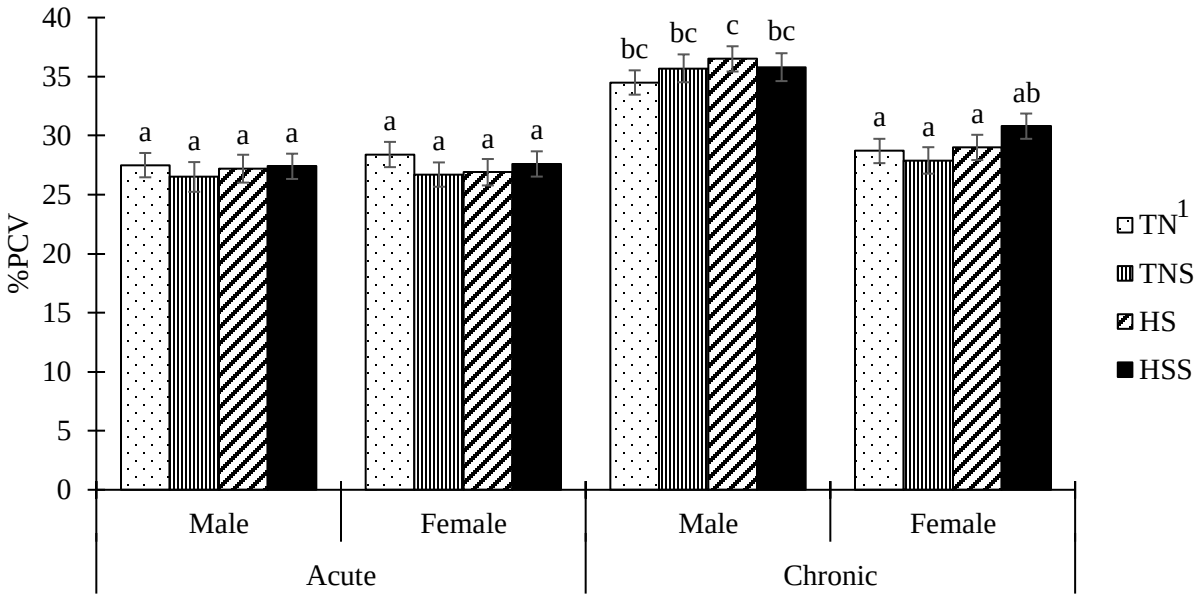

Figure 2.5. Hematocrit[2] of quail exposed to acute and chronic[3] temperatures.

[1] Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[2] Hematocrit was compared across treatment, length of exposure[2], sex, and their interactions.

[3] Acute, exposure to respective temperature for 4 hours; chronic, exposure to respective temperatures for 3 weeks.

[a-c] Superscripts indicate significant differences at $P \leq 0.05$.

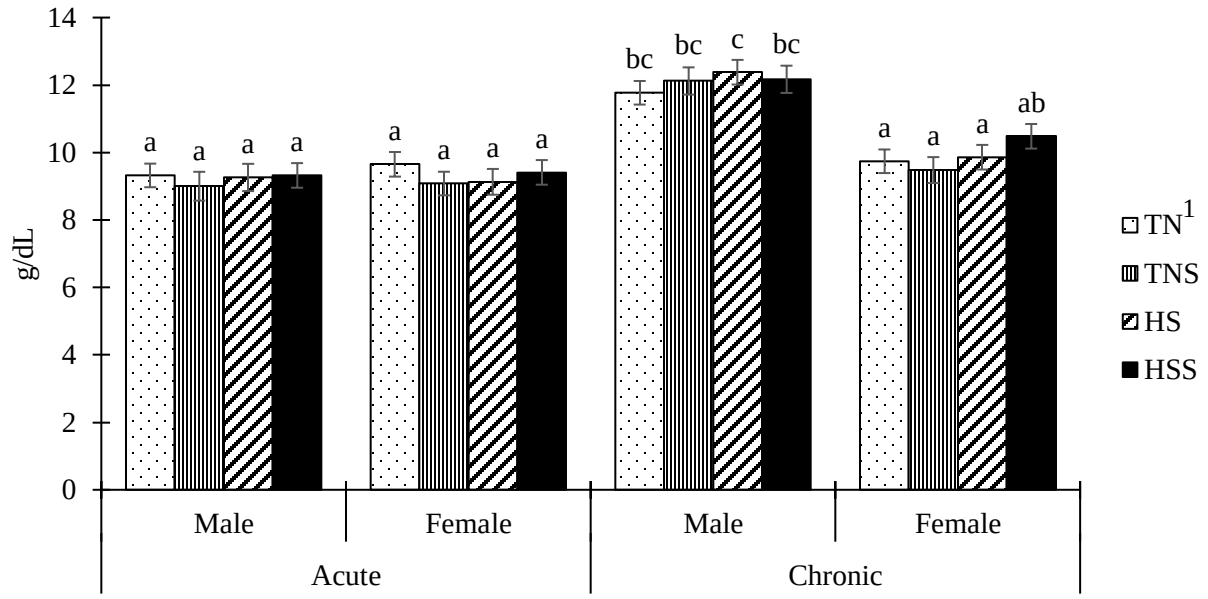

Figure 2.6. Hemoglobin[2] of quail exposed to acute and chronic[3] temperatures.

[1] Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[2] Hemoglobin was compared across treatment, length of exposure[2], sex, and their interactions.

[3] Acute, exposure to respective temperature for 4 hours; chronic, exposure to respective temperatures for 3 weeks.

[a-c] Superscripts indicate significant differences at $P \leq 0.05$.

Table 2.1. Percentage of infertile, embryo death, deformed/twins at the 10th generation of Japanese quail for respective treatments[1].

Treatment[1]	Infertile	SE	Total Embryo Death	SE	Deformed/Twins	SE
TN	0.069	0.016	0.075	0.021	0.0000	0.0047
TNS	0.046		0.089		0.0069	
HS	0.069		0.038		0.0000	
HSS	0.044		0.081		0.0063	

[1] Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

Superscripts indicate significant differences at $P<0.05$.

Table 2.2. FCR[1] within treatments.

Treatment[2]	Sex	AFI (g)[3]	ADG (g)[4]	FCR
HS	Male	17.30±0.46[a]	0.19±0.14[a]	1.09±4.65[a]
HS	Female	22.60±0.45[b]	2.74±0.13[b]	7.71±4.71[b]
HSS	Male	20.30±0.39[a]	2.39±0.13[a]	9.59±0.48[a]
HSS	Female	21.50±0.37[b]	3.04±0.13[b]	7.38±0.46[b]

FCR[1], AFI[3], and ADG[4] were compared within treatment by sex.

[1] FCR, feed conversion ratio, calculated by AFI/ADG; HS male (n=43); HS female (n=42); HSS male (n=44); HSS female (n=47).

[2] HS, heat stress; HSS, heat stressed siblings

[3] AFI, average feed intake; HS male (n=49); HS female (n=51); HSS male (n=44); HSS female (n=47).

[4] ADG, average daily gain; HS male (n=47); HS female (n=51); HSS male (n=42); HSS female (n=46).

[a-b] Superscripts indicate significant differences at $P \leq 0.05$.

Table 2.3. Blood gases[1] of quail exposed to acute[2] and chronic[2] temperatures.						
Blood gas	Length of exposure	Sex	TN[3]	TNS[3]	HS[3]	HSS[3]
PCO_2[4] (mmHg)	Acute	Male	44.30±1.26^{abcd}	45.20±1.55^{abcd}	40.30±1.46^{abc}	46.90±1.32^{cd}
		Female	42.60±1.32^{abcd}	44.70±1.26^{abcd}	38.90±1.38^{a}	47.40±1.32^{d}
	Chronic	Male	40.00±1.26^{ab}	42.20±1.38^{abcd}	47.40±1.32^{d}	46.60±1.46^{bcd}
		Female	40.70±1.26^{abc}	42.60±1.32^{abcd}	43.00±1.26^{abcd}	44.00±1.32^{abcd}
PO_2[4] (mmHg)	Acute	Male	39.40±1.63^{abc}	37.90±1.99^{abc}	34.10±1.88^{ab}	31.70±1.70^{a}
		Female	39.50±1.70^{abc}	36.10±1.63^{abc}	33.70±1.78^{ab}	35.20±1.70^{abc}
	Chronic	Male	43.00±1.63^{c}	40.70±1.88^{bc}	35.90±1.70^{abc}	40.40±1.88^{abc}
		Female	40.80±1.63^{bc}	38.90±1.70^{abc}	36.10±1.70^{abc}	35.90±1.70^{abc}
HCO_3[4] (mmol/L)	Acute	Male	27.90±0.74^{abc}	28.50±0.90^{abc}	27.70±0.85^{abc}	30.90±0.77^{c}
		Female	29.00±0.77^{abc}	29.30±0.74^{abc}	27.50±0.81^{abc}	30.10±0.77^{bc}
	Chronic	Male	27.40±0.74^{abc}	26.40±0.81^{ab}	29.20±0.77^{abc}	28.40±0.85^{abc}
		Female	28.00±0.74^{abc}	26.00±0.77^{a}	30.60±0.74^{c}	29.10±0.77^{abc}
TCO_2[4] (mmol/L)	Acute	Male	29.20±0.76^{abc}	29.90±0.92^{abc}	28.90±0.87^{abc}	32.30±0.79^{c}
		Female	30.40±0.79^{abc}	30.80±0.76^{abc}	28.80±0.83^{abc}	31.60±0.79^{bc}
	Chronic	Male	28.80±0.76^{abc}	27.70±0.83^{ab}	30.60±0.79^{abc}	29.90±0.87^{abc}
		Female	29.20±0.76^{abc}	27.20±0.79^{a}	31.90±0.76^{c}	30.50±0.79^{abc}
sO_2[4] (%)	Acute	Male	73.00±2.90^{abc}	71.00±3.55^{abc}	67.60±3.35^{abc}	60.70±3.03^{a}
		Female	75.70±3.03^{bc}	65.60±2.90^{ab}	66.20±3.18^{abc}	66.10±3.03^{abc}
	Chronic	Male	80.10±2.90^{c}	75.80±3.35^{abc}	66.80±3.03^{abc}	73.90±3.35^{abc}
		Female	77.00±2.90^{bc}	71.60±3.03^{abc}	71.20±3.03^{abc}	68.90±3.03^{abc}

[1] Blood gases were compared across treatment, length of exposure[2], sex, and their interactions.

[2] Acute, exposure to respective temperature for 4 hours; chronic, exposure to respective temperatures for 3 weeks.

[3] Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[4] PCO_2, carbon dioxide partial pressure; PO_2, oxygen partial pressure; HCO_3, bicarbonate; TCO_2, total carbon dioxide; sO_2, oxygen saturation.

[a-d] Superscripts indicate significant differences at $P \leq 0.05$.

Table 2.4. Blood electrolytes[1] of quail exposed to acute and chronic[2] temperatures.						
(mmol/L)	Length of exposure	Sex	TN[3]	TNS[3]	HS[3]	HSS[3]
		Male	144.00±0.76^{abc}	143.00±0.93^{abc}	143.00±0.88^{ab}	142.00±0.80^{a}
	Acute	Female	142.00±0.80^{a}	143.00±0.76^{ab}	143.00±0.83^{ab}	142.00±0.80^{a}
		Male	150.00±0.80^{f}	148.00±0.83^{def}	146.00±0.80^{abcde}	146.00±0.88^{bcdef}
Na	Chronic	Female	149.00±0.80^{ef}	147.00±0.80^{cdef}	145.00±0.80^{abcde}	145.00±0.80^{abcd}
		Male	5.23±0.18	5.35±0.22	5.26±0.21	5.29±0.19
	Acute	Female	5.32±0.19	5.09±0.18	5.17±0.20	5.32±0.19
		Male	4.71±0.18	4.77±0.20	5.49±0.19	5.57±0.21
K	Chronic	Female	4.70±0.18	4.77±0.19	4.81±0.18	4.81±0.19
		Male	1.52±0.02^{ab}	1.51±0.03^{ab}	1.49±0.03^{ab}	1.50±0.02^{ab}
	Acute	Female	1.53±0.02^{ab}	1.48±0.02^{ab}	1.48±0.02^{ab}	1.49±0.02^{ab}
		Male	1.46±0.02^{ab}	1.45±0.02^{ab}	1.45±0.02^{ab}	1.49±0.03^{ab}
Ionized Ca	Chronic	Female	1.53±0.02^{b}	1.48±0.02^{ab}	1.42±0.02^{a}	1.49±0.02^{ab}

[1] Blood electrolytes were compared across treatment, length of exposure[2], sex, and their interactions.

[2] Acute, exposure to respective temperature for 4 hours; chronic, exposure to respective temperatures for 3 weeks.

[3] Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[a-f] Superscripts indicate significant differences at $P \leq 0.05$.

CHAPTER 3

Fatty acid composition of multigenerational heat stressed adult Japanese quail (*Coturnix coturnix japonica)*

Abstract: As above average heat events are rising across the globe, it is imperative to understand the effects of heat stress on poultry species raised outdoors, especially for lipids, a class of nutrients that are integral to their health. Fatty acids are a major component of lipids and have been shown to be significantly affected by heat stress in poultry. Therefore, the effect of heat stress on the fatty acid composition of various organs was investigated in Japanese quail as a model. Treatments included quail that were (1) random-bred in a thermoneutral temperature (22.2˚C, TN), (2) random-bred in heat stress (31.1˚C, HS), (3) selected for low FCR in heat stress and not exposed to heat stress (22.2˚C, TNS), and (4) selected for low FCR in heat stress and exposed to heat stress (31.1˚C, HSS). It was hypothesized that HS and HSS would have less PUFA in all organs due to degradation. It was also hypothesized that n-6:n-3 PUFA would be high in the adult tissues due to high concentrations of corn in their diet. Data were analyzed using treatment, sex, and their interaction as the main effects. Significance was determined at $P \leq 0.05$. Results showed that of all organs analyzed, livers experienced the most variations in concentrations of fatty acids when compared by treatment and sex. Males had significantly more stearic acid in the brain, kidney, and thigh and significantly more PA in all tissues than females. TN brains had significantly less PUFA than that in both TNS and HSS. TN or TNS had significantly more long chain PUFA such as DPA n-6, DPA n-3, DHA, and ARA and significantly less SFA such as PA or stearic acid than that in HS or HSS across all tissues. Thus, it can be concluded that selection for low FCR in heat stress could possibly reduce oxidation of PUFA or increases retention of PUFA in the brain. However, heat stressed quail, regardless of selective breeding, had significantly less health-promoting fatty acids such as DHA and DPA. Future studies should include temperatures from 30 to 35 ˚C or above to determine how fatty acid composition of quail and other commercial poultry are affected when subjected to selective breeding to withstand increasing temperatures during climate change.

Also of interest is the relationship between the liver and skeletal muscle fatty acids and determination of differences for fatty acid partitioning within various avian tissues under heat stress. More knowledge on how heat stress affects cognitive ability through the oxidation of fatty acids is equally as important.

1. Introduction

As temperatures increase due to climate change, livestock are expected to experience heat stress more frequently (Sejian, et al., 2015). In heat stressed animals, it is energetically more efficient to deposit fat rather than protein (Renaudeau, et al., 2012). Lipids are a class of nutrients that are particularly susceptible to heat stress induced oxidation (Noble and Cocchi, 1990). During temperature stress, even cold stressed rats have been reported to have oxidative damage to the brain, heart, kidney, and liver, ultimately causing metabolic disruptions in both females and their offspring (Ren, et al., 2018; Surai, et al., 2016). Heat stress in poultry species has been investigated and caused reduction in performance such as lower body weight and higher feed conversion ratio (Renaudeau, et al., 2012; Slimen, et al., 2016; Kumari and Nath, 2018). High fat retention in heat stressed poultry could be due to decreased peripheral lipolysis and decreased lipogenesis. n-3 Fatty acids affect the expression of hepatic fatty acid synthase and can cause a decrease in triacylglyceride and adipose fat accumulation (Taouis, et al., 2001). Therefore, amounts of various fatty acids present in the liver are important for overall poultry health. Possibly, degradation of fatty acids in poultry could impact human health due to dietary essential fatty acids such as various n-6 and n-3 polyunsaturated fatty acids (PUFA), often provided by poultry such as quail meat and eggs in various parts of the world (Alagawany, et al., 2019). The ideal human n-6:n-3 should be 1:1; however, current food consumption norms in economically developing countries where quail are consumed have a 20:1 n-6:n-3 (Alagawany, et al., 2019).

Several researchers have studied nutritional strategies to combat heat stress in poultry. These strategies include maintaining water balance, low fiber, low crude protein, higher fat, and higher amino acids in diets to partially decrease diet heat increment (Renaudeau, et al., 2012). The composition of dietary fat can influence the bird's response to it during heat stress (Renaudeau, et al., 2012). For example, n-3 PUFA were less resistant to oxidative damage than n-6 PUFA (Laviola and Macrì, 2013). In chicken, α-linolenic acid (ALA; C18:3 n-3) and linoleic acid (LA; C18:2 n-6) are dietary essential fatty acids because chickens lack the desaturase to insert a double bond beyond the -9 carbon (Cherian, 2015). However, some researchers discovered that LA is not an essential fatty acid if ALA is present in high enough quantity (Murai, et al., 1995). With the current imbalance of n-6 to n-3 in current poultry diets, it was important to know if heat stress further changed their ratio. Of particular interest were arachidonic acid (ARA; C20:4 n-6) and docosahexaenoic acid (DHA; C22:6 n-3) due to their essentiality in many functions of the birds' body, particularly the brain and kidneys. Their essential functions include membrane structure, proper neural development, and visual acuity (Cherian, 2011; Cherian, 2015).

While the effect of different poultry diets on brain fatty acid composition have been studied, few have studied the effect of heat stress and sex on brain or kidney fatty acid composition, especially in Japanese quail (Cherian, 2015; Tavaniello, et al., 2017). The brain is composed of 8.5% lipid and approximately 23.5% of the lipids are PUFA (Durairaj, 1971). Therefore, it is expected that the brain will have high levels of oxidation during heat stress.

Avian kidneys are also highly susceptible to oxidative damage during temperature stress because they are one of the major organs for acid-base homeostasis (Ren, et al., 2018; Farag and Alagawany, 2018). Approximately 50 to 70% of the energy use in mammalian kidneys is dedicated to the sodium pump (Szabó, et al., 2010). Also, it was shown that lipid composition, particularly

DHA, in the kidney membrane can influence the activity of the sodium pump (Szabó, et al., 2010). Due to the kidneys' vital role for maintaining the acid-base balance during times of stress, understanding its fatty acid composition during heat stress in poultry is important (Sejian, et al., 2015).

As proposed in Chapters 1 and 2, quail are an excellent model for studying heat stress in commercial poultry. It is expected that there will be less PUFA in samples of various organs obtained from heat stressed quail due to degradation. PUFA levels may also decrease due to utilization in stress responses such as an increase in pro-inflammatory eicosanoids, which are derivatives of ARA. Despite the increased use of PUFA, it is expected that there will be adequate levels of ARA, DHA, and eicosapentaenoic acid (EPA, C20:5 n-3) in the liver and subsequently the brain. The levels of these long chain PUFA are expected to be maintained through desaturation of LA and ALA, respectively. n-6:n-3 PUFA is also expected to be high in the adult tissues due to high concentrations of corn in their diet.

2. Materials and Methods

2.1 Experimental design (Chapter 2, Figure 1).

The detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. The four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

2.2 Sample collection for birds and collection/analysis of feed

Animal care and use was approved by the Institutional Animal Care and Use Committee at the University of California Davis (Protocols #19473 and 21370; Davis, CA). Adult birds (16.86 weeks-old) were euthanized using cervical dislocation and harvested for the brain, liver, kidneys, and thighs. Tissues were placed into Whirlpak bags and immediately submerged into liquid nitrogen after collection. Samples were then transferred to a -80˚C freezer until further analyses.

After purchase of Purina® Gamebird Maintenance Feed (Purina Animal Nutrition, LLC, Arden Hills, MN 55126) 4 samples of feed were either analyzed immediately or stored (8 samples) at -20˚C. Before analysis, stored feed (~200 g for each group of 4 samples) was placed into feeders and subjected to either 22.2˚C or 31.1˚C for 48 hours to approximate the amount of time remaining in the feeders before consumption by birds in chambers.

2.3 Lipid extraction

For adult brain, liver, and kidneys, 8 samples per treatment per sex were analyzed for fatty acid composition. For thighs, sample size varied (HS males = 8, HS females = 7, HSS males = 8, HSS females = 8, TN males = 7, TN females = 5, TNS males = 6, TNS females = 5).

Lipids were extracted using a modified Folch method (Folch et al., 1957; Zhang et al., 2017). For lipid extraction, samples were weighed according to their respective total % lipid to obtain 2 mg of total lipids. Trinonadecanoin (C19:0 triacylglycerol, Nu Chek Inc, Elysian, MN; Cat# T-165) was added as an internal standard for thigh and liver samples and 1,2-dinonadecanoyl-sn-glycero-3-phosphocholine (C19:0 phosphatidylcholine, Avanti Polar Lipids, Alabaster, Alabama; Cat #850367P) was added as an internal standard for brain and kidney samples (Tokuşoğlu, 2006; Gecgel et al., 2015). Samples were then homogenized in 1 mL of methanol (for HPLC, ≥99.9%, Sigma-Aldrich, St. Louis, MO, USA; Cat #34860; BioSpec, Tissue Tearor 398).

The total contents of the homogenate were transferred to an 8 mL glass screw top test tube and 2 mL of chloroform and 0.75 mL of deionized water were added to each test tube. The mixture was vortexed and centrifuged at 458 × g for 10 minutes (Allegra 6 centrifuge with GH–3.8A rotor, Beckman Coulter, 128 Palo Alto CA). The bottom chloroform layer was transferred to a second test tube. Another 2 mL of chloroform were added to the first tube, vortexed, centrifuged, and the chloroform layer was again transferred to the previously mentioned second test tube. The chloroform containing the total extracted lipid was evaporated under N_2 and reconstituted in 400 µl of toluene (ACS reagent, ≥99.5%, Sigma-Aldrich, St. Louis, MO, USA; Cat #179418).

2.4 Fatty acid composition

For fatty acid transesterification, 3 mL of methanol were added to the sample containing extracted lipids and toluene, followed by the addition of 600 µl of 8% HCl (ACS reagent, 37%, Sigma-Aldrich, St. Louis, MO, USA; Cat #320331) in methanol. The sample was vortexed and heated at 90°C on a dry heating block for 60 minutes. Samples were cooled to room temperature (~23°C); 1 mL each of hexane (for HPLC, ≥97.0%, Sigma-Aldrich, St. Louis, MO, USA; Cat #34859) and deionized water were added, then vortexed. Phase separation was allowed to occur for approximately 15 minutes and 900 µL of the top hexane layer were transferred to micro-centrifuge tubes containing 450 µl of deionized water. Micro-centrifuge tubes were centrifuged at 16,627 × g for 2 minutes at 4°C and the hexane layer was transferred to another set of tubes; solvents were evaporated under N_2 and the remaining content reconstituted with 100 µL hexane.

Reconstituted samples were transferred to amber gas chromatography vials containing inserts. Fatty acid methyl esters were analyzed on a Perkin-Elmer Clarus 500 gas chromatography system coupled to a flame ionization detector (PerkinElmer, Inc., Shelton, Connecticut, US). A DB-FFAP nitroterephthalic-acid-modified polyethylene glycol (PEG) capillary column (FFAP; 30

m × 0.25 mm inner diameter, 0.25 µm film thickness; Agilent Technologies, Santa Clara, California, US) was used. The injector and detector temperatures were 240°C and 300°C, respectively, and the initial oven temperature was 80°C. After 2 minutes, the oven temperature increased by 10°C/minute to 185°C at the time of sample injection. The temperature increased by 5°C/minute to 240°C and was held for 13 minutes for a total run time of 36.5 min. Helium was the carrier gas at a flow rate of 1.3 mL/minute. The injection volume was 1 µL and the split ratio was set to 10:1. TotalChrom software (version 6.3.2.0646; PerkinElmer, Inc., Shelton, Connecticut, US) software was used for data collection and peak area integration. Peaks were identified based on their retention times, determined through injection of a standard mixture containing 29 fatty acid methyl esters. Concentration of fatty acids were calculated using Equation 1 below.

Equation 1. $$\frac{\left(\frac{Amount\ of\ triheptadecanoic\ acid\ (C17{:}0)(mg)}{Area\ of\ triheptadecanoic\ acid\ (C17{:}0)}\right)\times Area\ of\ FA\ peak}{Weight\ of\ sample\ (g)} = Concentration\ of\ FA\ \left(\frac{mg}{g}\right)$$

The concentrations were then added together and individually divided by the total to obtain % of total FA as the final unit for statistical comparison.

2.5 Statistical analysis

Analyses of data were performed in R 4.0.0 (R Core Team, 2020; RStudio Team, 2022) to determine significance ($P \leq 0.05$). The main effects for the adult tissues were treatment, sex, and their interactions. Differences among treatments were determined using an ANOVA for the main effects and their interactions. Analysis of the feed was reported as an average with standard deviation. Appropriate post-hoc analysis was used if the interaction was significant at $P \leq 0.05$. If an effect had significant differences, pairwise contrasts were made with confidence levels of 0.95 using Tukey's method for comparing estimates.

3. Results

3.1 Feed (Table 1)

ANOVA results showed that there were no temperature effects on any of the fatty acids except stearic acid (C18:0; p=0.030). However, when post hoc analysis was performed, there were significant differences for EPA. Feed stored at -20˚C had 3.54% less stearic acid (p=0.036) and 0.035% less EPA (p=0.049) than feed stored at 31.1˚C for 48 hours.

Temperature had a significant effect on total SFA (p=0.024). Feed stored at -20˚C had 3.54% less total SFA than feed in 31.1˚C for 48 hours (p=0.022). There was also a significant effect on total MUFA (p=0.0056). Feed stored at -20˚C had significantly higher levels of total MUFA than that in 22.2˚C for 48 hours (p=0.0098) and in 31.1˚C for 48 hours (p=0.018). There were no significant temperature effects on total PUFA, n-6 PUFA, n-3 PUFA, SFA:PUFA, and n-6:n-3.

3.2 Adult brains

3.2.1 Sex by treatment effects (Table 2)

TN versus TNS. TN females had significantly more oleic acid, elaidic acid, arachidic acid, EDA, total MUFA, and SFA:PUFA ($P \leq 0.005$), but significantly less myristoleic acid, PA, stearic acid, ARA, DPA n-6, DHA, and total PUFA than TN males ($P \leq 0.05$). TN males had significantly more myristoleic and margaric acid than TNS males ($P \leq 0.0006$). TN males also had significantly more myristoleic acid, PA, margaric acid, stearic acid, DHA, and total n-3 than TNS females ($P \leq 0.05$). TN females had significantly less total PUFA and SFA:PUFA ($P < 0.0001$), but significantly more oleic acid, elaidic acid, arachidic acid, EDA, and total MUFA than TNS females ($P \leq 0.007$). TN females had significantly less stearic acid, ARA, total PUFA, and total SFA

(P≤0.05), but significantly more margaric acid, oleic acid, elaidic acid, arachidic acid, EDA, total MUFA, and SFA:PUFA than TNS males (P≤0.007).

TN versus HS. TN males had significantly more myristoleic and margaric acid than HS males (P≤0.003) and significantly more myristic acid, myristoleic acid, PA, stearic acid, DHA, and total n-3 than HS females (P≤0.005). TN females had significantly less erucic acid, and total PUFA (P≤0.05), but significantly more palmitoleic, oleic acid, and SFA:PUFA than HS females (P≤0.02). TN females also had significantly less pentadecylic acid, stearic acid, ARA, total PUFA, and total SFA (P≤0.05) and significantly more myristoleic acid, palmitoleic acid, margaric acid, oleic acid, elaidic acid, arachidic acid, EDA, total MUFA, and SFA:PUFA than HS males (P≤0.05).

TNS versus HS. HS females had significantly more erucic acid (p=0.0016) and significantly less DHA than TNS females (p=0.038). TNS males had significantly less margaric and elaidic acid (P≤0.02), but significantly more stearic acid, DHA, and total n-3 than HS females (P≤0.0008).

TN versus HSS. TN males had significantly more myristoleic and margaric acid than HSS males (P≤0.003). TN males also had significantly more myristoleic acid, PA, margaric acid, stearic acid, DHA, and total n-3 than HSS females (P≤0.03). TN females had significantly less pentadecylic acid, DPA n-6, total MUFA, and total PUFA (P≤0.05), but significantly more myristoleic acid, palmitoleic acid, oleic acid, elaidic acid, arachidic acid, EDA, and SFA:PUFA than HSS females (P≤0.05). TN females had significantly less pentadecylic acid, stearic acid, DPA n-6, total PUFA, and total SFA (P≤0.03), but significantly more palmitoleic acid, oleic acid, elaidic acid, arachidic acid, EDA, total MUFA, and SFA:PUFA than HSS males (P≤0.01). HSS males had significantly more pentadecylic acid than TNS females (P≤0.05). Both HSS and HS males had significantly more stearic acid, DHA, and total n-3 than HS females (P≤0.05 and ≤0.008,

respectively). However, HSS and HS males had significantly less elaidic acid and EDA than HS females (P≤0.04 and ≤0.008, respectively).

3.2.2 Sex effects (Table 3)

Males had significantly less oleic acid, elaidic acid, LA, arachidic acid, EDA, DPA n-3, EPA, total MUFA, and SFA:PUFA than females (P≤0.03). However, males had significantly more pentadecylic acid, PA, stearic acid, ARA, DHA, total n-3, total PUFA, and total SFA than females (P≤0.04).

3.2.3 Treatments effects (Table 3)

TN had significantly less total PUFA (p=0.0062) and significantly more myristoleic, margaric, and elaidic acid than TNS (P≤0.003). TN had significantly more myristic acid, myristoleic acid, palmitoleic acid, and DHA (P≤0.01) and significantly less pentadecylic acid than HS (p=0.0024). TN had significantly less pentadecylic acid and total PUFA (P≤0.03) and significantly more myristoleic acid, palmitoleic acid, margaric acid, elaidic acid, and MUFA than HSS (P≤0.02). TNS had significantly less pentadecylic acid than HSS (p=0.023). HSS had significantly more myristic acid than HS (p=0.029).

3.3 Adult kidneys

There were no significant differences in myristoleic acid, pentadecylic acid, elaidic acid, ARA, 13,16 docosadienonic acid (C22:2), and DHA when compared by treatment, sex, and treatment by sex effects.

3.3.1 Sex by treatment effects (Table 4)

TN versus TN. TN Females had significantly more margaric acid, EDA, and DPA n-6 (P≤0.02) and significantly less stearic acid than TN males (p=0.0062).

TN versus TNS. TN males had significantly more stearic acid and EPA ($P \leq 0.002$) and significantly less margaric acid than TNS males ($p=0.032$). TN males had significantly more palmitoleic acid, stearic acid, and EPA ($P \leq 0.0004$) and significantly less margaric acid, LA, gondoic acid, EDA, DPA n-6, total SFA, total n-6, SFA:PUFA, and n-6:n-3 than TNS females ($P \leq 0.008$). TN females had significantly more DPA n-6 than TNS males ($P < 0.0001$).

TN versus HS. TN males had significantly less n-6:n-3 ($p=0.0013$) and significantly more EPA than HS males ($p=0.012$). TN males had significantly less margaric acid, EDA, DPA n-6, and n-6:n-3 ($P \leq 0.03$) and significantly more PA, palmitoleic acid, EPA, and SFA:PUFA than HS females ($P \leq 0.03$). TN females had significantly more DPA n-6 than HS males ($P < 0.0001$). HS males had significantly less margaric acid, EDA, 11,14,17-eicosatrienoic acid, and DPA n-6 ($P \leq 0.007$) and significantly more palmitoleic acid and EPA than HS females ($P \leq 0.05$).

TN versus HSS. TN males had significantly more EPA ($p=0.0011$) and significantly less margaric acid than HSS males ($p=0.023$). TN males had significantly more palmitoleic acid, stearic acid, EPA, and SFA:PUFA ($P \leq 0.005$) and significantly less margaric acid, gondoic acid, EDA, DPA n-6, and n-6:n-3 than HSS females ($P \leq 0.05$). TN females had significantly more 11,14,17-eicosatrienoic acid, DPA n-6, and SFA:PUFA and significantly less total SFA than HSS males ($P \leq 0.05$). TNS males had significantly less stearic acid and total SFA than HSS males ($P \leq 0.04$).

TNS versus HS. TNS males had significantly less EDA and DPA n-6 than TNS females ($P \leq 0.03$). As well, TNS males had significantly less margaric acid, EDA, DGLA, 11,14,17-eicosatrienoic acid, DPA n-6, and n-6:n-3 than HS females ($P \leq 0.05$). However, TNS males had significantly more palmitoleic acid than HS females ($p=0.024$). TNS females had significantly more LA, 11,14,17-eicosatrienoic acid, and DPA n-6 ($P \leq 0.05$) and significantly less palmitoleic and stearic acid than HS males ($P \leq 0.05$).

TNS versus HSS. TNS males had significantly less stearic acid and total SFA than HSS males (P≤0.04). As well, TNS males had significantly less EDA, 11,14,17-eicosatrienoic acid, and DPA n-6 than HSS females (P≤0.03). TNS females had significantly more LA, arachidic acid, EDA, 11,14,17-eicosatrienoic acid, and DPA n-6 (P≤0.04) and significantly less PA, palmitoleic acid, stearic acid, total SFA, and SFA:PUFA than HSS males (P≤0.02). TNS males had significantly less EDA, 11,14,17-eicosatrienoic acid, and DPA n-6 than HSS females (P≤0.03). TNS females had significantly more LA, arachidic acid, EDA, 11,14,17-eicosatrienoic acid, and DPA n-6 (P≤0.04) and significantly less PA, palmitoleic acid, stearic acid, total SFA, and SFA:PUFA than HSS males (P≤0.02).

HS versus HSS. HS females had significantly more margaric acid, EDA, 11,14,17-eicosatrienoic acid, and DPA n-6 (P≤0.04) and significantly less PA, palmitoleic acid, and SFA:PUFA than HSS males (P≤0.04). HS males had significantly less EDA, 11,14,17-eicosatrienoic acid, and DPA n-6 and significantly more SFA:PUFA than HSS females (P≤0.04).

HSS versus HSS. HSS males had significantly less EDA, 11,14,17-eicosatrienoic acid, and DPA n-6 and significantly more total SFA and SFA:PUFA than HSS females (P≤0.03).

3.3.2 Sex effects (Table 5)

Males had significantly less margaric acid, LA, ALA, EDA, DGLA, 11,14,17-eicosatrienoic acid, DPA n-6, total n-6, and n-6:n-3 (P≤0.004) and significantly more myristic acid, PA, palmitoleic acid, stearic acid, EPA, DPA n-3, total SFA, total MUFA, and SFA:PUFA than females (P≤0.03).

3.3.3 Treatment effects (Table 5)

TN had significantly more stearic acid and EPA(P≤0.007) and significantly less behenic and gondoic acid than TNS (P≤0.03). TN had significantly more EPA and total n-3 (P≤0.05) and

significantly less n-6:n-3 than HS (p=0.0002). TN had significantly less gondoic acid (p=0.03) and significantly more EPA than HSS (p=0.0043). TNS had significantly less stearic acid (p=0.0015) and significantly more arachidic acid, behenic acid, and total n-3 than HS (P≤0.04). TNS had significantly less stearic acid and total SFA (P≤0.02) and significantly more arachidic acid than HSS (p=0.038).

3.4 Adult livers

3.4.1 Sex by treatment effects (Table 6)

There were no significant treatment, sex, or treatment by sex effects on arachidic acid and behenic acid. ANOVA showed a significant treatment by sex on gamma-linolenic acid; however, there was no significant difference when post hoc analysis was performed.

TN versus TN. When TN males were compared to TN females, males had significantly more myristic acid, myristoleic acid, gondoic acid, EDA, DGLA, and EPA than females (P≤0.05).

TN versus TNS. TN males had significantly more myristic acid, myristoleic acid, oleic acid, and total MUFA (P≤0.02), but significantly less ALA, DHA, and total n-3 than TNS males (P≤0.003). TN males had significantly more myristic acid, myristoleic acid, gondoic acid, EDA, DGLA, EPA, 13,16-docosadienoic acid, and total MUFA than TNS females (P≤0.01). TN males also had significantly less pentadecylic acid, stearic acid, total SFA, and SFA:PUFA than TNS females. TN females had significantly less ALA, gondoic acid, EDA, DGLA, ARA, EPA, and DHA than TNS males (P≤0.04). TN females also had significantly less stearic acid, total SFA, and SFA:PUFA (P≤0.002), but significantly more total MUFA than TNS females (p=0.0027).

TN versus HS. TN males had significantly more EDA, ARA, and total n-3 and significantly less PA than HS males (P≤0.05). TN males had significantly more gondoic acid, EDA, DGLA, DHA, and total n-3 than HS females (P≤0.04). TN females had significantly more margaric acid

and DPA n-6 (P≤0.02) and significantly less myristic acid, myristoleic acid, PA, palmitoleic acid, and erucic acid than HS males (P≤0.05).

TN versus HSS. TN males had significantly less myristic acid and significantly more EDA than HSS males (P≤0.05). TN males had significantly more myristoleic acid, gondoic acid, EDA, DGLA, EPA, and total n-3 than HSS females (P≤0.03). TN females had significantly less myristic acid, myristoleic acid, PA, and DGLA than HSS males (P≤0.04).

TNS versus TNS. TNS females had significantly more stearic acid, total SFA, and SFA:PUFA (P≤0.002), but significantly less ALA, gondoic acid, EDA, DGLA, ARA, EPA, DPA n-3, DHA, total PUFA, total n-6, and total n-3 than TNS males (P≤0.03).

TNS versus HS. TNS males had significantly more LA, ALA, gondoic acid, ARA, EPA, DPA n-3, DHA, total PUFA, total n-6, and total n-3 (P≤0.05), but significantly less myristic acid, myristoleic acid, PA, palmitoleic acid, oleic acid, and total MUFA than HS males (P≤0.0008). TNS males had significantly less oleic acid (P<0.0001), but significantly more LA, ALA, gondoic acid, EDA, DGLA, ARA, EPA, DPA n-3, DHA, total PUFA, total n-6, and total n-3 than HS females (P≤0.04). TNS females had significantly less oleic acid, 13,16-docosadienoic acid, total MUFA, and n-6:n-3 (P≤0.02), but significantly more stearic acid than HS females (P≤0.03). TNS females had significantly more pentadecylic and stearic acid (P≤0.0002), but significantly less myristic acid, myristoleic acid, PA, palmitoleic acid, oleic acid, DGLA, and erucic acid than HS males (P≤0.05).

TNS versus HSS. TNS males had significantly more ALA, ARA, DPA n-3, DHA, total PUFA, and total n-3 (P≤0.03), but significantly less myristic acid, myristoleic acid, PA, palmitoleic acid, oleic acid, and total MUFA than HSS males (P≤0.002). TNS males also had significantly more LA, ALA, gondoic acid, EDA, DGLA, ARA, EPA, DPA n-3, DHA, total PUFA, total n-6,

and total n-3 (P≤0.02), but significantly less oleic acid and total MUFA than HSS females (P≤0.0001). TNS females had significantly less oleic acid, total MUFA, and n-6:n-3 (P≤0.03), but significantly more stearic acid and total SFA than HSS females (P≤0.04). TNS females had significantly more pentadecylic acid, stearic acid, and total SFA (P≤0.03), but significantly less myristic acid, myristoleic acid, palmitoleic acid, oleic acid, DGLA, 13,16-docosadienoic acid, and total MUFA than HSS males (P≤0.02).

HS versus HS. HS females had significantly more margaric acid and DPA n-6 (P≤0.05), but significantly less myristic acid, myristoleic acid, PA, and erucic acid than HS males (P≤0.04).

HS versus HSS. HS males had significantly more myristic acid, myristoleic acid, PA, and palmitoleic acid (P≤0.02), but significantly less pentadecylic and margaric acid than HSS females (P≤0.04). HS females had significantly less myristic acid, myristoleic acid, PA, and DGLA than HSS males (P≤0.03).

HSS versus HSS. HSS males had significantly more myrstic acid, myristoleic acid, and DGLA than HSS females (P≤0.02).

3.4.2 Sex effect (Table 7)

Males had significantly less pentadecylic acid, margaric acid, stearic acid, DPA n-6, and SFA:PUFA (P≤0.05), but significantly more myristic acid, myristoleic acid, PA, palmitoleic acid, ALA, arachidic acid, gondoic acid, EDA, DGLA, EPA, erucic acid, DHA, total PUFA, total n-6, and total n-3 than females (P≤0.04).

3.4.3 Treatment effect (Table 7)

TN versus TNS. TN had significantly less pentadecylic acid, stearic acid, ALA, 13,16-docosadienoic acid, and total SFA (P≤0.04), but significantly more myristic acid, palmitoleic acid, oleic acid, and total MUFA than TNS (P≤0.02).

TN versus HS. TN had significantly less oleic acid and total MUFA (P≤0.04), but significantly more ARA than HS (P≤0.05).

TN versus HSS. TN had significantly less oleic acid and total MUFA (P≤0.04).

TNS versus HS. TNS had significantly more pentadecylic acid, LA, ALA, ARA, 13,16-docosadienoic acid, DPA n-3, DHA, and total n-3 (P≤0.04), but significantly less myristic acid, myristoleic acid, PA, palmitoleic acid, oleic acid, total MUFA, and n-6:n-3 than HS (P≤0.04).

TNS versus HSS. TNS had significantly more stearic acid, LA, ALA, ARA, 13,16-docosadienoic acid, DHA, and total n-3 (P≤0.05), but significantly less myristic acid, myristoleic acid, palmitoleic acid, oleic acid, erucic acid, and total MUFA than HSS (P≤0.03).

3.5 Adult thighs

3.5.1 Sex by treatment effects (Table 8)

There was no significant difference in effects on myristoleic acid, elaidic acid, arachidic acid, EDA, DGLA, ARA, erucic acid, 13,16 docosadienonic acid, DHA, total PUFA, total n-3, and n-6:n-3. The ANOVA showed significant treatment by sex differences for oleic acid (p=0.034), behenic acid (p=0.028), and MUFA (p=0.048); however, there were no significant differences when post hoc analysis was performed. The ANOVA showed no significant treatment by sex effect on total n-6; however, there were significant effects when post hoc analysis was performed.

TN versus TN. TN males had significantly less LA, ALA, and SFA:PUFA (P≤0.008), but had significantly more PA and total SFA then TN females (P≤0.05).

TN versus TNS. TN females had significantly more ALA (P<0.003). TN males had significantly less LA, ALA, and total n-6 and significantly more SFA:PUFA than TNS females (P≤0.04).

TN versus HS. TN males had significantly less myristic acid than HS males (p=0.0022). TN males also had significantly less margaric acid, LA, ALA, gondoic acid, and total n-6 than HS females (P≤0.04); however, TN males had significantly more total SFA and SFA:PUFA than HS females (p=0.02). TN females had significantly more LA and ALA (P≤0.008), but significantly less myristic acid, PA, stearic acid, DPA n-3, total SFA, and SFA:PUFA than HS males (P≤0.04).

TN versus HSS. TN males had significantly less myristic acid than HSS males (p=0.0006). TN males also had significantly less margaric acid, LA, ALA, and DPA n-6 and significantly more SFA:PUFA than HSS females (P≤0.02). TN females had significantly more LA and ALA (P≤0.05), but significantly less myristic acid, PA, total SFA, and SFA:PUFA than HSS males (P≤0.002). TN females had significantly more palmitoleic acid than HSS females (p=0.036).

TNS versus TNS. TNS females had significantly more LA and ALA (P≤0.002).

TNS versus HS. TNS males had significantly less LA and ALA and significantly more SFA:PUFA than HS females (P≤0.04). TNS females had significantly more LA, ALA, and total n-6 (P≤0.05), but significantly less myristic acid, PA, total SFA, and SFA:PUFA than HS males (P≤0.04).

TNS versus HSS. TNS females had significantly more LA, ALA, and total n-6 (P≤0.02), but significantly less myristic acid, PA, total SFA, and SFA:PUFA than HSS males (P≤0.009). TNS females also had significantly more ALA than HSS females (p=0.037).

HS versus HS. HS males had significantly less LA, ALA, and total n-6 (P≤0.03), but significantly more myristic acid, PA, stearic acid, DPA n-3, total SFA, and SFA:PUFA than HS females (P≤0.03).

HS versus HSS. HS females had significantly more margaric acid, LA, ALA, gondoic acid, and total n-6 (P≤0.04), but significantly less myristic acid, PA, total SFA, and SFA:PUFA than

HSS males (P≤0.002). HS males had significantly more myristic acid, stearic acid, total SFA, and SFA:PUFA (P≤0.03), but significantly less LA than HSS females (P≤0.006).

HSS versus HSS. HSS males had significantly more myristic acid, total SFA, and SFA:PUFA (P≤0.03), but significantly less margaric acid, DPA n-6, and total n-6 than HSS females (P≤0.05).

3.5.2 Sex effect (Table 9)

Males had significantly less margaric acid, LA, ALA, gondoic acid, DPA n-6, total PUFA, and total n-6 (P≤0.002), but significantly more myristic acid, PA, palmitoleic acid, stearic acid, DPA n-3, total SFA, and SFA:PUFA than females (P≤0.05).

3.5.3 Treatment effect (Table 9)

TN had significantly less myristic and myristoleic acid than HS (P≤0.05), significantly less myristic acid than HSS (P≤0.04), and significantly more palmitoleic acid than HSS (p=0.011).

4. Discussion

4.1 Feed

Purina® Gamebird Maintenance Feed (Purina Animal Nutrition, LLC, Arden Hills, MN 55126) had a high level of stearic acid that contributed 10.5% to 14.0% of the total fatty acids in the feed. These values were higher than those in quail feed containing soybean oil (Szabó, et al., 2010; Donaldson, et al., 2016). The high level of stearic acid was closer to values when lard or animal fat is used in feed formulation (Donaldson, et al., 2016). Although not listed as lard or animal fat, the porcine meat and bone meal, listed as the 3rd ingredient on the feed label, was most likely the origin of this high level of stearic acid.

PA is synthesized by the animal and is greatly affected by the diet, whereas LA must be provided entirely in the diet (Wood, et al., 2008). The diet used in this study did not have as much

PA as reported by others (Szabó, et al., 2010; Donaldson, et al., 2016). LA in the feed was similar to that in reports for breeder hen rations containing approximately 50% LA (Cherian, 2015; Table 9). In the current study, the 50% LA was due to the corn/soy based diet fed to quail. The feed also contained 11.3% to 12% ALA which was higher than that reported in other quail diets (Szabó, et al., 2010). A high level of ALA was also found when analyzing blood samples from 12-week-old Japanese quail fed soybean oil in their diet (Güçlü, et al., 2008).

DHA and ARA were not significantly affected by temperature which suggested that eggs hatched from both heat stress and thermo-neutral treatments likely had equal levels of these fatty acids. This is important because DHA and ARA levels in the egg are dependent on the diet (Cherian, 2005). There were no significant differences in total PUFA among temperatures which could be due to antioxidants, such as vitamin E, zinc oxide, and sodium selenite that were feed additives. However, the antioxidant protection of PUFA does not explain the significant temperature effects on total SFA and total MUFA because feed stored at -20˚C had less total SFA, but more total MUFA.

4.2 Adult Brain

The amount of PA in the current study was lower than that reported (Szabó, et al. 2010). However, the quantities of all other fatty acids agreed with that reported by Szabó, et al. (2010). Tallima and El Ridi (2018) reported that DHA and ARA were 20% of the human brain dry weight, comparable to that in the current study. Additionally, DHA is 50-70% of the fatty acids in brain synaptosome membranes which contributes to membrane fluidity, signal transduction, protein signaling, and gene expression (Cherian, 2005). Therefore, high abundance of DHA in the brain was to be expected.

Also as expected, TN had higher levels of ARA, DHA, and DPA than HS or HSS; but, TN was not expected to have higher levels of elaidic acid, margaric acid, oleic acid, PA, stearic acid, total SFA, and SFA:PUFA than HS or HSS. Because the brain does not have desaturase activity, all the DHA present in the brain was transported via plasma from either the diet or after synthesis in the liver (Catala, 2012). This reasoning may factor into the significantly higher level of DHA in the TN brain compared to that in all other treatments due to less oxidation of DHA from the liver. Long chain PUFA are particularly susceptible to oxidation when an animal is experiencing heat stress (Noble and Cocchi, 1990). The results from the present study showed that TN brains had lower total PUFA than TNS and HSS, which was opposite expectations. The higher level of total PUFA in TNS and HSS could indicate that quail in these treatments adapted to heat stress by increasing antioxidant response to protect compounds, including lipids, from oxidation.

When DHA is not in adequate quantities, DPA takes its place in the brain (Catala, 2012). Thus, males in the current study may have experienced more lipid oxidation resulting in significantly higher level of both SFA and DPA and lower levels of DHA. In contrast, the lower SFA:PUFA in female brains than in male brains could be due to the high susceptibility of the brain to lipid oxidation (Yigit, et al., 2014). The liver can preferentially transport DHA and other PUFA to the yolk rather than to the female brain leading to less lipid oxidation in females (Speake, et al., 1998). Contrary to the findings in this current study, others found that Japanese quail subjected to 42˚C did not have significant lipid changes in the brain (Durairaj, 1971).

4.3 Adult Kidneys

Other investigators have found that chronically heat stressed male broilers had decreased activity of antioxidants in the kidney, suggesting that heat stress caused oxidation in the kidneys (Sejian, et al., 2015; Akbarian, et al., 2016; Saeed, et al., 2019). The decrease in antioxidant activity

in male kidneys could be the reason for males having significantly higher levels of PA, palmitoleic acid, and stearic acid than females. The higher levels of these fatty acids could contribute to males having more total SFA, MUFA, and SFA:PUFA than females.

The reason for females having more LA, ALA, total PUFA, total n-6 PUFA, and n-6:n-3 could be due to sexually mature females maintaining mineral balances for egg laying. Studies in rats have shown that n-3 PUFA can prevent changes in calcium to maintain bone health (Ahmed and Abd EL Samad, 2013). Additionally, the diet in the present study contained soybean meal; others who fed soybean oil to Japanese quail found a higher retention of n-6 fatty acids in bone lipids (Liu, et al., 2003). The Japanese quail fed high n-6 diets had impaired bone formation rates compared to those fed high n-3 diets (Liu, et al., 2003). Therefore, the lower amount of renal n-3 in proportion to n-6 could indicate less retention of calcium due to laying. However, there were no significant ionized calcium differences between males and females (Chapter 2, Table 4).

There was no treatment or sex effect in DHA or ARA which are the long chain PUFA that are important for membrane fluidity to ensure proper sodium pump function (Szabó, et al., 2010). Because kidneys play an important role in regulating homeostasis, changes in their fatty acid profile could indicate stress in the animal (Alabdallah Ahmad, et al., 2021). Thus, in the present work, there were higher levels of stearic acid and total SFA in HSS than TNS. Quail that were chosen to have low FCR in heat stress and then exposed to heat stress likely had more renal stress than those that were chosen to have low FCR in heat stress but not later exposed to heat stress. The renal stress likely influenced the higher n-6:n-3 in HS than TN. This observation was also supported by the lower level of n-3 in HS than TN and TNS.

4.4 Adult Livers

When female chickens become sexually mature, they have a 2- to 3-fold increase in total lipid concentration in the liver (Noble and Cocchi, 1990). The change in fatty acid composition in the liver will reflect the fatty acid composition in the yolk (Noble and Cocchi, 1990). In the present work, female livers also had significantly less total PUFA, total n-6 PUFA, and total n-3 PUFA than males, indicating that these fatty acids were being deposited into the yolk. In the present work, females also had a higher total SFA and SFA:PUFA than males indicating that there may have been more β-oxidation for energy in the females than in males as noted by Poureslami, et al. (2010). These investigators reported that fatty acids are not utilized equally for β-oxidation and SFA and are preferentially oxidized over MUFA (Poureslami, et al., 2010). However, this may not fully explain the difference in SFA:PUFA observed between males and females in the present work as others have also found that preferential oxidation occurs on fatty acids that are present in larger quantities in the diet (Poureslami, et al., 2010).

There is preferential deposition of n-3 fatty acids over n-6 fatty acids from the liver into the yolk (Noble and Cocchi, 1990; Speake, et al., 1998). This maternal control over fatty acids deposited into the yolk can influence the inflammation response in chicks because lower n-6:n-3 have been observed in decreased inflammation (Cherian, 2011). In the current study, results showed that HS had significantly less n-6 and n-3 than TN or TNS, indicating that inflammation could be less in HS than TN or TNS. Likely, an adaptation of the HS to be less reactive to oxidative stress occurred over generations. Alternatively, heat stress may have degraded long-chain PUFA that are typically n-6 or n-3; thus, there was less in HS when compared to TN or TNS. However, for the n-6:n-3, TNS had a significantly lower ratio than HS and HSS, indicating that TNS had a

more desirable balance of n-6:n-3. n-6 PUFA are also more pro-inflammatory, thus, HS and HSS may have experienced more inflammation than TN or TNS.

The higher level of LA in HS and HSS indicated a possible suppression of the conversion of ALA to EPA or DHA; therefore, a lower amount of n-3 was found in quail from the HS and HSS groups (Alagawany, et al., 2019). The conversion of ALA to ARA could also explain the significantly higher levels of ARA in TN and TNS over HS and HSS and the significantly lower levels of ALA in TNS males (Murai, et al., 1995).

With increased environmental temperatures, there is an increase in oxidation in the liver (Hosseini-Vashan and Raei-Moghadam, 2019). The liver is also the source of elongation and desaturation of fatty acids in avian species (Cherian, 2011). Heat stress in other studies have produced increased SFA, particularly PA, in abdominal and subcutaneous fat in male broilers (Baziz, et al., 1996). Heat stressed cows have also produced increased PA and stearic acid (Sejian, et al., 2015). However, in the current study, female quail had higher levels of stearic acid and SFA:PUFA than males, but males had higher PA than females.

4.5 Adult Thighs

The recommended n-6:n-3 is 1 to 4:1; however, the current western human diet has a ratio of 10:1 to 20:1 (Patterson, et al. 2012). The current study had similar findings with adult quail thighs having a n-6:n:3 of 23.03:1 to 29.72:1 (Table 1). However, the present study had lower amounts of ARA than that reported in human skeletal muscle (Tallima and El Ridi, 2018).

Other researchers showed that tissue fat of fasting birds have an increase in SFA concentrations due to the preferential metabolism of unsaturated fatty acids (Ben-Hamo, et al., 2013). Researchers also note that shorter chain unsaturated fatty acids are selectively mobilized for energy but in membranes, n-6 fatty acids such as ARA and LA are preferentially retained and

may increase in concentration during fasting (Ben-Hamo, et al., 2013). However, there was no clear treatment effect indicating that HS and HSS had significantly less shorter chain unsaturated fatty acids, such as LA and ALA, than TN or TNS. Birds tend to decrease feed intake during heat stress to decrease their heat increment from metabolizing feed (Kumari and Nath, 2018). HSS had significantly more total SFA than TN and TNS in quail thigh meat, possibly due to oxidation of long chain PUFA.

Males had significantly more PA than females which is in line with reports of others (Gecgel, et al., 2015). Similarly, results of the current study agreed with those of other studies reporting that males had significantly more palmitoleic acid, stearic acid, and total SFA than females (Tavaniello, et al., 2017). Females had significantly more LA, ALA, DPA n-6, total PUFA, and total n-6 PUFA which was in accord with reports of other researchers (Gecgel, et al., 2015). Others have found no sex effect on SFA and MUFA metabolism which is contrary to the findings of the current study with higher levels of SFA and MUFA in males and higher levels of PUFA in females (Poureslami, et al., 2010).

5. Conclusions

The most notable differences were:

1. Overall, of all the organs analyzed, livers experienced the most variations in concentrations of fatty acids when compared by treatment and sex which was expected because it is the source of desaturation and elongation of fatty acids in avian species.
2. Most significant differences observed in the adult livers was reflected in the adult thighs, which was expected because the fatty acid composition of skeletal muscle is directly related to that in the diet and liver.

3. Males had significantly more stearic acid in brains, kidneys, and thighs and significantly more PA in all tissues than females.
4. TN brains had significantly less PUFA than that for both TNS and HSS, possibly indicating that selection for lower FCR in heat stressed environments reduced oxidation of PUFA or increased retention of PUFA in the brain.
5. TN or TNS had significantly more long chain PUFA such as DPA n-6, DPA n-3, DHA, and ARA and significantly less SFA such as PA or stearic acid than HS or HSS across all tissues.

Future studies should include higher temperatures from 30˚C and above to determine how fatty acid composition of quail and other commercial poultry are affected when subjected to selective breeding to withstand increasing temperatures during climate change. Of interest also are further explorations of the relationship between the liver and skeletal muscle fatty acids and determination of the differences in fatty acid partitioning within various avian tissues under heat stress. As well, it is important to continue the work of others to know if heat stress affects cognitive ability through the oxidation of fatty acids using Japanese quail as a model (Laviola and Macrì, 2013; Tallima and El Ridi, 2018).

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Table 3.1. Fatty acid (%) composition[1] of stored feed[2].

	Fatty Acids of Feed (%)				
CODE	-20.0˚C[2,3]	22.2˚C[2,3]	31.1˚C[2,3]	P value	SD[4]
C14:0	0.33	0.34	0.34	0.53	0.02
C15:0	0.08	0.08	0.08	0.83	0.01
C16:0	2.50	2.53	2.52	0.19	0.46
C16:1	0.49	0.51	0.53	0.72	0.08
C17:0	0.16	0.17	0.16	0.67	0.01
C18:0	10.50[a]	10.90[ab]	14.00[b]	0.03	2.89
C18:1 n-9 cis	16.50	15.20	14.90	0.20	1.78
C18:2 n-6	43.80	43.70	44.50	0.30	1.03
C18:3 n-6	11.40	11.30	12.00	0.59	1.37
C20:0	0.29	0.28	0.29	0.43	0.01
C20:1 n-9	0.58	0.60	0.60	0.45	0.03
C20:2 n-6	0.17	0.18	0.18	0.37	0.01
C20:3 n-6	0.01	0.02	0.02	0.15	0.01
C20:4 n-6	0.20	0.18	0.20	0.33	0.03
C20:5 n-3	0.01[a]	0.03[ab]	0.05[b]	0.06	0.03
C22:0	0.21	0.21	0.21	0.76	0.01
C22:1	0.06	0.06	0.07	0.48	0.01
C22:2 n-3	0.13	0.13	0.13	0.57	0.01
C22:5 n-6	0.05	0.04	0.04	0.37	0.01
C22:6 n-3	0.21	0.21	0.21	0.69	0.01
∑SFA[5]	26.40[a]	28.50[ab]	29.90[b]	0.02	2.19
∑MUFA[5]	17.60[a]	15.70[b]	15.70[b]	0.01	1.38
∑PUFA[5]	56.20	55.80	57.20	0.18	1.43
∑n-6 PUFA[5]	55.80	55.50	56.90	0.20	1.50
∑n-3 PUFA[5]	0.35	0.36	0.39	0.28	0.04
∑SFA:PUFA[5]	0.47	0.50	0.44	0.53	0.09
∑n-6:n-3[5]	161.00	155.00	146.00	0.27	15.44

[a-b] Superscripts indicate significant differences at P≤0.05.

[1] For the main effect, fatty acids were compared by temperature.

[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[3]-20˚C, feed storage temperature; 22.2˚C, feed kept at temperature of thermoneutral and thermoneutral siblings' treatments for 48 hours; 31.1˚C, feed kept at temperature of heat stress and heat stress siblings' treatments for 48 hours.
[4] SD, standard deviation.
[5] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6, omega-6 PUFA; n-3, omega-3 PUFA.

Table 3.2. Fatty acid (%) composition[1] of adult brains compared by treatment × sex.

	Fatty Acids of Adult Brains (%)									
	TN[2]		TNS[2]		HS[2]		HSS[2]			
CODE	M	F	M	F	M	F	M	F	P value	SD[3]
C14:0	0.30^{a}	0.21^{ab}	0.22^{ab}	0.22^{ab}	0.18^{ab}	0.15^{b}	0.23^{ab}	0.24^{ab}	0.22	0.08
C14:1	0.14^{a}	0.08^{b}	0.07^{bc}	0.07^{bc}	0.06^{c}	0.07^{bc}	0.06^{bc}	0.06^{c}	0.00	0.03
C15:0	1.97^{abc}	1.79^{a}	2.21^{abc}	2.14^{ab}	2.56^{bc}	2.29^{abc}	2.61^{c}	2.36^{bc}	0.76	0.36
C16:0	25.50^{a}	21.90^{b}	23.60^{ab}	23.00^{b}	23.50^{ab}	21.70^{b}	23.30^{ab}	22.60^{b}	0.01	1.63
C16:1	0.67^{ab}	0.92^{b}	0.61^{ab}	0.66^{ab}	0.54^{a}	0.54^{a}	0.51^{a}	0.54^{a}	0.38	0.24
C17:0	9.70^{a}	6.46^{ab}	4.16^{c}	4.94^{bcd}	4.65^{cd}	6.41^{abd}	4.72^{bcd}	5.49^{bcd}	0.01	1.46
C18:0	21.80^{a}	18.00^{b}	20.70^{ac}	19.00^{bc}	20.30^{ac}	18.10^{b}	20.20^{ac}	19.50^{bc}	0.01	1.61
C18:1 trans	9.73^{ab}	13.85^{c}	9.49^{a}	10.28^{ab}	9.09^{a}	12.06^{bc}	9.04^{a}	10.13^{ab}	0.01	2.08
C18:1 cis	4.91^{a}	7.21^{b}	5.05^{a}	5.67^{a}	4.96^{a}	5.73^{a}	4.95^{a}	5.48^{a}	0.01	1.05
C18:2 n-6	0.60	0.89	0.66	0.83	0.50	0.91	0.55	0.79	0.57	0.32
C20:0	0.14^{a}	0.43^{b}	0.19^{a}	0.15^{a}	0.15^{a}	0.32^{ab}	0.15^{a}	0.24^{a}	0.01	0.14
C20:2 n-6	0.33^{ab}	0.67^{c}	0.34^{ab}	0.35^{ab}	0.29^{a}	0.60^{bc}	0.33^{a}	0.38^{ab}	0.01	0.20
C20:4 n-6	10.50^{a}	9.12^{b}	10.30^{a}	9.82^{ab}	10.31^{a}	9.28^{ab}	10.02^{ab}	9.74^{ab}	0.2	0.94
C20:5 n-3	0.26	0.38	0.30	0.30	0.23	0.34	0.29	0.39	0.6	0.18
C22:1	0.35^{ab}	0.31^{a}	0.36^{ab}	0.30^{a}	0.34^{ab}	0.43^{b}	0.33^{ab}	0.37^{ab}	0.00	0.07
C22:2 n-3	2.99^{ab}	nd[4]	3.07^{ab}	3.31^{ab}	2.94^{a}	3.49^{b}	3.02^{ab}	2.97^{ab}	0.04	0.36
C22:5 n-6	5.46^{a}	3.65^{b}	4.80^{ab}	5.24^{ab}	5.23^{ab}	4.72^{ab}	5.63^{a}	5.35^{a}	0.04	1.17
C22:5 n-3	0.40	0.53	0.40	0.42	0.45	0.50	0.41	0.46	0.34	0.09
C22:6 n-3	14.40^{a}	12.60^{bc}	13.90^{ac}	12.70^{c}	13.30^{ac}	11.20^{b}	12.90^{ac}	12.50^{bc}	0.06	1.25
∑SFA[5]	49.90^{ab}	47.90^{a}	51.10^{b}	49.40^{ab}	51.00^{b}	49.00^{ab}	51.00^{b}	50.10^{ab}	0.79	2.04
∑MUFA[5]	14.90^{a}	22.40^{b}	15.60^{a}	16.90^{a}	15.00^{a}	18.80^{ab}	14.80^{a}	16.60^{a}	0.01	3.45
∑PUFA[5]	33.70^{a}	27.60^{b}	32.90^{a}	32.70^{a}	33.00^{a}	30.80^{a}	32.90^{a}	32.10^{a}	0.00	2.46
∑n-6 PUFA[5]	17.00	14.20	16.10	16.10	16.10	15.30	16.30	16.30	0.13	1.76
∑n-3 PUFA[5]	15.00^{a}	13.30^{abc}	14.50^{ab}	13.30^{bc}	13.90^{ab}	12.00^{c}	13.50^{ab}	13.20^{bc}	0.12	1.24
∑SFA:PUFA[5,6]	1.47^{a}	1.74^{b}	1.55^{a}	1.51^{a}	1.55^{a}	1.59^{a}	1.55^{a}	1.56^{a}	<0.0001	0.04
∑n-6:n-3[5]	1.15	1.08	1.11	1.22	1.16	1.27	1.22	1.24	0.49	0.17

[a-d] Superscripts indicate significant differences at P≤0.05.

[1] For the main effects, fatty acids were compared by treatment, sex, and their interaction.

[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[3] SD, standard deviation.
[4] nd, not detected.
[5] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6, omega-6 PUFA; n-3, omega-3 PUFA.
[6] Transformation of 1/(x) was used in the analysis but presented means and SD are presented in their original scale.

Table 3.3. Fatty acid (%) composition[1] of adult brains compared by sex and treatment[2].

	Fatty Acids of Adult Brains (mg/g)								
	Sex			Treatment					
CODE	M	F	P value	TN	TNS	HS	HSS	P value	SD[3]
C14:0	0.23	0.20	0.29	0.25[a]	0.22[ab]	0.16[b]	0.23[a]	0.01	0.08
C14:1	0.08	0.07	0.12	0.11[a]	0.07[b]	0.06[b]	0.06[b]	0.00	0.03
C15:0	2.41[a]	2.19[b]	0.03	1.85[a]	2.18[ab]	2.42[bc]	2.49[c]	0.00	0.36
C16:0	23.90[a]	22.30[b]	0.00	23.40	23.30	22.60	23.00	0.49	1.63
C16:1	0.58	0.67	0.16	0.80[a]	0.63[ab]	0.54[b]	0.53[b]	0.00	0.24
C17:0	4.72[a]	5.82[b]	0.00	6.82[a]	4.55[b]	5.53[ab]	5.11[b]	0.00	1.46
C18:0	20.70[a]	18.70[b]	0.00	19.60	19.90	19.20	19.80	0.66	1.61
C18:1 trans	9.31[a]	11.58[b]	0.00	12.09[a]	9.89[b]	10.58[ab]	9.58[b]	0.00	2.08
C18:1 cis	4.97[a]	6.02[b]	0.00	6.14	5.36	5.34	5.21	0.06	1.05
C18:2 n-6	0.59[a]	0.85[b]	0.00	0.74	0.74	0.74	0.69	0.91	0.32
C20:0	0.16[a]	0.28[b]	0.00	0.32[a]	0.17[b]	0.25[ab]	0.20[ab]	0.03	0.14
C20:2 n-6	0.32[a]	0.50[b]	0.00	0.52	0.35	0.44	0.36	0.06	0.20
C20:4 n-6	10.26[a]	9.49[b]	0.00	9.66	10.06	9.80	9.88	0.62	0.94
C20:5 n-3	0.27[a]	0.35[b]	0.03	0.32	0.30	0.29	0.33	0.81	0.18
C22:1	0.34	0.35	0.70	0.33	0.33	0.38	0.35	0.10	0.07
C22:2	3.00[a]	3.27[b]	0.01	2.99	3.21	3.21	3.00	0.28	0.36
C22:5 n-6	5.27	4.74	0.07	4.49	5.02	4.98	5.49	0.13	1.17
C22:5 n-3	0.42[a]	0.48[b]	0.01	0.47	0.41	0.48	0.44	0.17	0.09
C22:6 n-3	13.60[a]	12.20[b]	0.00	13.40[a]	13.30[ab]	12.20[b]	12.70[ab]	0.03	1.25
∑SFA[4]	50.80[a]	49.10[b]	0.00	48.70	50.30	50.00	50.50	0.08	2.04
∑MUFA[4]	15.10[a]	18.60[b]	0.00	19.20[a]	16.20[ab]	16.90[ab]	15.70[b]	0.03	3.45
∑PUFA[4]	33.10[a]	30.80[b]	0.00	29.90[a]	32.80[b]	31.90[ab]	32.50[b]	0.01	2.46
∑n-6 PUFA[4]	16.30	15.50	0.06	15.30	16.10	15.70	16.30	0.41	1.76
∑n-3 PUFA[4]	14.20[a]	13.00[b]	0.00	14.10	13.90	13.00	13.40	0.06	1.24
∑SFA:PUFA[4]	1.54[a]	1.60[b]	0.02	1.57	1.53	1.56	1.65	0.08	0.04
∑n-6:n-3[4]	1.16	1.20	0.39	1.11	1.17	1.22	1.23	0.22	0.17

[a-c] Superscripts indicate significant differences at P≤0.05.

[1] For the main effects, fatty acids were compared by treatment and sex.

[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (Coturnix coturnix japonica)** blood gases and electrolytes in response to multigenerational heat stress. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[3] SD, standard deviation.

[4] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6, omega-6 PUFA; n-3, omega-3 PUFA.

Table 3.4. Fatty acid (%) composition[1] of adult kidneys compared by treatment × sex.

	Fatty Acids of Adult Kidneys (%)									
	TN[2]		TNS[2]		HS[2]		HSS[2]			
CODE	M	F	M	F	M	F	M	F	P value	SD[3]
C14:0	0.29	0.21	0.26	0.22	0.29	0.23	0.29	0.25	0.64	0.06
C14:1[4]	0.04	0.06	0.04	0.06	0.04	0.04	0.04	0.05	0.75	0.02
C15:0	1.28	1.63	1.43	1.64	1.53	1.50	1.48	1.42	0.30	0.33
C16:0	18.00^{ab}	17.10^{abc}	17.70^{abc}	16.90^{ac}	17.40^{abc}	16.70^{c}	18.20^{b}	17.20^{abc}	0.92	0.82
C16:1	2.88^{a}	1.92^{abcd}	2.27^{acd}	1.39^{bc}	2.35^{ad}	1.23^{b}	2.49^{ad}	1.54^{bcd}	0.95	0.79
C17:0	0.51^{a}	1.64^{bc}	1.17^{b}	1.49^{bc}	1.01^{ab}	1.85^{c}	1.19^{b}	1.61^{bc}	0.03	0.55
C18:0	21.20^{a}	18.40^{bcd}	18.40^{bc}	17.40^{b}	19.90^{acd}	19.30^{abcd}	20.40^{ad}	18.50^{bcd}	0.11	1.67
C18:1 trans	16.90	15.10	16.30	15.40	16.50	16.10	16.50	16.50	0.75	2.17
C18:2 n-6	19.40^{a}	23.10^{ab}	22.30^{ab}	24.80^{b}	20.50^{a}	22.40^{ab}	20.40^{a}	23.60^{ab}	0.81	3.06
C18:3 n-3	0.47	0.79	0.65	0.80	0.47	0.63	0.52	0.77	0.71	0.08
C20:0	0.23^{ab}	0.24^{ab}	0.26^{ab}	0.28^{b}	0.22^{ab}	0.23^{ab}	0.21^{a}	0.25^{ab}	0.51	0.04
C20:1 n-9	0.24^{a}	0.25^{ab}	0.26^{ab}	0.28^{b}	0.26^{ab}	0.27^{ab}	0.27^{ab}	0.27^{b}	0.78	0.03
C20:2 n-6	0.83^{a}	1.24^{bcd}	0.94^{ab}	1.30^{cd}	0.99^{abc}	1.42^{d}	0.91^{ab}	1.34^{d}	0.96	0.29
C20:3 n-6	0.84^{ab}	0.97^{ab}	0.80^{a}	0.93^{ab}	0.94^{ab}	1.06^{b}	0.82^{ab}	1.01^{ab}	0.92	0.17
C20:3 n-3	0.11^{abcd}	0.12^{bcd}	0.09^{abc}	0.13^{cd}	0.07^{ab}	0.14^{d}	0.05^{a}	0.14^{d}	0.01	0.03
C20:4 n-6	12.90	12.90	12.90	12.20	13.60	12.50	12.30	11.30	0.90	2.23
C20:5 n-3[4]	0.30^{a}	0.15^{abc}	0.12^{bc}	0.08^{bc}	0.15^{b}	0.09^{c}	0.13^{bc}	0.07^{bc}	0.77	0.09
C22:0	0.38	0.36	0.51	0.46	0.37	0.40	0.37	0.45	0.27	0.11
C22:2 n-3	0.45	0.45	0.56	0.55	0.51	0.49	0.55	0.52	0.99	0.11
C22:5 n-6	0.29^{a}	0.77^{b}	0.21^{a}	0.59^{b}	0.25^{a}	0.66^{b}	0.26^{a}	0.61^{b}	0.40	0.23
C22:5 n-3	0.29	0.26	0.31	0.25	0.25	0.26	0.30	0.26	0.28	0.06
C22:6 n-3	2.31	2.20	2.35	2.04	2.03	1.93	2.08	1.95	0.89	0.43
∑SFA[5]	41.80^{ab}	39.20^{ac}	39.70^{ac}	38.60^{c}	40.70^{abc}	39.90^{abc}	42.20^{b}	39.50^{ac}	0.23	1.81
∑MUFA[5]	19.80	17.60	19.00	17.30	19.50	17.90	19.50	18.50	0.93	2.67
∑PUFA[5]	38.20^{a}	42.90^{ab}	41.20^{ab}	43.60^{b}	39.70^{ab}	41.50^{ab}	38.30^{a}	41.50^{ab}	0.60	3.44
∑n-6 PUFA[5]	34.30^{a}	39.00^{ab}	37.20^{ab}	39.80^{b}	36.30^{ab}	38.00^{ab}	34.70^{a}	37.80^{ab}	0.51	3.18
∑n-3 PUFA[5]	3.46	3.42	3.49	3.26	2.92	3.02	3.04	3.13	0.57	0.41
∑SFA:PUFA[5]	1.12^{ab}	0.92^{acd}	0.97^{abcd}	0.87^{cd}	1.04^{abd}	0.92^{cd}	1.11^{b}	0.85^{c}	0.16	0.14
∑n-6:n-3[5]	10.00^{a}	11.40^{abc}	10.80^{ab}	12.20^{bc}	12.50^{bc}	12.70^{c}	11.60^{abc}	12.20^{bc}	0.32	1.40

[a-d] Superscripts indicate significant differences at P≤0.05.

[1] For the main effects, fatty acids were compared by treatment, sex, and their interaction.

[2] Detailed experimental design is delineated in Chapter 2: Changes in Japanese Quail (Coturnix coturnix japonica) blood gases and electrolytes in response to multigenerational heat stress. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively.

TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.
[3] SD, standard deviation.
[4] Transformation of log(x) was used in the analysis but presented means and SD are presented in their original scale.
[5] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6, omega-6 PUFA; n-3, omega-3 PUFA.

Table 3.5. Fatty acid (%) composition[1] of adult kidneys compared by sex and treatment[2].

	Fatty Acids of Adult Kidney (mg/g)								
	Sex			Treatment					
CODE	M	F	P value	TN	TNS	HS	HSS	P value	SD[3]
C14:0	0.28[a]	0.23[b]	0.00	0.25	0.24	0.26	0.27	0.59	0.06
C14:1	0.04	0.05	0.06	0.05	0.05	0.04	0.04	0.43	0.02
C15:0	1.43	1.55	0.17	1.43	1.54	1.51	1.45	0.79	0.33
C16:0	17.80[a]	17.00[b]	0.00	17.60	17.30	17.10	17.70	0.17	0.82
C16:1	2.49[a]	1.49[b]	0.00	2.43	1.83	1.79	2.01	0.12	0.79
C17:0	0.97[a]	1.65[b]	0.00	0.95	1.33	1.43	1.40	0.07	0.55
C18:0	19.90[a]	18.40[b]	0.00	19.90[a]	17.90[b]	19.60[a]	19.50[a]	0.00	1.67
C18:1 trans	16.50	15.80	0.18	16.10	15.80	16.30	16.50	0.84	2.17
C18:2 n-6	20.70[a]	23.50[b]	0.00	21.00	23.60	21.40	22.00	0.09	3.06
C18:3 n-3	0.53[a]	0.75[b]	0.00	0.61	0.73	0.55	0.65	0.25	0.08
C20:0	0.23	0.25	0.08	0.23[ab]	0.27[b]	0.23[a]	0.23[a]	0.01	0.04
C20:1 n-9	0.26	0.27	0.07	0.24[a]	0.27[b]	0.26[ab]	0.27[b]	0.01	0.02
C20:2 n-6	0.91[a]	1.33[b]	0.00	1.00	1.11	1.20	1.12	0.34	0.29
C20:3 n-6	0.85[a]	0.99[b]	0.00	0.90	0.87	1.00	0.91	0.17	0.17
C20:3 n-3	0.09[a]	0.13[b]	0.00	0.11	0.12	0.12	0.12	0.96	0.03
C20:4 n-6	12.90	12.20	0.17	12.90	12.50	13.00	11.80	0.42	2.23
C20:5 n-3	0.17[a]	0.09[b]	0.00	0.27[a]	0.11[b]	0.12[b]	0.12[b]	0.00	0.09
C22:0	0.41	0.42	0.61	0.37[a]	0.48[b]	0.38[a]	0.41[ab]	0.02	0.11
C22:2	0.52	0.51	0.68	0.45[a]	0.56[b]	0.50[ab]	0.53[ab]	0.05	0.11
C22:5 n-6	0.25[a]	0.65[b]	<0.0001	0.50	0.40	0.45	0.44	0.73	0.23
C22:5 n-3	0.29[a]	0.26[b]	0.03	0.28	0.28	0.26	0.28	0.60	0.06
C22:6 n-3	2.19	2.02	0.11	2.26	2.19	1.98	2.02	0.20	0.43
∑SFA[4]	41.10[a]	39.30[b]	0.00	40.50[ab]	39.20[a]	40.30[ab]	41.00[b]	0.04	1.81
∑MUFA[4]	19.40[a]	17.80[b]	0.02	18.80	18.20	18.60	19.00	0.84	2.67
∑PUFA[4]	39.40[a]	42.30[b]	0.00	40.20	42.40	40.60	39.90	0.16	3.44
∑n-6 PUFA[4]	35.60[a]	38.60[b]	0.00	36.30	38.50	37.10	36.30	0.17	3.18
∑n-3 PUFA[4]	3.23	3.19	0.73	3.44[a]	3.37[ab]	2.97[c]	3.08[bc]	0.00	0.41
∑SFA:PUFA[4]	1.05[a]	0.89[b]	0.00	1.02	0.92	0.97	0.98	0.34	0.14
∑n-6:n-3[4]	11.20[a]	12.20[b]	0.01	10.60[a]	11.50[ab]	12.60[b]	11.90[b]	0.00	1.40

[a-c] Superscripts indicate significant differences at P≤0.05.

[1] For the main effects, fatty acids were compared by treatment and sex.

[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (Coturnix coturnix japonica)** blood gases and electrolytes in response to multigenerational heat stress. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C,

respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.
[3] SD, standard deviation.
[4] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6, omega-6 PUFA; n-3, omega-3 PUFA.

Table 3.6. Fatty acid (%) composition[1] of adult livers compared by treatment × sex.

	Fatty Acids of Adult Livers (%)									
	TN[2]		TNS[2]		HS[2]		HSS[2]			
CODE	M	F	M	F	M	F	M	F	P value	SD[3]
C14:0	0.58^{ab}	0.37^{c}	0.24^{c}	0.32^{c}	0.79^{bd}	0.39^{ac}	0.80^{d}	0.41^{ac}	0.00	0.23
C14:1	0.12^{ab}	0.05^{c}	0.03^{c}	0.03^{c}	0.17^{b}	0.059^{ac}	0.16^{b}	0.05^{c}	0.00	0.07
C15:0	0.02^{ab}	0.03^{abc}	0.04^{abc}	0.05^{c}	0.02^{a}	0.03^{abc}	0.03^{ab}	0.04^{bc}	0.91	0.01
C16:0	26.80^{ab}	24.60^{a}	23.20^{a}	26.70^{ab}	31.10^{c}	24.00^{a}	29.20^{bc}	26.50^{ab}	0.00	3.48
C16:1	3.52^{abc}	2.69^{ab}	1.88^{a}	1.95^{a}	4.79^{c}	3.27^{abc}	4.35^{bc}	2.87^{ab}	0.16	1.37
C17:0	0.10^{ab}	0.14^{b}	0.09^{ab}	0.11^{ab}	0.08^{a}	0.14^{b}	0.10^{ab}	0.15^{b}	0.42	0.04
C18:0	24.60^{a}	29.20^{a}	27.00^{a}	45.70^{b}	26.90^{a}	31.40^{a}	25.10^{a}	29.60^{a}	0.02	9.31
C18:1 cis	18.10^{ab}	16.60^{abc}	10.60^{c}	11.20^{ac}	20.30^{b}	22.50^{b}	20.40^{b}	22.90^{b}	0.50	5.79
C18:2 n-6	13.26^{ab}	13.05^{ab}	17.99^{b}	16.57^{ab}	9.57^{a}	10.51^{a}	11.26^{ab}	10.74^{a}	0.91	4.79
C18:3 n-6	0.18	0.25	0.27	0.15	0.14	0.22	0.19	0.19	0.01	0.08
C18:3 n-3	0.23^{a}	0.20^{a}	0.45^{b}	0.26^{a}	0.19^{a}	0.22^{a}	0.25^{a}	0.16^{a}	0.03	0.12
C20:0[4]	0.08	0.09	0.09	0.05	0.06	0.06	0.08	0.05	0.16	0.04
C20:1 n-9	0.22^{ab}	0.14^{c}	0.24^{b}	0.11^{c}	0.14^{ac}	0.13^{c}	0.16^{abc}	0.09^{c}	0.04	0.07
C20:2 n-6	0.35^{a}	0.15^{b}	0.31^{ac}	0.10^{b}	0.22^{bc}	0.16^{b}	0.22^{bc}	0.11^{b}	0.04	0.11
C20:3 n-6	0.48^{a}	0.18^{bc}	0.55^{a}	0.09^{b}	0.36^{ac}	0.17^{bc}	0.47^{a}	0.15^{bc}	0.24	0.23
C20:4 n-6	6.87^{ab}	5.91^{ac}	9.65^{b}	4.44^{ac}	3.36^{c}	4.21^{ac}	4.51^{ac}	4.02^{ac}	0.00	2.80
C20:5 n-3	0.24^{ab}	0.11^{c}	0.25^{b}	0.07^{c}	0.15^{ac}	0.11^{ac}	0.17^{abc}	0.09^{c}	0.06	0.09
C22:0	0.12	0.13	0.16	0.09	0.09	0.08	0.09	0.07	0.25	0.06
C22:1	0.25^{ab}	0.21^{a}	0.22^{ab}	0.21^{a}	0.26^{b}	0.21^{a}	0.26^{ab}	0.25^{ab}	0.10	0.04
C22:2 n-3[4]	0.13^{a}	0.09^{ab}	0.07^{ab}	0.04^{b}	0.10^{ab}	0.11^{a}	0.12^{a}	0.09^{ab}	0.31	0.06
C22:5 n-6	0.52^{ab}	0.78^{b}	0.62^{ab}	0.41^{ab}	0.25^{a}	0.73^{b}	0.38^{ab}	0.52^{ab}	0.02	0.32
C22:5 n-3	0.15^{ab}	0.14^{ab}	0.24^{b}	0.13^{a}	0.08^{a}	0.11^{a}	0.13^{a}	0.12^{a}	0.02	0.07
C22:6 n-3	2.43^{a}	1.76^{ab}	4.23^{c}	1.37^{ab}	1.15^{ab}	1.08^{b}	1.67^{ab}	1.21^{ab}	0.00	1.26
∑SFA[5]	52.30^{a}	51.50^{a}	59.90^{a}	80.80^{b}	78.80^{b}	78.10^{b}	55.40^{a}	56.80^{a}	0.02	15.75
∑MUFA[5]	22.17^{a}	19.66^{a}	3.56^{b}	3.84^{b}	7.82^{b}	4.59^{b}	25.26^{a}	25.83^{a}	0.62	10.48
∑PUFA[5]	24.80^{ab}	22.50^{ab}	33.00^{b}	15.30^{a}	13.40^{a}	17.20^{a}	19.30^{a}	17.30^{a}	0.00	9.39
∑n-6 PUFA[5]	21.60^{ab}	20.20^{ab}	27.90^{b}	13.50^{a}	11.70^{a}	15.70^{a}	17.00^{ab}	15.70^{a}	0.00	8.26
∑n-3 PUFA[5]	3.54^{a}	2.41^{ab}	5.35^{c}	1.97^{ab}	1.87^{b}	1.72^{b}	2.57^{ab}	1.73^{b}	0.00	1.51
∑SFA:PUFA[5]	2.39^{a}	2.42^{a}	1.67^{a}	10.47^{b}	7.58^{ab}	5.07^{ab}	3.00^{a}	4.17^{a}	0.00	4.48
∑n-6:n-3[5]	1.87^{ab}	2.18^{b}	1.79^{ab}	1.60^{a}	1.90^{ab}	2.21^{b}	1.92^{ab}	2.26^{b}	0.09	2.73

[a-d] Superscripts indicate significant differences at P≤0.05.

[1] For the main effects, fatty acids were compared by treatment, sex, and their interaction.

[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring

evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.
[3] SD, standard deviation.
[4] Transformation of log(x) was used in the analysis but presented means and SD are presented in their original scale
[5] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6, omega-6 PUFA; n-3, omega-3 PUFA.

Table 3.7. Fatty acid (%) composition[1] of adult livers compared by sex and treatment[2].

	Fatty Acids of Adult Livers (mg/g)								
	Sex			Treatment					
CODE	M	F	P value	TN	TNS	HS	HSS	P value	SD[3]
C14:0	0.61[a]	0.37[b]	0.00	0.48[a]	0.28[b]	0.59[a]	0.60[a]	0.00	0.23
C14:1	0.13[a]	0.05[b]	0.00	0.09[ab]	0.03[a]	0.12[b]	0.10[b]	0.00	0.07
C15:0	0.03[a]	0.04[b]	0.00	0.03[a]	0.04[b]	0.03[a]	0.03[ab]	0.01	0.01
C16:0	27.70[a]	25.50[b]	0.01	25.80	25.10	27.60	27.80	0.08	3.48
C16:1	3.73[a]	2.71[b]	0.00	3.10[a]	1.91[b]	4.03[a]	3.61[a]	0.00	1.37
C17:0	0.09[a]	0.14[b]	0.00	0.12	0.10	0.11	0.13	0.42	0.04
C18:0	25.90[a]	34.00[b]	0.00	26.90[a]	37.00[b]	29.20[ab]	27.50[a]	0.01	13.98
C18:1 cis	17.50	18.80	0.38	17.40[a]	10.90[b]	21.40[c]	21.70[c]	0.00	6.40
C18:2 n-6	13.10	12.20	0.47	13.20[a]	17.50[b]	10.00[a]	11.00[a]	0.00	3.86
C18:3 n-6	0.19	0.20	0.98	0.21	0.21	0.17	0.19	0.50	0.08
C18:3 n-3	0.28[a]	0.21[b]	0.02	0.22[a]	0.35[b]	0.20[a]	0.20[a]	0.00	0.12
C20:0	0.08[a]	0.06[b]	0.03	0.08	0.06	0.06	0.07	0.45	0.04
C20:1 n-9	0.20[a]	0.12[b]	0.00	0.18	0.17	0.13	0.12	0.07	0.07
C20:2 n-6	0.28[a]	0.13[b]	0.00	0.25	0.19	0.19	0.16	0.15	0.11
C20:3 n-6	0.46[a]	0.15[b]	0.00	0.32	0.31	0.27	0.30	0.94	0.23
C20:4 n-6	6.03	4.64	0.05	6.39[ab]	6.87[b]	3.79[c]	4.25[ac]	0.00	2.80
C20:5 n-3	0.20[a]	0.09[b]	0.00	0.19	0.15	0.14	0.15	0.46	0.09
C22:0	0.11	0.10	0.20	0.13	0.12	0.08	0.08	0.04	0.06
C22:1	0.25[a]	0.22[b]	0.00	0.23[ab]	0.22[a]	0.24[ab]	0.26[b]	0.02	0.03
C22:2 n-3	0.11	0.08	0.14	0.05[a]	0.11[b]	0.11[a]	0.10[a]	0.00	0.06
C22:5n-6	0.44[a]	0.61[b]	0.04	0.65	0.51	0.49	0.46	0.36	0.32
C22:5 n-3	0.15	0.13	0.23	0.15[ab]	0.18[b]	0.09[a]	0.12[ab]	0.01	0.07
C22:6 n-3	2.39[a]	1.36[b]	0.00	2.09[ab]	2.80[b]	1.12[a]	1.43[a]	0.00	1.26
∑SFA[4]	54.50	59.40	0.09	51.90[a]	62.70[b]	57.60[ab]	56.20[ab]	0.07	15.75
∑MUFA[4]	21.60	20.50	0.56	20.90[a]	11.50[b]	25.90[c]	25.60[c]	0.00	10.48
∑PUFA[4]	23.30[a]	18.20[b]	0.03	23.60	24.20	16.50	18.30	0.04	9.39
∑n-6 PUFA[4]	20.70[a]	16.30[b]	0.03	20.90	21.80	14.90	16.30	0.03	8.26
∑n-3 PUFA[4]	3.10[a]	1.82[b]	0.00	2.72[ab]	3.49[b]	1.62[a]	1.96[a]	0.00	1.51
∑SFA:PUFA[4]	2.98[a]	4.83[b]	0.05	2.41	5.54	4.25	3.62	0.12	4.48
∑n-6:n-3[4]	8.24	9.29	0.37	8.88[ab]	6.37[a]	10.61[b]	9.15[ab]	0.08	2.73

[a-c] Superscripts indicate significant differences at P≤0.05.

[1] For the main effects, fatty acids were compared by treatment and sex.

[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (Coturnix coturnix japonica)** blood gases and electrolytes in response to multigenerational heat stress. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C,

respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.
[3] SD, standard deviation.
[4] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6, omega-6 PUFA; n-3, omega-3 PUFA.

Table 3.8. Fatty acid (%) composition[1] of adult thighs compared by treatment × sex.

	Fatty Acids of Adult Thighs (%)									
	TN[2]		TNS[2]		HS[2]		HSS[2]			
	M	F	M	F	M	F	M	F	P value	SD[3]
C14:0	0.58^{ab}	0.37^{c}	0.24^{c}	0.32^{c}	0.79^{bd}	0.39^{ac}	0.80^{d}	0.41^{ac}	0.00	0.10
C14:1	0.05	0.07	0.09	0.09	0.13	0.13	0.15	0.16	0.15	0.10
C16:0	18.70^{ab}	15.70^{c}	18.10^{abc}	16.40^{ac}	19.20^{b}	16.30^{ac}	19.70^{b}	17.40^{abc}	0.66	2.00
C16:1	4.39^{a}	4.65^{a}	4.38^{a}	2.94^{ab}	3.70^{ab}	3.66^{ab}	3.77^{ab}	2.56^{b}	0.13	1.20
C17:0	0.11^{a}	0.15^{ab}	0.13^{ab}	0.18^{ab}	0.16^{ab}	0.18^{b}	0.11^{a}	0.19^{b}	0.23	0.10
C18:0	11.22^{ab}	8.93^{a}	10.73^{ab}	10.09^{ab}	12.75^{b}	9.51^{a}	12.12^{ab}	9.56^{a}	0.32	2.10
C18:1 cis	27.30	31.70	29.70	27.50	24.80	29.20	28.40	26.10	0.03	4.10
C18:1 trans	1.63	1.00	1.41	1.18	1.56	1.32	1.13	1.34	0.28	0.30
C18:2 n-6	24.70^{a}	30.00^{bc}	26.50^{abd}	32.30^{c}	24.80^{a}	31.20^{c}	25.70^{ad}	29.10^{bcd}	0.28	3.40
C18:3 n-3	0.72^{a}	1.28^{bc}	0.86^{ad}	1.32^{c}	0.82^{ad}	1.25^{c}	0.91^{ad}	1.03^{bd}	0.00	0.30
C20:0	0.16	0.16	0.16	0.18	0.18	0.19	0.17	0.16	0.88	0.10
C20:1 n-9	0.23^{a}	0.32^{ab}	0.26^{ab}	0.32^{ab}	0.28^{ab}	0.38^{b}	0.27^{a}	0.32^{ab}	0.68	0.10
C20:2 n-6	0.15	0.13	0.15	0.13	0.16	0.15	0.15	0.16	0.83	0.00
C20:3 n-6	0.14	0.11	0.18	0.15	0.19	0.15	0.16	0.15	0.79	0.10
C20:4 n-6	6.02	3.58	5.13	4.44	5.96	4.22	4.76	5.28	0.28	2.10
C22:0	0.10	0.20	0.22	0.14	0.26	0.12	0.13	0.15	0.03	0.10
C22:1	0.22	0.17	0.24	0.25	0.37	0.30	0.18	0.25	0.38	0.10
C22:2 n-3	0.25	0.28	0.20	0.30	0.32	0.33	0.25	0.37	0.70	0.10
C22:5 n-6[4]	0.36^{a}	0.61^{ab}	0.28^{a}	0.53^{ab}	0.44^{ab}	0.61^{ab}	0.30^{a}	0.74^{b}	0.52	0.30
C22:5 n-3	0.32^{ab}	0.20^{a}	0.34^{ab}	0.22^{ab}	0.40^{b}	0.21^{a}	0.35^{ab}	0.29^{ab}	0.32	0.10
C22:6 n-3	1.93	1.17	1.49	1.56	2.05	1.15	1.54	1.92	0.06	0.70
∑SFA[5]	31.30^{ab}	25.60^{c}	29.80^{abc}	27.50^{ac}	33.10^{b}	26.90^{c}	33.00^{b}	29.10^{ac}	0.14	3.33
∑MUFA[5]	32.90	37.10	34.90	31.70	29.40	34.00	32.80	30.30	0.048	4.73
∑PUFA[5]	34.50	37.30	34.90	39.80	35.10	39.10	34.10	39.00	0.86	3.74
∑n-6 PUFA[5]	31.30^{ab}	34.40^{abc}	32.10^{abc}	36.50^{c}	31.50^{ab}	36.30^{c}	31.00^{a}	35.40^{bc}	0.88	3.35
∑n-3 PUFA[5]	3.14	2.92	2.86	3.29	3.60	2.84	3.09	3.67	0.11	0.81
∑SFA:PUFA[5]	0.95^{a}	0.69^{bc}	0.86^{ac}	0.70^{bc}	0.90^{a}	0.69^{b}	0.97^{a}	0.72^{bc}	0.54	0.14
∑n-6:n-3[5]	10.40	11.96	12.14	11.40	9.42	13.12	10.40	10.00	0.08	2.57

[a-d] Superscripts indicate significant differences at P≤0.05.

[1] For the main effects, fatty acids were compared by treatment, sex, and their interaction.

[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[3] SD, standard deviation.

[4] Transformation of log(x) was used in the analysis but presented means and SD are presented in their original scale.

[5] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6, omega-6 PUFA; n-3, omega-3 PUFA.

Table 3.9. Fatty acid (%) composition[1] of adult thighs compared by sex and treatment[2].

	Fatty Acids of Adult Thighs (mg/g)								
	Sex			Treatment					
CODE	M	F	P value	TN	TNS	HS	HSS	P value	SD[3]
C14:0	0.71[a]	0.55[b]	0.00	0.55[a]	0.62[ab]	0.67[b]	0.67[b]	0.03	0.12
C14:1	0.11	0.12	0.57	0.08	0.13	0.14	0.11	0.08	0.06
C16:0	19.00[a]	16.60[b]	0.00	17.60	17.30	17.80	18.60	0.40	1.96
C16:1	4.04[a]	3.31[b]	0.02	4.49[a]	3.73[ab]	3.68[ab]	3.16[b]	0.03	1.17
C17:0	0.13[a]	0.18[b]	0.00	0.12	0.17	0.17	0.15	0.13	0.05
C18:0	11.78[a]	9.55[b]	0.00	10.30	10.40	11.10	10.80	0.76	2.06
C18:1 cis	27.40	28.20	0.48	28.90	28.70	26.90	27.20	0.50	4.14
C18:1 trans	1.50	1.27	0.05	1.47	1.27	1.46	1.31	0.59	0.27
C18:2 n-6	25.40[a]	30.50[b]	0.00	26.70	29.10	27.80	27.40	0.39	3.42
C18:3 n-3	0.83[a]	1.20[b]	0.00	0.92	1.07	1.02	0.97	0.57	0.25
C20:0	0.17	0.17	0.78	0.16	0.17	0.18	0.16	0.52	0.05
C20:1 n-9	0.26[a]	0.34[b]	0.00	0.26	0.30	0.33	0.30	0.16	0.07
C20:2 n-6	0.15	0.15	0.69	0.14	0.13	0.16	0.16	0.68	0.04
C20:3 n-6	0.17	0.14	0.11	0.13	0.17	0.17	0.16	0.29	0.05
C20:4 n-6	5.47	4.52	0.10	5.13	4.86	5.15	5.02	0.99	2.07
C22:0	0.19	0.15	0.17	0.16	0.19	0.19	0.14	0.47	0.07
C22:1	0.26	0.25	0.88	0.20[ab]	0.25[ab]	0.34[b]	0.22[a]	0.03	0.13
C22:2 n-3	0.26	0.33	0.09	0.26	0.25	0.33	0.31	0.51	0.13
C22:5n-6	0.35[a]	0.64[b]	0.00	0.45	0.39	0.52	0.52	0.58	0.26
C22:5 n-3	0.36[a]	0.24[b]	0.00	0.27	0.29	0.31	0.32	0.75	0.12
C22:6 n-3	1.76	1.50	0.20	1.65	1.52	1.63	1.73	0.92	0.74
∑SFA[4]	32.00[a]	27.40[b]	0.00	29.00	28.70	30.00	31.20	0.23	3.33
∑MUFA[4]	32.30	32.80	0.71	34.40	33.40	31.50	31.60	0.33	4.73
∑PUFA[4]	34.60[a]	38.90[b]	0.00	35.50	37.10	37.00	36.60	0.74	3.74
∑n-6 PUFA[4]	31.40[a]	35.70[b]	0.00	32.50	34.10	33.70	33.20	0.69	3.35
∑n-3 PUFA[4]	3.20	3.22	0.90	3.06	3.06	3.25	3.38	0.69	0.81
∑SFA:PUFA[4]	0.92[a]	0.70[b]	0.00	0.85	0.79	0.81	0.84	0.68	0.14
∑n-6:n-3[4]	10.50	11.50	0.15	11.00	11.80	11.10	10.20	0.46	2.57

[a-b] Superscripts indicate significant differences at P≤0.05.

[1] For the main effects, fatty acids were compared by treatment and sex.

[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (Coturnix coturnix japonica)** blood gases and electrolytes in response to multigenerational heat stress. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[3] SD, standard deviation.

[4] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6, omega-6 PUFA; n-3, omega-3 PUFA.

CHAPTER 4
Fatty acid composition of multigenerational heat stressed embryo Japanese quail (*Coturnix coturnix japonica)*

Abstract: The fatty acid composition of eggs and the subsequent embryos are highly determined by maternal deposition before ovulation and lay. Fatty acid composition of organs can be critical to the overall health of the offspring and certain fatty acids (such as DHA and DPA) can significantly promote cognitive ability or visual acuity. However, heat stress in female quail can alter fatty acids of the offspring. Therefore, the objective of this study was to determine if yolk and embryo fatty acid compositions are affected when adults are exposed to heat stress. Four treatments were developed: (1) random bred quail in thermoneutral temperature (TN, 22.2˚C), (2) random bred quail in heat stress temperature (HS, 31.1˚C), (3) quail selected for low FCR in heat stress and not exposed to heat stress (TNS, 22.2˚C) and (4) quail selected for low FCR in heat stress and exposed to heat stress (HSS, 31.1˚C). As incubation progressed, yolks as well as embryo brains, kidneys, and livers were analyzed for fatty acid composition. Hypotheses were that (1) liver and brain would have high levels of DHA and oleic acid, (2) overall, HS or HSS would have lower total PUFA than TN or TNS, and (3) embryos from HSS would have higher levels of PUFA than HS. Significant ($P \leq 0.05$) results showed that: (1) for yolk, HSS had higher levels of LA and ARA than TN, (2) for brain, DHA, total PUFA, and stearic acid increased over the duration of incubation as expected for proper brain development, (3) TNS had more LA, ALA, DPA n-3, and total n-3 than TN and significantly more stearic acid and SFA than HSS, (4) HS kidneys/gonads had more total SFA than TNS and HSS, (5) HS livers had more stearic acid, SFA:PUFA, and total SFA than TN and TNS, (6) TN and TNS had more PUFA than HSS.

Overall, results indicated that TNS likely had less fatty acid oxidation compared to other treatments, suggesting that selective breeding for low FCR in heat stress may incur a fitness advantage for Japan quail. Future studies should include analysis of fatty acids in the yolk sac and its membrane to determine the changes occurring during incubation and the percentage of transfer

of fatty acids from the hen to embryos. Determining if PUFA experience more degradation in heat stressed environments before reaching the embryo could be the focus of another study. An increase in the environmental temperature of heat stressed quail would be appropriate to adequately determine effects of exposure as they are subjected to increasingly higher temperatures during climate change.

1. Introduction

An increase in incidences of heat stress is expected in poultry species as temperatures rise due to climate change, particularly during times of lay (Sejian, et al., 2015; Rojas-Downing, et al., 2017). When females experience environmental or nutritional stress, there can be an overall decrease in total yolk lipid content, but, generally, the lipid composition will not change (Noble and Cocchi, 1990). However, changing feed fatty acid profiles may influence lipid composition in the yolk. Others have found that changing saturated fatty acids (SFA) in the hen's diet will have little effect on the yolk fatty acid profile, but alterations in monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA) will have substantial effects on yolk fatty acid profile (Noble and Cocchi, 1990). Due to high levels of quail egg consumption in many areas of the world, it is important to know if heat stress will alter quail egg fatty acid composition (Gecgel, et al., 2015). If fatty acid composition of highly consumed quail eggs is altered by heat stress, ultimately the health of humans consuming quail eggs may be affected.

Avian females have control over fatty acid deposition in eggs (Laviola and Macrì, 2013). Thus, changes in the fatty acid composition of a female's diet can influence the fatty acid composition of embryo tissues, impacting the long-term health of off-springs (Cherian, 2015). It is known that oxidative stress within the embryo can increase lipid oxidation of the quail brain, liver, and heart as incubation progresses (Tsunekage and Ricklefs, 2015). Thus, female control over

deposition of fatty acids may have a direct effect on the susceptibility of oxidative stress in embryo tissues. If heat stress can alter lipids of diets, female tissues, and deposition/oxidation of lipids in eggs/embryos, changes in fatty acids of embryo tissues may be directly associated with heat stress of layers. However, little is known about the fatty acid profiles of key organs during the embryonic stage; particularly, the progression of fatty acid composition of embryo tissue from heat stressed females (Su, et al., 2020). Delineating the influences of heat stress on the development of the embryo will provide guidance for possible fatty acid supplementation or lipid protection in the laying hen's diet during times of heat stress.

PUFA are highly susceptible to oxidation due to their more vulnerable molecular structure (Frankel, 2005). The brain and kidney have high concentrations of PUFA to ensure the fluidity of membranes (Cherian, 2015). However, there are low concentrations of various antioxidants in the brain as compared to other tissue in quail (Tsunekage and Ricklefs, 2015; Surai, et al., 2016). This inverse relationship between high PUFA and low antioxidant may expose the brain to higher levels of fatty acid degradation during times of heat stress (Yigit, et al., 2014). Therefore, one of the aims of the current study is to determine if maternal heat stress influences the fatty acid composition of various embryo tissues, particularly the brain and liver.

The last 7 days of the 21-day chicken egg incubation period is the most intense period of lipid metabolism and embryonic growth. The equivalent time span for Japanese quail is the last 6 days of the 17-day Japanese quail egg incubation period. During this time, approximately 80% of the lipid content of the yolk is mobilized, absorbed into embryonic tissue, and utilized for energy (Noble and Cocchi, 1990). Therefore, it is important to know if heat stressed hens are depositing adequate levels of fatty acids into the yolk to support proper embryonic development and health.

Whether exposed to heat stress or not, it is hypothesized that the embryonic liver and brain will have high levels of DHA and oleic acid as they are highly associated with phospholipids and cholesteryl esters of the yolk sac membrane. It is also hypothesized that heat stressed hens will deposit adequate levels of fatty acids into the yolk; however, long chain PUFA will be in lower quantities compared to yolk from hens in a thermoneutral environment. To test if genetic selection influences fatty acid deposition, quail were selected for low feed conversion ratio (FCR) at a heat stress temperature (HSS; 31.1˚C) and their siblings were bred in a thermoneutral environment (TNS; 22.2˚C). It is hypothesized that embryos from HSS will have higher levels of PUFA than those that were not selected for low FCR in a heat stress environment due to a possible increase in anti-oxidation response by HSS.

2. Materials and Methods

2.1 Experimental design

The detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. The four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

2.2 Sample collection

Animal care and use was approved by the Institutional Animal Care and Use Committee at the University of California Davis (Protocols #19473 and 21370; Davis, CA). Fertilized eggs from

all treatments were collected weekly for 4 weeks. Unincubated eggs laid in week 2 were stored at 4˚C for 2 weeks, broken out for yolk isolation, then stored at -80˚C until fatty acid analysis. Eggs for week 4 were stored for a maximum of 7 days at 12.78˚C, then all were incubated together. To obtain quail embryos from week 4 egg collection, 60 eggs were incubated for sampling on days 11, 13, and 15 (d11, d13, and d15, respectively). Embryos (20) per incubation day per treatment were euthanized using cervical dislocation, then sampled for brain, liver, and kidneys/gonads. Incubated eggs were not candled to assess embryo viability prior to sampling; therefore, samples per treatments are provided below. Kidneys and gonads were physically close in proximity and difficult to harvest separately; therefore, they were harvested, homogenized, and analyzed together and are referred to as kidney/gonads. Upon harvesting, brains, livers, and kidneys/gonads were placed in snap-cap microtubes filled with physiological saline buffer with 10% DMSO and stored in -80˚C until analyzed, following procedures of Tsunekage and Ricklefs (2015).

2.3 Lipid extraction

For embryo brain and liver, 8 samples per treatment per day of incubation were analyzed for fatty acid composition. Embryo kidneys/gonads had to be pooled due to low sample weight to obtain 3 pooled samples per treatment per day of incubation except for d13 TNS, HS, HSS and d15 TN and TNS, there were 2 pooled samples per treatment per day.

Lipids were extracted using a modified Folch method (Folch, et al., 1957; Zhang et al., 2017). For lipid extraction, samples were weighed according to their respective total % lipid to obtain 2 mg of total lipids. Trinonadecanoin (C19:0 triacylglycerol, Nu Chek Inc, Elysian, MN; Cat# T-165) was added as an internal standard for liver and yolk samples and 1,2-dinonadecanoyl-sn-glycero-3-phosphocholine (C19:0 phosphatidylcholine, Avanti Polar Lipids, Alabaster, Alabama; Cat #850367P) was added as an internal standard for brain and kidney samples

(Tokuşoğlu, 2006; Gecgel et al., 2015). Samples were homogenized in 1 mL of methanol (for HPLC, ≥99.9%, Sigma-Aldrich, St. Louis, MO, USA; Cat #34860; BioSpec, Tissue Tearor 398). The homogenate was transferred to an 8 mL glass screw top test tube (test tube A) and 2 mL of chloroform and 0.75 mL of deionized water were added. The mixture was vortexed and centrifuged at 458 × g force for 10 minutes (Allegra 6 centrifuge with GH–3.8A rotor, Beckman Coulter, 128 Palo Alto CA). The bottom chloroform layer was transferred to a new test tube (test tube B). Another 2 mL of chloroform was added to test tube A, vortexed, centrifuged, and the chloroform layer was again transferred to test tube B. The chloroform containing the total extracted lipid was evaporated under N_2 and reconstituted in 400 µl of toluene (ACS reagent, ≥99.5%, Sigma-Aldrich, St. Louis, MO, USA; Cat #179418).

2.4 Fatty acid composition

For fatty acid transesterification, 3 mL of methanol was added to the sample containing extracted lipids and toluene, followed by the addition of 600 µl of 8% HCl (ACS reagent, 37%, Sigma-Aldrich, St. Louis, MO, USA; Cat #320331) in methanol. The sample was vortexed and heated at 90°C on a dry heating block for 60 minutes. Samples were cooled to room temperature (~23°C); 1 mL each of hexane (for HPLC, ≥97.0%, Sigma-Aldrich, St. Louis, MO, USA; Cat #34859) and deionized distilled water were added, then vortexed. Phase separation was allowed to occur for approximately 15 minutes, then 900 µL of the top hexane layer was transferred to micro-centrifuge tubes containing 450 µl of deionized water. Micro-centrifuge tubes were centrifuged at 16,627 × g for 2 minutes at 4°C and the hexane layer was transferred to another set of tubes; solvents were evaporated under N_2 and the remaining content reconstituted with 100 µL hexane.

Reconstituted samples were transferred to amber gas chromatography vials containing inserts. Fatty acid methyl esters were analyzed on a Perkin-Elmer Clarus 500 gas chromatography

system coupled to a flame ionization detector (PerkinElmer, Inc., Shelton, Connecticut, US). A DB-FFAP nitroterephthalic-acid-modified polyethylene glycol (PEG) capillary column (FFAP; 30 m × 0.25 mm inner diameter, 0.25 µm film thickness; Agilent Technologies, Santa Clara, California, US) was used. The injector and detector temperatures were 240°C and 300°C, respectively, and the initial oven temperature was 80°C and was programmed as follows. After 2 minutes, the oven temperature increased by 10°C/minute to 185°C at the time of sample injection. The temperature increased by 5°C/minute to 240°C and was held for 13 minutes for a total run time of 36.5 minutes. Helium was the carrier gas at a flow rate of 1.3 mL/minute. The injection volume was 1 µL and the split ratio was set to 10:1. TotalChrom (version 6.3.2.0646; PerkinElmer, Inc., Shelton, Connecticut, US) software was used for data collection and peak area integration. Peaks were identified based on their retention times, determined through injection of a standard mixture containing 29 fatty acid methyl esters (Nu-Chek Prep, Elysian, MN, USA). Concentration of fatty acids were calculated as shown in Equation 1.

Equation 1. $$\frac{\left(\frac{Amount\ of\ triheptadecanoic\ acid\ (C17{:}0)(mg)}{Area\ of\ triheptadecanoic\ acid\ (C17{:}0)}\right)\times Area\ of\ FA\ peak}{Weight\ of\ sample\ (g)} = Concentration\ of\ FA\ \left(\frac{mg}{g}\right)$$

The concentrations were then added together and individually divided by the total to obtain % of total FA as the final unit for statistical comparison.

2.5 Statistical analysis

Analyses of data were performed in R 4.0.0 (R Core Team, 2020; RStudio Team, 2022) to determine significance ($P \leq 0.05$). The main effects of the embryo tissues were treatment, day of incubation, and their interaction. The differences among treatments were determined using one-way repeated measures ANOVA for the main effects and their interactions. Appropriate post-hoc analysis was used if the interaction was significant. If an effect had significant differences, pairwise contrasts were made with confidence levels of 0.95 using Tukey's method for comparing estimates.

3. Results

3.1 Yolks (Table 1)

There were no significant effects on myristoleic acid (C14:1), palmitic acid (PA; C16:0), palmitoleic acid (C16:1), margaric acid (C17:0), oleic acid (C18:1 cis), linoleic acid (LA; C18:2 n-6), behenic acid (C22:0), 11, 14-eicosadienoic acid (EDA; C20:2 n-6), dihomo-γ-linolenic acid (DGLA; C20:3 n-6), docosapentaenoic acid n-6 (DPA n-6; C22:5 n-6), docosahexaenoic acid (DHA; C22:6 n-3), total PUFA, total n-6 PUFA, and SFA:PUFA.

3.1.1 Treatment effects

TN had significantly less pentadecylic acid, ALA, DPA n-3, and total n-3 ($P \leq 0.04$), but significantly more n-6:n-3 than TNS ($p=0.0005$). TN had significantly less DPA n-3 than HS ($p=0.0018$). TN had significantly less myristic acid, ARA, 13,16-docosadienoic acid, and DPA n-3 ($P \leq 0.04$) and significantly more erucic acid than HSS ($p=0.015$). TNS had significantly more stearic acid, erucic acid, and SFA than HSS ($P \leq 0.02$). HS had significantly less ARA then HSS ($p=0.023$).

3.2 Embryo brains

3.2.1 Day within treatment and day by treatment effects (Table 2)

TN versus TNS. TN d11 had significantly more myristic acid and SFA:PUFA ($P \leq 0.008$) and significantly less total PUFA than TN d13 ($p=0.014$). TN d11 had significantly more myristic acid, PA, margaric acid, and SFA:PUFA ($P \leq 0.03$) and significantly less stearic acid, DGLA, EDA, DPA n-6, and total PUFA than TN d15 ($P \leq 0.04$). TN d13 had significantly more margaric acid ($p=0.013$) and significantly less stearic acid than TN d15 ($P<0.0001$).

TN d11 had significantly more myristic acid, erucic acid, and 13,16-docosadienoic ($P \leq 0.05$) and significantly less DGLA and EDA then TNS d13 ($P \leq 0.04$). TN d11 had significantly

more myristic acid, PA, palmitoleic acid, margaric acid, erucic acid, SFA, and SFA:PUFA (P≤0.03) and significantly less stearic acid, gondoic acid, DGLA, EDA, DPA n-6, and total PUFA than TNS d15 (P≤0.02). TN d13 had significantly more stearic acid, total n-3, and total PUFA (P≤0.05) and significantly less myristic acid and SFA:PUFA than TNS d11 (P≤0.02). TN d13 had significantly more erucic acid than TNS d13 (p=0.0093). TN d13 had significantly more myristic acid, margaric acid, LA, and erucic acid (P≤0.02) and significantly less stearic acid, gondoic acid, and EDA than TNS d15 (P≤0.007). TN d15 had significantly less myristic acid, PA, palmitoleic acid, and margaric acid (P≤0.05) and significantly more stearic acid, DPA n-6, total n-3, and total PUFA than TNS d11 (P≤0.04). TN d15 had significantly more stearic acid and erucic acid than TNS d13 (P≤0.002). TN d15 had significantly more erucic acid (p=0.0001) and significantly less gondoic acid and total MUFA than TNS d15 (P≤0.03).

TN versus HS. TN d11 had significantly less myristoleic acid, ARA, total MUFA, total n-6, and n-6:n-3 (P≤0.04) and significantly more erucic acid and total SFA than HS d11 (P≤0.003). TN d11 had significantly more myristic acid, erucic acid, and SFA:PUFA (P≤0.002) and significantly less myristoleic acid than HS d13 (p=0.0027).

TN d11 had significantly more myristic acid, PA, margaric acid, erucic acid, and SFA:PUFA (P≤0.03) and significantly less myristoleic acid, stearic acid, DPA n-6, and total PUFA than HS d15 (P≤0.05). TN d13 had significantly more erucic acid, DHA, and total n-3 (P≤0.02) and significantly less myristic acid, myristoleic acid, ARA, and n-6:n-3 than HS d11 (P≤0.05). TN d13 had significantly more erucic acid (P<0.0001) and significantly less myristoleic acid and pentadecylic acid than HS d13 (P≤0.05). TN d13 had significantly more LA and erucic acid (P≤0.005) and significantly less stearic acid than HS d15 (p=0.0005). TN d15 had significantly more stearic acid, erucic acid, DHA, total n-3, and total PUFA (P≤0.05) and significantly less

myristic acid, margaric acid, LA, total MUFA, and n-6:n-3 than HS d11 (P≤0.04). TN d15 had significantly more erucic acid (P<0.0001) and significantly less margaric acid than HS d13 (P≤0.005). TN d15 had significantly more erucic acid than HS d15 (P<0.0001).

TN versus HSS. TN d11 had significantly less myristoleic acid and total MUFA (P≤0.02) and significantly more erucic acid than HSS d11 (p=0.025). TN d11 had significantly more myristic acid (p=0.0002) and significantly less myristoleic acid, EDA, and erucic acid than HSS d13 (P≤0.03). TN d11 had significantly less myristoleic acid, stearic acid, DGLA, EDA, and DPA n-6 and significantly more myristic acid, PA, margaric acid, erucic acid, and 13,16-docosadienoic than HSS d15 (P≤0.03). TN d13 had significantly less myristic acid, myristoleic acid, and pentadecylic acid (P≤0.02) and significantly more stearic acid, erucic acid, and total PUFA than HSS d11 (P≤0.03). TN d13 had significantly less myristoleic acid, pentadecylic acid, and erucic acid than HSS d13 (P≤0.02) and significantly less myristoleic acid, stearic acid, and EDA than HSS d15 (P≤0.006). TN d15 had significantly less myristic acid, pentadecylic acid, margaric acid, and total MUFA (P≤0.05) and significantly more stearic acid, erucic acid, and total PUFA than HSS d11 (P≤0.02). TN d15 had significantly less myristoleic acid, margaric acid, erucic acid, and total MUFA (P≤0.05) and significantly more stearic acid than HSS d13 (P<0.0001). TNS d15 had significantly less myristoleic acid (p=0.046) and significantly more erucic acid than HSS d15 (P<0.0001).

TNS versus HS. TNS d11 had significantly more myristic acid than TNS d13 (p=0.0022). TNS d11 had significantly more myristic acid, PA, palmitoleic acid, margaric acid, LA, and SFA:PUFA (P≤0.02) and significantly less stearic acid, gondoic acid, EDA, and total PUFA than TNS d15 (P≤0.005). TNS d13 had significantly more myristic acid, PA, and LA (P≤0.02) and significantly less stearic acid and gondoic acid than TNS d15 (P≤0.0003).

TNS d11 had significantly more erucic acid than HS d11 (p=0.022). TNS d11 had significantly less total PUFA (p=0.024) and significantly more myristic acid, erucic acid, and SFA:PUFA than HS d13 (P≤0.04). TNS d11 had significantly less stearic acid, DHA, total n-3, and total PUFA (P≤0.01) and significantly more myristic acid, PA, palmitoleic acid, margaric acid, LA, and erucic acid than HS d15 (P≤0.03). TNS d13 had significantly less myristic acid and ARA than HS d11 (P≤0.05). TNS d13 had significantly more PA and LA (P≤0.02) and significantly less stearic acid than HS d15 (P≤0.02). TNS d15 had significantly more stearic acid, gondoic acid, EDA, and total n-3 (P≤0.04) and significantly less myristic acid, palmitoleic acid, margaric acid, LA, and ARA than HS d11 (P≤0.03). TNS d15 had significantly less myristic acid, palmitoleic acid, and margaric acid and significantly more gondoic acid, DGLA, and EDA than HS d13 (P≤0.02). TNS d15 had significantly more gondoic acid than HS d15 (p=0.0093).

TNS versus HSS. TNS d11 had significantly less erucic acid (P<0.0001) and significantly more myristic acid than HSS d13 (p=0.0039). TNS d11 had significantly more myristic acid, PA, palmitoleic acid, margaric acid, LA, and erucic acid and significantly less stearic acid and DPA n-6 than HSS d15 (P≤0.03). TNS d13 had significantly less myristic acid than HSS d11 (P≤0.007). TNS d13 had significantly less myristoleic acid and erucic acid than HSS d13 (P≤0.03). TNS d13 had significantly less stearic acid (P≤0.002) and significantly more PA and LA than HSS d15 (P≤0.03). TNS d15 had significantly less myristic acid, PA, palmitoleic acid, and margaric acid (P≤0.003) and significantly more stearic acid, DGLA, EDA, and total PUFA than HSS d11 (P≤0.04). TNS d15 had significantly less myristic acid, PA, palmitoleic acid, margaric acid, and erucic acid (P≤0.005) and significantly more stearic acid and gondoic acid than HSS d13 (P≤0.003).

HS versus HS. HS d11 had significantly more myristic acid than HS d13 (p=0.0017). HS d11 had significantly more myristic acid, margaric acid, LA, ARA, and n-6:n-3 ($P \leq 0.05$) and significantly less stearic acid, DHA, and total n-3 than HS d15 ($P \leq 0.0004$). HS d13 had significantly more LA (p=0.037) than HS d15.

HS versus HSS. HS d11 had significantly more ARA than HSS d11 (p=0.042). HS d11 had significantly more myristic acid (p=0.0074) and significantly less erucic acid than HSS d13 ($P < 0.0001$). HS d11 had significantly more myristic acid, margaric acid, LA, ARA, and n-6:n-3 and significantly less stearic acid, DHA, and total n-3 than HSS d15 ($P \leq 0.05$). HS d13 had significantly less myristic acid than HSS d11 (p=0.0001) and significantly less erucic acid than HSS d13 ($P < 0.0001$). HS d13 had significantly less DGLA ($P \leq 0.03$) than HSS d15. HS d15 had significantly more stearic acid, DHA, and total n-3 and significantly less myristic acid and PA than HSS d11 ($P \leq 0.03$). HS d15 had significantly more stearic acid ($P < 0.0001$) and significantly less margaric acid, LA, and erucic acid than HSS d13 ($P \leq 0.03$).

HSS versus HSS. HSS d11 had significantly more myristic acid (p=0.0004) and significantly less erucic acid than HSS d13 ($P < 0.0001$). HSS d11 had significantly more myristic acid and PA ($P \leq 0.02$) and significantly less stearic acid and DGLA than HSS d15 ($P \leq 0.05$). HSS d13 had significantly more myristic acid, margaric acid, and erucic acid and significantly less stearic acid and DPA n-6 than HSS d15 ($P \leq 0.05$).

3.2.2 Day effect (Table 3)

When fatty acids were compared by day, myristic acid and palmitoleic acid significantly increased with d11 > d13 > d15. However, stearic acid significantly decreased with d11 < d13 < d15 ($P < 0.0001$). PA and margaric acid were significantly higher in d11 and d13 compared to d15 ($P < 0.0001$). Oleic acid, LA, gondoic acid, EDA, and DPA n-6 were significantly lower in d11 and

13 compared to d15 (P<0.009). DGLA, DHA, total PUFA, and total n-3 PUFA were significantly lower on d11 than d13 and d15 and SFA:PUFA was significantly higher on d11 than on d13 and 15 (P<0.0001). Elaidic acid, ARA, 13,16-docosadienoic acid, and n-6:n-3 had significantly higher abundance on d11 than d15 (P≤0.04). Erucic acid had significantly higher abundance on d13 compared to d15 (p=0.02).

3.2.3 Treatment effect (Table 3)

TN and TNS had significantly less myristoleic acid than HS and HSS (P<0.0001). Elaidic acid and total MUFA were significantly lower in TN than all other treatments (P<0.009). TN had significantly less pentadecylic acid than HS and HSS (P<0.009). TN had significantly less EDA than TNS and HSS (P<0.009). TN also had significantly more total SFA than HS (p=0.03). TN had significantly more erucic acid than TNS and HS; HS had significantly less erucic acid than HSS (P<0.0001).

3.3 Embryo kidneys/gonads

As seen in Table 6, there were no significant effects on myristic, pentadecylic, margaric, EDA, DGLA, EPA, erucic acid, 13, 16-docosadienoic acid, DPA n-6, DPA n-3, and SFA:PUFA.

3.3.1 Day within treatment and day by treatment effects (Table 4)

TN versus TN. TN d11 had significantly more oleic acid (P<0.009) and significantly less arachidic acid and LA than TN d13 (p=0.042). TN d11 had significantly more total SFA (P<0.02) and significantly less LA and arachidic acid than TN d15 (p=0.0005).

TN versus TNS. TN d11 had significantly more oleic acid than TNS d11 (p=0.0066). TN d11 had significantly more stearic acid and oleic acid than TNS d13 (P<0.05). TN d11 had significantly more PA and oleic acid and significantly less LA, arachidic acid, ARA, behenic acid, total PUFA, and total n-6 than TNS d15 (P<0.02). TN d13 had significantly more elaidic acid and

total MUFA (P<0.05) and significantly less ARA than TNS d15 (p=0.0027). TN d15 had significantly more arachidic acid than TNS d11 (p=0.0099), significantly more behenic acid and n-6:n-3 than TNS d13 (P<0.03).

TN versus HS. TN d11 had significantly less elaidic acid, arachidic acid, and n-6:n-3 than HS d13 (P<0.03). TN d11 had significantly less LA, arachidic acid, and behenic acid than HS d15 (P<0.04). TN d13 had significantly less oleic acid and significantly more LA than HS d11 (P<0.003). TN d15 had significantly more LA, arachidic acid, and ARA than HS d11 (P<0.05). TN d15 had significantly less PA and significantly more ARA than HS d13 (P<0.03).

TN versus HSS. TN d11 had significantly less elaidic acid and total MUFA than HSS d11 (P<0.05). TN d11 had significantly less LA and gondoic acid (p=0.014) and significantly more stearic acid and total SFA than HSS d13 (P<0.006). TN d11 had significantly less arachidic acid than HSS d15 (p=0.019). TN d13 had significantly more behenic acid than HSS d13 (p=0.028). TN d15 had significantly less elaidic acid and significantly more arachidic acid and behenic acid than HSS d11 (P<0.02). TN d15 had significantly more behenic acid than HSS d13 (p=0.0058).

TNS versus TNS. TNS d11 had significantly less arachidic acid, ARA, and behenic acid than TNS d15 (P<0.04). TNS d13 had significantly less behenic acid and total n-6 than TNS d15 (P<0.03).

TNS versus HS. TNS d11 had significantly less oleic acid than HS d11 (p=0.0024), significantly less arachidic acid than HS d13 (p=0.012), and significantly less arachidic acid and behenic acid than HS d15 (P<0.03). TNS d13 had significantly less oleic acid than HS d11 (P<0.05), significantly less behenic acid and n-6:n-3 than HS d13 (P<0.04), and significantly less behenic acid than HS d15 (P<0.03). TNS d15 had significantly less PA, oleic acid, and total MUFA and significantly more LA, arachidic acid, ARA, behenic acid, total PUFA, and total n-6 than HS

d11 (P<0.02). TNS d15 had significantly less PA, oleic acid, elaidic acid, and total MUFA (P<0.04) and significantly more ARA, total PUFA, total n-6, and total n-3 than HS d13 (P<0.02).

TNS versus HSS. TNS d13 had significantly less oleic acid than HSS d11 (p=0.014). TNS d15 had significantly less oleic acid, elaidic acid, and total MUFA (P<0.005) and significantly more arachidic acid, ARA, and behenic acid than HSS d11 (P<0.003). TNS d15 had significantly more behenic acid than HSS d13 (P<0.0001).

HS versus HS. HS d11 had significantly less LA and arachidic acid than HS d13 (P<0.02). HS d11 had significantly more oleic acid and total MUFA (P<0.03) and significantly less LA and arachidic acid than HS d15 (p=0.0072). HS d13 had significantly more elaidic acid and total MUFA than HS d15 (P<0.04).

HS versus HSS. HS d11 had significantly less LA than HSS d11 (p=0.020). HS d13 had significantly more behenic acid than HSS d13 (P<0.04). HS d15 had significantly more arachidic acid and behenic acid (P<0.03) and significantly less elaidic acid and total MUFA than HSS d11 (P<0.007). HS d15 had significantly more behenic acid than HSS d13 (p=0.0007).

HSS versus HSS. For the comparison of HSS by day, HSS d11 had significantly more total MUFA than d13 (p=0.015). HSS d11 had significantly less arachidic acid than HSS d15 (p=0.027).

3.3.2 Day effect (Table 5)

D11 had significantly more stearic acid and n-6:n-3 and significantly less arachidic acid than d13 (P<0.03). D11 had significantly more myristic acid, PA, oleic acid, elaidic acid, DPA n-6, total MUFA, and SFA:PUFA and significantly less LA, ARA, EPA, behenic acid, total PUFA, total n-6, and n-6:n-3 than d15 (P<0.04). d13 had significantly more gondoic acid and total MUFA (P<0.03) and significantly less arachidic acid, DGLA, ARA, behenic acid, total PUFA, and total n-6 than d15 (P<0.01).

3.3.3 Treatment effect (Table 5)

TN had significantly more elaidic acid than TNS (p=0.027). TNS had significantly less PA and margaric acid (P<0.04) and significantly more DHA and total n-3 than HS (P<0.02). HS had significantly more behenic acid and total SFA than HSS (P<0.05).

3.4 Embryo livers

3.4.1 Day within treatment and day by treatment effects (Table 6)

As seen in Table 7, there were no significant effects on myristic acid, ALA, DGLA, ARA, erucic acid, 13, 16-docosadienoic acid, total MUFA, total n-3 PUFA, and n-6:n-3.

TN versus TN. TN d11 had significantly more PA, DPA n-6, and DHA than TN d13 (P<0.005). TN d11 had significantly more pentadecylic acid, PA, margaric acid, DPA n-6, and DHA (P<0.04) and significantly less LA, arachidic acid, and EPA than TN d15 (P<0.004). TN d13 had significantly more PA and significantly less arachidic acid than TN d15 (P<0.02).

TN versus TNS. TN d11 had significantly more PA, DPA n-6, DHA, and total SFA and significantly less LA than TNS d13 (P<0.03). TN d11 had significantly more pentadecylic acid, PA, DPA n-6, DPA n-3, DHA, total SFA, and SFA:PUFA and significantly less oleic acid, LA, and EPA than TNS d15 (P<0.04). TN d13 had significantly more LA and significantly less PA and margaric acid than TNS d11 (P<0.009). TN d13 had significantly more pentadecylic acid and PA than TNS d15 (P<0.02). TN d15 had significantly more palmitoleic acid, oleic acid, LA, and arachidic acid and significantly less pentadecylic acid, PA, margaric acid, and DHA than TNS d11 (P<0.03). TN d15 had significantly more palmitoleic acid and arachidic acid and significantly less PA and margaric acid than TNS d13 (P<0.006). TN d15 had significantly more palmitoleic acid than TNS d15 (p=0.039).

TN versus HS. TN d11 had significantly less margaric acid (p=0.032) and significantly more DHA than HS d11 (p=0.0078). TN d11 had significantly more PA and DHA than HS d13 (P<0.004). TN d11 had significantly less stearic acid and LA (P≤0.0004) and significantly more pentadecylic acid, PA, DPA n-6, DPA n-3, and DHA than HS d15 (P<0.04). TN d13 had significantly less PA, margaric acid, and DPA n-6 and significantly more total n-6 and LA than HS d11 (P<0.02). TN d15 had significantly less PA, margaric acid, total SFA, and SFA:PUFA (P<0.009) and significantly more oleic acid, LA, and total n-6 than HS d11 (P<0.04). TN d15 had significantly less pentadecylic acid and PA than HS d13 (P<0.0008).

TN versus HSS. TN d11 had significantly more PA, DPA n-6, and DHA (P<0.006) and significantly less stearic acid and LA than HSS d13 (P<0.05). TN d11 had significantly less oleic acid, LA, and behenic acid and significantly more PA, DPA n-6, DHA, and total PUFA than HSS d15 (P≤0.02). TN d13 had significantly less PA and margaric acid (P<0.006) and significantly more LA and total n-6 than HSS d11 (P<0.04). TN d13 had significantly less oleic acid (P<0.0003) and significantly more PA and behenic acid than HSS d15 (p=0.022). TN d15 had significantly less pentadecylic acid, PA, margaric acid, and DHA (P<0.008) and significantly more total n-6, palmitoleic acid, oleic acid, LA, and arachidic acid than HSS d11 (P<0.04). TN d15 had significantly less pentadecylic acid, PA, and margaric acid (P<0.03) and significantly more EPA and EDA than HSS d13 (p=0.038). TN d15 had significantly more behenic acid and significantly less oleic acid than HSS d15 (p=0.03).

TNS versus TNS. TNS d11 had significantly less LA and significantly more PA than TNS d13 (P≤0.0001). TNS d11 had significantly more pentadecylic acid, PA, margaric acid, DPA n-6, DPA n-3, DHA, and total SFA (P<0.04) and significantly less oleic acid and LA than TNS d15

(P≤0.0008). TNS d13 had significantly more pentadecylic acid, PA, and margaric acid than TNS d15 (P<0.01).

TNS versus HS. TNS d11 had significantly less stearic acid and behenic acid (P<0.03) and significantly more PA than HS d13 (p=0.0022). TNS d11 had significantly less stearic acid, oleic acid, and LA (P<0.03) and significantly more pentadecylic acid, PA, margaric acid, and DHA than HS d15 (P<0.05). TNS d13 had significantly less PA, DPA n-6, total SFA, and SFA:PUFA (P<0.009) and significantly more total n-6 and LA than HS d11 (P<0.03). TNS d13 had significantly less total SFA than HS d13 (p=0.019) and significantly more PA than HS d15 (p=0.0021). TNS d15 had significantly less PA, margaric acid, DPA n-6, total SFA, and SFA:PUFA (P<0.0001) and significantly more oleic acid and LA than HS d11 (P<0.003). TNS d15 had significantly less pentadecylic acid, PA, DPA n-6, total SFA, and SFA:PUFA (P<0.02) and significantly more oleic acid than HS d13 (p=0.028). TNS d15 had significantly less stearic acid, total SFA, and SFA:PUFA than HS d15 (P<0.04).

TNS versus HSS. TNS d11 had significantly less stearic acid and LA (P<0.05) and significantly more PA, behenic acid, and DHA than HSS d13 (P<0.009). TNS d11 had significantly less oleic acid and LA and significantly more PA, behenic acid, and DHA than HSS d15 (P≤0.0004). TNS d13 had significantly less PA, DPA n-6, total SFA, and SFA:PUFA (P<0.02) and significantly more LA than HSS d11 (P<0.0001). TNS d13 had significantly less SFA:PUFA than HSS d13 (p=0.019). TNS d13 had significantly less oleic acid (P<0.04) and significantly more PA, behenic acid, DHA, and total PUFA than HSS d15 (P<0.02). TNS d15 had significantly less pentadecylic acid, PA, margaric acid, DPA n-6, DPA n-3, DHA, total SFA, and SFA:PUFA (P<0.05) and significantly more oleic acid and LA than HSS d11 (P<0.003). TNS d15 had

significantly less pentadecylic acid, PA, margaric acid, DPA n-3, total SFA, and SFA:PUFA ($P<0.02$).

HS versus HS. HS d11 had significantly more PA and margaric acid ($P<0.006$) and significantly less LA than HS d13 ($p=0.021$). HS d11 had significantly more PA, margaric acid, gondoic acid, and DPA n-6 ($P<0.003$) and significantly less LA than HS d15 ($P<0.03$). HS d13 had significantly more pentadecylic acid, PA, and DPA n-6 than HS d15 ($P<0.03$).

HS versus HSS. HS d11 had significantly more PA and DPA n-6 and significantly less LA than HSS d13 ($P\leq0.0005$). HS d11 had significantly more PA, margaric acid, behenic acid, DPA n-6, DHA, and total SFA ($P<0.05$) and significantly less oleic acid and LA than HSS d15 ($P<0.03$). HS d13 had significantly less PA ($p=0.0001$) and significantly more LA than HSS d11 ($p=0.0037$). HS d13 had significantly more DPA n-6 than HSS d13 ($p=0.0067$). HS d13 had significantly more PA and DPA n-6 ($P<0.03$) and significantly less oleic acid than HSS d15 ($P<0.0001$). HS d15 had significantly more stearic acid and LA ($P<0.0008$) and significantly less pentadecylic acid, PA, margaric acid, DPA n-6, and DHA than HSS d11 ($P<0.02$). HS d15 had significantly less pentadecylic acid and PA than HSS d13 ($P<0.004$) and significantly less oleic acid and significantly more behenic acid than HSS d15 ($P<0.05$).

HSS versus HSS. HSS d11 had significantly less stearic acid and LA ($P<0.02$) and significantly more PA and DPA n-6 than HSS d13 ($P<0.004$). HSS d11 had significantly more PA, behenic acid, DPA n-6, and DHA ($P<0.02$) and significantly less oleic acid and LA than HSS d15 ($P<0.003$). HSS d13 had significantly more PA and significantly less oleic acid than HSS d15 ($P<0.0001$).

3.4.2 Day effect (Table 7)

D11 had significantly more myristic acid, PA, margaric acid, behenic acid, DPA n-6, DHA, and total SFA and significantly less stearic acid, oleic acid, LA, ARA, and total n-6 than d13 ($P<0.04$). D11 had significantly more myristic acid, pentadecylic acid, PA, margaric acid, gondoic acid, DPA n-6, DPA n-3, DHA, total SFA, and SFA:PUFA ($P<0.04$) and significantly less palmitoleic acid, stearic acid, oleic acid, LA, arachidic acid, gondoic acid, EDA, EPA, and total n-6 than d15 ($P<0.02$). D13 had significantly more pentadecylic acid, PA, margaric acid, EDA, ARA, DHA, and total PUFA ($P<0.05$) and significantly less oleic acid, LA, arachidic acid, and EDA than d15 ($P<0.005$).

3.4.3 Treatment effect (Table 7)

TN had significantly more palmitoleic acid, total SFA, and total MUFA than TNS ($P<0.04$). TN had significantly less margaric acid, stearic acid, and SFA:PUFA than HS ($P<0.03$). TN had significantly more EDA, behenic acid, total PUFA and total n-6 ($P<0.02$) and significantly less margaric acid than HSS ($P<0.04$). HS had significantly more palmitoleic acid, stearic acid, DPA n-6, total SFA, total MUFA, and SFA:PUFA than TNS ($P<0.05$). TNS had significantly more ALA, behenic acid, erucic acid, and total PUFA and significantly less palmitoleic acid, DPA n-3, total SFA, total MUFA, and SFA:PUFA than HSS. HS had significantly less oleic acid and significantly more DPA n-6 than HSS ($P<0.05$).

4. Discussion

4.1 Yolk

Although plasma fatty acid composition was not tested in the current study, it was expected that the composition of lipids in the yolk would be very similar to those that are in the plasma (Noble and Cocchi, 1990). Others have also reported that PA (22 to 26%) and stearic acid (8-10%)

are the main SFA in yolk (Cherian, 2005; da Silva, et al. 2009; Cherian and Quezada, 2016). Results of the current study were in agreement with the findings of these investigators because PA ranged from 21.8% to 22.9% and stearic acid ranged from 10.9% to 14.8% of the total yolk FA (Table 1).

Results of the current study also revealed total SFA were similar to that reported by others who found that MUFA comprises about 42-46% the chicken egg lipids and that the main MUFA are palmitoleic acid and oleic acid (Cherian, 2005; Cherian and Quezada, 2016). However, the amount of MUFA in egg are influenced by diet. Findings of the current study also agreed with findings of others indicating that the predominant MUFA are palmitoleic acid and oleic acid (Cherian, 2005; da Silva, et al., 2009). PUFA composition is also heavily influenced by the diet. Results of the current study agreed with results of others indicating that the most abundant PUFA in the quail yolks are LA and ARA (Cherian, 2005; da Silva, et al., 2009).

The main n-3 fatty acid is DHA and one of the main n-6 fatty acid is ARA. Results of studies noted that a deficiency in either impair visual acuity and result in reduced cognitive performance (Cherian, 2005; Tallima and El Ridi, 2018). However, when layer diets were supplemented with DHA and ARA, there was preferential incorporation into the yolk and, subsequently, into the chick especially in the last week of incubation (Cherian, 2005). In the current study, there was no significant treatment effect on DHA; however, HSS had significantly more ARA than TN and HS which could indicate that when chosen for low FCR in heat stress, more inflammation may occur in the embryo brain. Higher levels of DPA n-3 in the embryo brains of TNS, HS, and HSS compared to TN could indicate that DPA n-3 was preferentially being used in the brain due to a deficiency of DHA in these treatments.

Additionally, birds fed fish oil in their diet had high levels of EPA and DHA which are n-3 long-chain fatty acids (Güçlü, et al., 2008). With higher levels of n-3 long-chain fatty acids, there was a decrease in total n-6 PUFA in egg yolk (Güçlü, et al., 2008). The concentration of n-6 and n-3 in the yolk can influence the metabolic and immune tissue functions during development and these effects may persist post-hatch (Cherian, 2011). In the present work, there was no treatment effect on n-6:n-3, thus, indicating that chicks in various treatments would not have significantly different metabolic and immune tissue functions during development.

When housed in a stable environment, the yolk composition and hepatic synthesis of yolk lipids is expected to be uniform (Noble and Cocchi, 1990). However, eggs laid by HSS females were not housed in a stable environment due to the daily cycling between 22.2˚C and 31.1˚C. This may have caused the differences observed between HSS and TNS where TNS had significantly more stearic acid, ALA, and total SFA (Table 1). This was opposite of expectations that HSS would have more stearic acid and SFA due to oxidation of PUFA in higher temperatures.

4.2 Embryo brains

In the present work, amounts of PA, palmitoleic acid, stearic acid, oleic acid, ARA, and DHA throughout incubation were similar to findings of others for newly hatched chicken (Cherian and Sim, 1992). However, results from the present study were most similar to the fatty acid composition of chick brains fed a high LA diet (Cherian and Sim, 1992). This is reflective of the high LA found in the diet from **Chapter 3: Fatty acid composition of multigenerational heat stressed adult Japanese quail (*Coturnix coturnix japonica)***.

As expected, during progressive incubation, there was an increase in PUFA such as DHA and DPA in the embryo brain (Noble and Cocchi, 1990; *Table 5*). The percentages of ARA and DHA in the current study do not agree with the 10-15% of total lipids in newly hatched chicks that

others have reported (Cherian, 2015). In the current study, ARA and DHA each accounted for approximately 10% of the total fatty acid content of the embryo brain throughout development (*Table 2*). Due to the high levels of Δ6-desaturase and Δ9-desaturase in the yolk sac membrane, there should be more arachidonic and oleic acids in the embryo compared to those fatty acids that were deposited into the yolk by the parent (Noble and Cocchi, 1990). However, it was not expected to see a higher amount of ARA and DPA n-6 in d11 than in d15 in the present study.

As incubation progressed, PA and LA decreased as expected due to utilization and elongation by enzymes in the liver (Su, et al., 2020). This could also explain the increase in PUFA and the decrease in SFA:PUFA as incubation progressed. There were also more n-3 and lower n-6:n-3 as incubation progressed, indicating more conversion of ALA to n-3 fatty acids as expected. Stearic acid also increased as incubation progressed which could be an indication that there was more conversion of stearic acid to oleic acid with increased Δ9-desaturase activity (Noble and Cocchi, 1990).

In humans that have deficient DHA during preweaning brain development, there can be excessive hypothalamus-pituitary-adrenal axis response to stress which causes anxiety-like behaviors in adults (Laviola and Macrì, 2013). Early life, especially in utero and in ovo, can significantly impact long-term adjustments to the new post birth and post hatch environment (Laviola and Macrì, 2013). Δ6-Desaturase converts LA to ARA; others have observed that chicks hatched from eggs enriched with n-3 have less Δ6-desaturase activity (Cherian and Sim, 2001). The higher levels of ARA and less n-3 in HS compared to TN could be a result of higher levels of Δ6-desaturase in HS (Su, et al., 2020). The lower levels of SFA in HS compared to TN was unexpected because quail subjected to heat stress were expected to have less SFA due to increased sensitivity to oxidation.

4.3 Embryo kidneys/gonads

To the author's knowledge, there is little published information about the fatty acid composition of embryonic quail kidneys (Surai, et al., 1999). However, studies on kidneys of gallus gallus domesticus chicks' fatty acid composition reported 11.84% LA, 19.90% ARA, and 6.25% DHA (Surai, et al., 1999). The current study had lower levels of LA, ARA, and DHA than those reported in the newly hatched chicks (Surai, et al., 1999). The amount of PUFA found in the kidneys/gonads was largely dependent on the levels of antioxidants and the availability of pro-oxidants such as Fe and Cu (Surai, et al., 1999).

The significantly higher levels of total SFA and PA and lower levels of ARA, DHA, and n-3 in HS as compared to TNS or TN may indicate that heat stress caused SFA to become more resistant to oxidative stress as has been suggested (Tsunekage, 2015). However, HS had higher levels of SFA than HSS, which may indicate that HSS did not develop adaptations to heat stress when compared to HS. HSS and TNS both had significantly lower levels of SFA than HS, which could be due to their relatedness to each other. Lack of significance between HSS and TNS could also suggest that regardless of heat stress, kidneys/gonads of embryos from genetically similar individuals do not change SFA levels.

Similar to adult kidneys, embryo kidneys are responsible for retention of H^+ during respiratory alkalosis which occurs when there is an increase in respiration (Sejian, et al., 2015). This can decrease Na^+ or K^+ in the blood during heat stress (Farag and Alagawany, 2018). Others have noted that chicken embryo kidneys start to function by day 4 of incubation, after blood circulation is established (Zemanová, et al., 2002). Due to the increase of kidney function after day 4 of incubation, it was expected that PUFA would increase due to the importance of PUFA (DHA) in kidney function. Results from the current study show that PUFA increased as incubation

progressed; however, there was no clear correlation between DHA levels and embryonic age, indicating that DHA was not preferentially deposited in kidneys as incubation progressed.

The significantly lower levels of PA, stearic acid, oleic acid, LA, and MUFA in 15-day-old embryos and significantly lower levels of oleic, LA, and n-3 in HS than TNS or HSS indicate that kidneys/gonads were sustaining more cell damage due to increase in toxin removal (Noels, et al., 2021). This coincided with the increase of n-6:n-3 and ARA levels as the quail embryo kidney aged because n-6 PUFA are associated with proinflammatory events (Noels, et al., 2021). Other studies on the effect of various fatty acids on renal function found that SFA such as PA triggered reactive oxygen species production and inflammatory responses (Noels, et al., 2021). Opposing renal reactions to SFA, MUFA, such as oleic acid, increased fatty acid β-oxidation for energy through activation of the AMP-activated protein kinase-acetyl-CoA carboxylase-carnitine palmitoyltransferase 1 axis (Soumara, et al., 2010). Oleic acid, n-3 PUFA, and EPA were shown to have protective effects on renal proximal tubular cells (Soumara, et al., 2010).

4.4 Embryo Livers

In chicken, the full development of the yolk sac membrane and chorioallantoic membrane does not occur until day 10 of incubation. Additionally, there is an increase in the absorptive area of the yolk sac membrane at day 18-19 of incubation leading to the internalization of the yolk sac into the abdominal cavity of the embryo before hatch. Due to the absorption of lipids from the yolk sac membrane, the yolk lipids do not necessarily equate to the embryonic lipid composition due to preferential absorption and use of specific lipids. PUFA is absorbed for membrane development and has preference for specific embryo tissues (van der Wagt, et al., 2020).

The yolk contents are absorbed through the abdominal cavity to be used as nutrients for hatch and there is a sharp increase in production of free radicals due to the increase in oxygen

intake (Yigit, et al., 2014). Therefore, expectations are that total PUFA decrease and SFA increase over the course of incubation as the chick adapts to aerobic energy production. As observed for the present results, there was a decrease in both DHA and PUFA while SFA increased as incubation progressed. These changes may have been due to the enzymatic conversion of ALA to DHA in the yolk sac membrane and increased utilization of DHA due to its importance as a fatty acid in many physiological functions (Cherian, 2011). Triacylglycerides in embryo livers are also reported to be high in DHA (Noble and Cocchi, 1990). Contrary to findings herein, others reported an increase in PUFA in chick embryonic tissue as incubation progressed (Surai, et al., 1999; Cherian and Sim, 2001).

LA is converted to ARA and ALA is converted to DHA; therefore, present results followed this enzymatic sequence with higher levels of LA and ARA as opposed to higher levels of ALA and DHA (da Silva, et al., 2009). The conversion of LA and ALA to longer chain PUFA could also be reflected in the lower SFA:PUFA found in d15 as opposed to d11 in the work reported here. As mentioned above, the diet was high in LA which, as noted by Su, et al. (2020), is converted to ARA (an n-6 PUFA) by Δ6-desaturase, possibly contributing to the higher levels of n-6 in d15 as opposed to d11. Across all 3 embryonic tissues analyzed in the current study, n-6 increased as incubation progressed.

The fatty acid composition of chicks reflected maternal diet up to 3 weeks post-hatch (Liu, 2000). High levels of Δ6-desaturase is expressed in the yolk sac membrane to catalyze linoleic acid to arachidonic acid with maximum activity during days 13 to 15 of incubation for the chicken (Speake, et., 1998). Δ6-Desaturase was observed to be highly expressed in the liver at the end of development (Speake, et al., 1998). As incubation progressed in the present study, LA and ARA

increased, indicating that more LA was being absorbed from the yolk sac as well as more conversion of LA to ARA by Δ6-desaturase.

The composition of fatty acids in the TAG in the yolk should remain unchanged during incubation; however, as reported by others, as incubation progressed, there were lower levels of PA, C20, and C22 PUFA and higher levels of oleic acid (C18:1) in the TAG compared to that in the yolk (Noble and Cocchi, 1990). The results from the current study also reflected these findings with d13 and d15 embryo livers having significantly more oleic acid than d11. As with the brain, stearic acid increased as incubation progressed due to greater mobilization of stearic acid conversion to oleic acid with increased Δ9-desaturase activity (Noble and Cocchi, 1990).

Throughout incubation, TN or HS embryo livers had significantly more n-6, DPA n-6, and n-6:n-3 than HSS or TNS, indicating there may have been more eicosanoids produced in TN than in HSS or TNS (Cherian and Sim, 2001). Eicosanoids can act as pro-inflammatory molecules and are derived from ARA (Tallima and El Ridi, 2018). There was also significantly less DPA n-6 and DPA n-3 as incubation progressed which could be explained by the increasing ARA. Due to the ALA (n-3) and LA (n-6) having competitive binding to Δ6-desaturase to produce either DPA n-3 or ARA, it was apparent that due to the high abundance, LA bound to Δ6-desaturase more, thereby decreasing DPA and increasing ARA (Cherian, 2015).

HSS had more oleic acid, total SFA, total MUFA, and SFA:PUFA, indicating that HSS had less fluid membranes than embryos from TN or TNS. This likely indicated that there was more oxidation of PUFA for energy or oxidative stress in HSS due to their selection for low FCR.

5. Conclusions

From this current study, the most notable results were:

1. In the yolk:

a. HSS had significantly higher levels of LA and ARA than TN, indicating that HSS may have experienced more inflammation from eicosanoids derived from ARA.
b. TNS had significantly more LA, ALA, DPA n-3, and total n-3 than TN and significantly more stearic acid and SFA than HSS indicating there may have been less susceptibility to oxidation of beneficial n-3 fatty acids in TNS.

2. In the embryo brain:
    a. DHA, total PUFA, and stearic acid increased over the duration of incubation as expected for proper brain development.
    b. PA decreased as incubation progressed, most likely due to its conversion to stearic acid.
    c. TN had significantly lower levels of MUFA than all other treatments.
3. In the embryo kidney/gonad:
    a. As was seen in the brain, PA decreased as incubation progressed.
    b. N-6:n-3 increased as incubation progressed.
    c. HS kidney/gonads had more total SFA than TNS and HSS.
4. In the embryo liver:
    a. LA increased as incubation progressed, possibly due to the allocation of decreasing fatty acids (PA, DPA n-6, DHA, and total SFA) to other organs like the brain.
    b. HS had significantly more stearic acid, SFA:PUFA, and total SFA than TN and TNS, possibly as a result of less PUFA availability from the female.
    c. TN and TNS had significantly more PUFA than HSS.

A future study should include analysis of fatty acids in the yolk sac and its membrane to determine the changes occurring during incubation of eggs (30℃ to 35℃). Introduction of labeled

fatty acids in the feed of the hen to determine the percentage of transfer to the embryos is also of interest. If heat stress does not increase PUFA degradation before reaching the embryo, it could indicate that there is enough control over lipid deposition into eggs that embryos of heat stressed quail do not have differing fatty acids than those that are not heat stressed. Thus, determining if PUFA experience more degradation in heat stressed environments before reaching the embryo could be the focus of another study.

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Table 4.1. Fatty acid composition[1] (%) of yolks.

CODE	TN[2]	TNS[2]	HS[2]	HSS[2]	P value	SD[3]
C14:0	0.30[a]	0.34[ab]	0.33[ab]	0.37[b]	0.05	0.05
C14:1	0.04	0.04	0.04	0.05	0.40	0.01
C15:0	0.03[a]	0.04[b]	0.03[ab]	0.03[ab]	0.02	0.01
C16:0	22.20	22.90	21.80	22.20	0.42	1.29
C16:1	2.62	2.53	2.80	2.81	0.61	0.47
C17:0	0.08	0.08	0.08	0.11	0.12	0.03
C18:0	12.70[ab]	14.80[b]	13.20[ab]	10.90[a]	0.02	2.67
C18:1 cis	34.40	33.30	35.60	35.40	0.30	2.68
C18:2 n-6	22.40	20.50	21.10	22.10	0.60	3.13
C18:3 n-3	0.16[a]	0.23[b]	0.20[ab]	0.23[b]	0.02	0.05
C18:3 n-6	0.22[a]	0.30[b]	0.22[a]	0.24[ab]	0.01	0.05
C20:0	0.11	0.11	0.12	0.10	0.75	0.03
C20:2 n-6	0.15	0.14	0.15	0.19	0.36	0.05
C20:3 n-6	0.13	0.14	0.16	0.14	0.35	0.03
C20:4 n-6	2.22[a]	2.27[ab]	2.22[a]	2.58[b]	0.01	0.27
C22:1	0.41[a]	0.41[a]	0.32[ab]	0.28[b]	0.004	0.09
C22:2 n-3	0.09[a]	0.1[ab]	0.12[ab]	0.13[b]	0.03	0.03
C22:5 n-3	0.10[a]	0.15[b]	0.15[b]	0.16[b]	0.0001	0.03
C22:5 n-6	0.11	0.11	0.12	0.10	0.79	0.03
C22:6 n-3	0.82	0.91	0.84	0.97	0.12	0.13
ΣSFA[4]	35.40[ab]	38.30[b]	35.50[ab]	33.70[a]	0.05	3.41
ΣMUFA[4]	37.40	35.90	38.70	38.50	0.18	2.84
ΣPUFA[4]	26.30	24.80	25.30	26.60	0.66	3.22
Σn-6 PUFA[4]	25.00	23.20	23.80	25.10	0.57	3.24
Σn-3 PUFA[4]	1.15[a]	1.45[b]	1.27[ab]	1.32[ab]	0.0046	0.18
ΣSFA:ΣPUFA[4]	1.38	1.55	1.43	1.31	0.35	0.28
Σn-6:Σn-3[4]	21.90[a]	16.10[b]	18.70[ab]	18.40[ab]	0.0011	3.20

[1] Each fatty acid was compared with treatment as the main effect.

[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[3] SD, standard deviation.

[4] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6, omega-6 PUFA; n-3, omega-3 PUFA.

[a-b] superscripts indicate significant differences at $P \leq 0.05$.

Table 4.2. Fatty acid composition[1] (%) of embryo brains compared by treatment × day.

	TN[2]			TNS[2]			HS[2]			HSS[2]				
CODE	d11[3]	d13[3]	d15[3]	d11	d13	d15	d11	d13	d15	d11	d13	d15	P value	SD[4]
C14:0	0.88^{a}	0.69^{b}	0.59^{bcd}	0.85^{a}	0.66^{bd}	0.46^{c}	0.85^{a}	0.65^{bd}	0.57^{bcd}	0.88^{a}	0.67^{b}	0.52^{cd}	0.44	0.16
C14:1	0.06^{a}	0.07^{ab}	0.07^{abc}	0.08^{abcde}	0.08^{abcd}	0.08^{abcde}	0.09^{cde}	0.09^{cde}	0.09^{bcde}	0.09^{cde}	0.10^{e}	0.10^{de}	0.63	0.02
C15:0	0.16^{abc}	0.10^{a}	0.12^{ab}	0.29^{abc}	0.18^{abc}	0.14^{abc}	0.17^{abc}	0.29^{bc}	0.26^{abc}	0.32^{c}	0.32^{bc}	0.23^{abc}	0.06	0.13
C16:0	35.10^{a}	33.40^{abcd}	32.40^{bcd}	34.90^{a}	34.50^{ad}	31.30^{b}	33.40^{abcd}	33.40^{abcd}	32.10^{bc}	34.40^{ad}	34.00^{acd}	32.00^{bc}	0.09	1.67
C16:1	1.59^{abc}	1.48^{abd}	1.29^{ad}	1.76^{bc}	1.50^{abd}	1.19^{d}	1.85^{a}	1.58^{abc}	1.40^{ad}	1.92^{a}	1.61^{abc}	91.38^{ad}	0.60	0.29
C17:0	0.24^{a}	0.22^{abc}	0.18^{de}	0.25^{a}	0.21^{abcd}	0.17^{d}	0.25^{a}	0.23^{bc}	0.19^{bcde}	0.22^{abc}	0.25^{a}	90.18^{bde}	0.02	0.04
C18:0	15.80^{ab}	16.20^{b}	17.70^{c}	15.30^{a}	15.90^{ab}	17.70^{c}	15.30^{ab}	15.80^{abc}	17.50^{c}	15.20^{a}	15.80^{ab}	17.79^{c}	0.81	1.12
C18:1 trans	15.20^{a}	15.00^{abc}	14.10^{abcd}	15.50^{ab}	15.30^{a}	15.60^{d}	16.40^{abcd}	15.80^{abc}	15.00^{bcd}	15.80^{abcd}	15.60^{abc}	15.49^{cd}	0.15	0.93
C18:1 cis	2.31^{abc}	2.39^{ab}	2.63^{a}	2.32^{bc}	2.21^{abc}	2.93^{bc}	2.49^{c}	2.42^{bc}	2.73^{ab}	2.63^{bc}	2.36^{bc}	2.89^{abc}	0.08	0.33
C18:2 n-6	1.84^{abcd}	2.08^{cd}	1.62^{abc}	2.06^{cd}	2.06^{cd}	1.53^{ab}	2.15^{d}	1.99^{bcd}	1.49^{a}	1.81^{abcd}	2.00^{bcd}	1.54^{ab}	0.27	0.36
C20:1 n-9	0.28^{a}	0.30^{a}	0.33^{a}	0.26^{a}	0.28^{a}	0.54^{b}	0.34^{a}	0.29^{a}	0.34^{a}	0.37^{ab}	0.26^{a}	0.37^{ab}	0.00	0.12
C20:2 n-6	0.52^{a}	0.56^{ab}	0.80^{bcd}	0.68^{abc}	0.80^{bcd}	1.04^{d}	0.72^{abc}	0.70^{abc}	0.78^{abcd}	0.69^{abc}	0.80^{bcd}	0.92^{cd}	0.25	0.20
C20:3 n-6	0.22^{a}	0.35^{abc}	0.38^{bc}	0.26^{abc}	0.38^{bc}	0.40^{c}	0.27^{abc}	0.25^{ab}	0.30^{abc}	0.26^{ab}	0.29^{abc}	0.43^{c}	0.13	0.10
C20:4 n-6	10.02^{a}	9.98^{a}	10.13^{ab}	10.45^{ab}	9.92^{a}	9.99^{a}	11.13^{b}	10.64^{ab}	9.81^{a}	10.05^{a}	10.39^{ab}	9.93^{a}	0.02	0.70
C22:1	0.46^{a}	0.46^{a}	0.47^{a}	0.42^{ab}	0.35^{bc}	0.33^{bc}	0.31^{c}	0.29^{c}	0.31^{c}	0.35^{bc}	0.62^{d}	0.29^{c}	0.00	0.11
C22:2 n-3	0.41^{a}	0.37^{ab}	0.29^{ab}	0.36^{ab}	0.24^{b}	0.34^{ab}	0.30^{ab}	0.30^{ab}	0.25^{ab}	0.32^{ab}	0.30^{ab}	0.23^{b}	0.14	0.10
C22:5n-6	3.23^{a}	3.93^{abcd}	4.16^{cd}	3.38^{ab}	3.87^{abcd}	4.09^{bcd}	3.52^{abcd}	3.68^{abcd}	4.01^{bcd}	3.83^{abcd}	3.41^{abc}	4.18^{d}	0.03	0.53
C22:5 n-3	0.47	0.57	0.59	0.48	0.50	0.53	0.51	0.52	0.53	0.57	0.57	0.59	0.50	0.09
C22:6 n-3	11.16^{abc}	11.63^{bc}	11.60^{bc}	10.07^{ab}	11.18^{abc}	11.43^{abc}	9.85^{a}	11.09^{abc}	12.09^{c}	10.28^{ab}	10.53^{abc}	11.44^{bc}	0.17	1.11
ΣSFA[5]	52.20^{a}	50.70^{ab}	51.30^{ab}	51.50^{ab}	51.40^{ab}	50.00^{b}	49.90^{b}	50.40^{ab}	50.90^{ab}	51.00^{ab}	51.20^{ab}	50.80^{ab}	0.01	1.19
ΣMUFA[5]	19.30^{ab}	19.60^{abc}	18.80^{a}	20.20^{abc}	19.70^{abc}	20.70^{bc}	21.20^{c}	20.50^{abc}	19.90^{abc}	21.20^{c}	20.60^{bc}	20.20^{abc}	0.07	1.17
ΣPUFA[5]	27.70^{ab}	29.40^{cd}	29.50^{d}	27.50^{a}	28.90^{abcde}	29.40^{cd}	28.00^{abce}	29.10^{bcde}	29.30^{cde}	27.80^{abe}	28.20^{abcde}	28.90^{abcde}	0.51	1.09
Σn-6 PUFA[5]	15.80^{a}	16.90^{ab}	17.00^{ab}	16.80^{ab}	17.00^{ab}	17.10^{ab}	17.60^{b}	17.20^{ab}	16.40^{ab}	16.60^{ab}	16.80^{ab}	16.80^{ab}	0.04	1.00
Σn-3 PUFA[5]	12.00^{abcd}	12.50^{cd}	12.40^{cd}	10.80^{ab}	11.90^{abcd}	12.30^{bcd}	10.40^{a}	11.90^{abcd}	12.90^{d}	11.20^{abc}	11.40^{abcd}	12.20^{bcd}	0.10	1.14
ΣSFA:ΣPUFA[5]	1.88^{a}	1.72^{b}	1.74^{bc}	1.87^{ac}	1.78^{abc}	1.71^{b}	1.79^{abc}	1.73^{b}	1.74^{bc}	1.84^{abc}	1.82^{abc}	1.76^{abc}	0.11	0.10

Σn-6:Σn-3[5]	1.32[a]	1.36[a]	1.38[a]	1.58[ab]	1.44[ab]	1.40[ab]	1.69[b]	1.45[ab]	1.29[a]	1.50[ab]	1.48[ab]	1.39[a]	0.03	0.20

[1] Each fatty acid was compared with treatment, incubation day[3], and their interaction as main effects.

[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[3] d11, 11th day of incubation; d13, 13th day of incubation; d15, 15th day of incubation.

[4] SD, standard deviation.

[5] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6, omega-6 PUFA; n-3, omega-3 PUFA.

[a-e] superscripts indicate significant differences at $P \leq 0.05$.

Table 4.3. Fatty acid composition[1] (%) of embryo brains compared by treatment and day.

	Day[2]				Treatment[3]					
CODE	11	13	15	P value	TN	TNS	HS	HSS	P value	SD[4]
C14:0	0.86[a]	0.67[b]	0.53[c]	<.0001	0.72	0.66	0.68	0.69	0.61	0.16
C14:1	0.08	0.08	0.08	0.83	0.07[a]	0.08[a]	0.09[b]	0.09[b]	<.0001	0.02
C15:0	0.24	0.22	0.19	0.32	0.12[a]	0.20[ab]	0.24[b]	0.29[b]	0.00	0.13
C16:0	34.40[a]	33.80[a]	32.00[b]	<.0001	33.60	33.60	32.90	33.50	0.49	1.67
C16:1	1.79[a]	1.54[b]	1.31[c]	<.0001	1.44	1.49	1.61	1.64	0.06	0.29
C17:0	0.24[a]	0.23[a]	0.18[b]	<.0001	0.21	0.21	0.22	0.22	0.55	0.04
C18:0	15.40[a]	15.90[b]	17.70[c]	<.0001	16.60	16.30	16.20	16.20	0.63	1.12
C18:1 trans	15.70[a]	15.40[ab]	15.00[b]	0.0095	14.80[a]	15.40[b]	15.70[b]	15.60[b]	0.00	0.93
C18:1 cis	2.44[a]	2.35[a]	2.77[b]	<.0001	2.45	2.49	2.55	2.60	0.40	0.33
C18:2 n-6	1.97[a]	2.03[a]	1.55[b]	<.0001	1.85	1.89	1.88	1.78	0.76	0.36
C20:1 n-9	0.32[a]	0.29[a]	0.40[b]	0.00	0.31	0.37	0.32	0.34	0.36	0.12
C20:2 n-6	0.65[a]	0.72[a]	0.88[b]	<.0001	0.63[a]	0.84[b]	0.74[ab]	0.80[b]	0.00	0.20
C20:3	0.25[a]	0.32[b]	0.37[b]	<.0001	0.32	0.35	0.28	0.31	0.08	0.10
C20:4 n-6	10.41[a]	10.23[ab]	9.97[b]	0.04	10.00	10.10	10.50	10.10	0.07	0.70
C22:1	0.39[ab]	0.43[a]	0.35[b]	0.02	0.46[a]	0.37[bc]	0.30[b]	0.42[ac]	<.0001	0.11
C22:2	0.35[a]	0.30[ab]	0.28[b]	0.04	0.36	0.31	0.28	0.28	0.06	0.10
C22:5n-6	3.49[a]	3.72[a]	4.11[b]	<.0001	3.77	3.78	3.74	3.81	0.98	0.53
C22:5 n-3	0.51	0.54	0.55	0.15	0.54	0.51	0.52	0.57	0.11	0.09
C22:6	10.30[a]	11.10[b]	11.60[b]	<.0001	11.50	10.90	11.00	10.80	0.13	1.11
∑SFA[5]	51.10	50.90	50.70	0.39	51.40[a]	51.00[ab]	50.40[b]	51.00[ab]	0.03	1.19
∑MUFA[5]	20.50	20.10	19.90	0.13	19.20[a]	20.20[b]	20.50[b]	20.60[b]	<.0001	1.17
∑PUFA[5]	27.80[a]	28.90[b]	29.30[b]	<.0001	28.90	28.60	28.80	28.30	0.28	1.09
∑n-6 PUFA[5]	16.70	17.00	16.80	0.50	16.60	16.90	17.10	16.70	0.34	1.00
∑n-3 PUFA[5]	11.10[a]	11.90[b]	12.40[b]	<.0001	12.30	11.70	11.70	11.60	0.10	1.14
∑SFA:PUFA[5]	1.84[a]	1.76[b]	1.74[b]	<.0001	1.78	1.79	1.75	1.80	0.32	0.10
∑n-6:n-3[5]	1.52[a]	1.43[ab]	1.37[a]	0.01	1.36	1.47	1.48	1.46	0.11	0.20

[a-c] superscripts indicate significant differences at P≤0.05.

[1] Each fatty acid was compared with treatment and incubation day as the main effects.

[2] d11, 11th day of incubation; d13, 13th day of incubation; d15, 15th day of incubation.

[3] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[4] SD, standard deviation.
[5] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6, omega-6 PUFA; n-3, omega-3 PUFA.

Table 4.4. Fatty acid composition[1] (%) of embryo kidneys/gonads compared by treatment × day.

	TN[2]			TNS[2]			HS[2]			HSS[2]				
CODE	d11[3]	d13[3]	d15[3]	d11	d13	d15	d11	d13	d15	d11	d13	d15	P value	SD[4]
C14:0	0.27	0.25	0.20	0.21	0.19	0.21	0.31	0.28	0.22	0.31	0.26	0.21	0.67	0.05
C15:0	0.09	0.07	0.06	0.07	0.04	0.06	0.09	0.09	0.07	0.08	0.05	0.06	0.45	0.02
C16:0	30.10^{ab}	28.80^{abc}	25.90^{ac}	28.00^{abc}	28.30^{abc}	25.90^{c}	30.40^{ab}	30.90^{b}	28.40^{abc}	28.20^{abc}	27.50^{abc}	28.30^{abc}	0.36	1.86
C17:0	0.31	0.31	0.28	0.31	0.30	0.28	0.35	0.33	0.31	0.32	0.31	0.31	0.91	0.03
C18:0	25.20^{a}	23.60^{ab}	22.20^{ab}	23.40^{ab}	22.50^{b}	22.90^{ab}	24.10^{ab}	23.10^{ab}	23.30^{ab}	23.00^{ab}	21.50^{b}	23.50^{ab}	0.26	1.27
C18:1 trans	15.90^{ab}	17.70^{bcd}	15.50^{abc}	17.70^{abcd}	17.60^{abcd}	15.60^{a}	17.50^{abcd}	18.20^{cd}	15.80^{ab}	19.10^{d}	17.20^{abcd}	17.50^{abcd}	0.00	1.19
C18:1 cis	1.99^{ab}	1.66^{cde}	1.69^{abcde}	1.66^{cde}	1.54^{cd}	1.49^{c}	2.04^{b}	1.78^{abde}	1.74^{acde}	1.86^{abe}	1.83^{abcde}	1.71^{abcde}	0.26	0.19
C18:2 n-6[5]	5.81^{ab}	7.69^{c}	7.94^{c}	6.67^{abc}	7.04^{ac}	7.87^{c}	5.46^{b}	7.09^{ac}	7.41^{c}	6.90^{ac}	8.33^{c}	7.19^{abc}	0.10	1.00
C20:0	0.40^{a}	0.64^{bcd}	0.81^{d}	0.47^{abc}	0.55^{abcd}	0.74^{d}	0.45^{abc}	0.77^{d}	0.76^{d}	0.41^{ab}	0.60^{abcd}	0.78^{cd}	0.22	0.16
C20:1 n-9	0.42^{a}	0.47^{ab}	0.44^{ab}	0.43^{ab}	0.48^{ab}	0.43^{ab}	0.49^{ab}	0.47^{ab}	0.43^{ab}	0.49^{ab}	0.51^{b}	0.45^{ab}	0.33	0.04
C20:2 n-6	0.70	0.64	0.71	0.70	0.71	0.73	0.67	0.612	0.69	0.63	0.69	0.70	0.69	0.06
C20:3 n-6	0.52	0.47	0.57	0.55	0.51	0.60	0.53	0.46	0.55	0.53	0.55	0.62	0.77	0.06
C20:4 n-6	11.70^{ab}	11.40^{ab}	15.40^{bc}	12.40^{ab}	12.90^{abc}	16.70^{c}	11.00^{a}	10.40^{a}	13.80^{abc}	11.70^{ab}	13.10^{abc}	13.20^{abc}	0.39	2.05
C20:5 n-3	0.21	0.20	0.28	0.22	0.31	0.35	0.17	0.20	0.27	0.27	0.31	0.32	0.92	0.08
C22:0	0.55^{abc}	0.74^{bcde}	0.84^{cde}	0.54^{abc}	0.48^{ab}	1.01^{e}	0.59^{abcd}	0.79^{cde}	0.86^{de}	0.48^{ab}	0.44^{a}	0.65^{abcde}	0.01	0.20
C22:1	0.32	0.37	0.22	0.38	0.30	0.34	0.41	0.22	0.29	0.35	0.32	0.33	0.33	0.09
C22:2 n-3	0.46	0.24	0.24	0.50	0.31	0.67	0.57	0.53	1.06	0.98	0.84	0.24	0.51	0.45
C22:5n-6	1.25	1.14	1.24	1.29	1.34	0.96	1.26	1.16	1.05	1.37	1.30	1.05	0.31	0.17
C22:5 n-3	0.24	0.40	0.48	0.27	0.32	0.44	0.29	0.18	0.24	0.28	0.36	0.28	0.15	0.11
C22:6 n-3	3.19^{ab}	2.83^{ab}	3.08^{ab}	2.99^{ab}	3.29^{ab}	3.29^{ab}	2.82^{ab}	2.37^{a}	2.87^{ab}	3.33^{b}	2.93^{ab}	2.67^{ab}	0.19	0.38
ΣSFA[6]	56.80^{a}	54.30^{abc}	50.40^{bc}	53.00^{abc}	52.30^{abc}	51.80^{abc}	56.20^{ac}	56.20^{ac}	53.70^{abc}	51.90^{abc}	50.40^{c}	53.80^{abc}	0.08	2.65
ΣMUFA[6]	19.10^{abc}	20.60^{bcd}	18.30^{abcd}	20.60^{abcd}	20.30^{abcd}	18.10^{a}	21.00^{cd}	20.90^{cd}	18.30^{ab}	21.80^{d}	18.70^{abc}	20.00^{abcd}	0.00	1.35
ΣPUFA[6]	24.00^{a}	25.00^{ab}	29.70^{ab}	25.60^{ab}	24.50^{ab}	31.60^{b}	22.80^{a}	22.90^{a}	28.00^{ab}	26.00^{ab}	28.40^{ab}	26.20^{ab}	0.28	3.30
Σn-6 PUFA[6]	20.00^{a}	21.30^{ab}	25.60^{ab}	21.70^{ab}	20.30^{a}	26.80^{b}	18.90^{a}	19.70^{a}	23.50^{ab}	21.20^{ab}	24.00^{ab}	22.70^{ab}	0.26	2.90
Σn-3 PUFA[6]	4.06^{ab}	3.90^{ab}	4.13^{ab}	3.96^{ab}	4.33^{ab}	4.68^{b}	3.81^{ab}	3.15^{a}	3.93^{ab}	4.34^{ab}	4.08^{ab}	3.88^{ab}	0.42	0.53
ΣSFA:ΣPUFA[6]	2.37	2.18	1.69	2.11	2.17	1.42	2.47	2.45	1.92	1.83	1.60	2.05	0.44	0.46
Σn-6:Σn-3[6]	4.94^{ab}	5.47^{abc}	6.20^{bc}	5.48^{abc}	4.71^{a}	5.78^{abc}	4.97^{abc}	6.26^{c}	5.99^{abc}	4.95^{ab}	5.91^{abc}	5.85^{abc}	0.01	0.64

[1] Each fatty acid was compared with treatment, incubation day, and their interaction as main effects.

[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[3] d11, 11th day of incubation; d13, 13th day of incubation; d15, 15th day of incubation.

[4] SD, standard deviation.

[5] Transformation of 1/(x) was used in the analysis but the mean and SD was presented in their original scale.

[6] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6, omega-6 PUFA; n-3, omega-3 PUFA.

[a-e] superscripts indicate significant differences at $P \leq 0.05$.

Table 4.5. Fatty acid composition[1] (%) of embryo kidneys/gonads compared by treatment and day.

	Day[2]				Treatment[3]					
CODE	11	13	15	P value	TN	TNS	HS	HSS	P value	SD[4]
C14:0	0.28^{a}	0.25^{ab}	0.21^{b}	0.03	0.25^{ab}	0.21^{a}	0.29^{b}	0.28^{b}	0.01	0.05
C15:0	0.08	0.06	0.06	0.08	0.07	0.06	0.08	0.06	0.06	0.02
C16:0	29.30^{a}	28.80^{ab}	27.00^{b}	0.01	28.80^{ab}	27.40^{a}	29.90^{b}	27.90^{ab}	0.02	1.86
C17:0	0.32	0.31	0.29	0.05	0.30^{ab}	0.30^{a}	0.33^{b}	0.32^{ab}	0.03	0.03
C18:0	24.00^{a}	22.70^{b}	22.90^{ab}	0.02	24.00	22.90	23.50	22.50	0.07	1.27
C18:1 trans	1.90^{a}	1.68^{b}	1.64^{b}	0.00	1.83^{a}	1.57^{b}	1.85^{a}	1.82^{a}	0.00	1.19
C18:1 cis	17.30^{a}	17.60^{a}	15.90^{b}	0.00	16.50	16.90	17.20	17.80	0.22	0.19
C18:2 n-6	6.27^{a}	7.70^{b}	7.65^{b}	<.0001	6.92	7.29	6.66	7.60	0.21	1.00
C20:0	0.43^{a}	0.63^{b}	0.77^{c}	<.0001	0.57	0.59	0.66	0.53	0.45	0.16
C20:1 n-9	0.45^{ab}	0.48^{b}	0.43^{a}	0.01	0.44^{a}	0.45^{ab}	0.46^{ab}	0.49^{b}	0.03	0.04
C20:2 n-6	0.67	0.67	0.71	0.28	0.68	0.71	0.66	0.66	0.29	0.06
C20:3	0.53^{ab}	0.50^{a}	0.58^{b}	0.01	0.51	0.56	0.52	0.55	0.29	0.06
C20:4 n-6	11.70^{a}	12.00^{a}	15.10^{b}	<.0001	12.40	14.00	11.70	12.50	0.12	2.05
C20:5 n-3	0.22^{a}	0.25^{ab}	0.31^{b}	0.04	0.23	0.30	0.21	0.29	0.04	0.08
C22:0	0.53^{a}	0.60^{a}	0.88^{b}	<.0001	0.69^{ab}	0.68^{ab}	0.74^{a}	0.48^{b}	0.02	0.20
C22:1	0.36	0.30	0.30	0.20	0.31	0.34	0.31	0.34	0.85	0.09
C22:2	0.64	0.51	0.66	0.69	0.34	0.49	0.72	0.84	0.07	0.45
C22:5n-6	1.29^{a}	1.24^{a}	1.06^{b}	0.01	1.21	1.19	1.16	1.30	0.33	0.17
C22:5 n-3	0.27	0.33	0.36	0.11	0.35	0.34	0.24	0.32	0.20	0.11
C22:6	3.11	2.86	3.04	0.23	3.05^{ab}	3.19^{a}	2.69^{b}	3.08^{ab}	0.02	0.38
∑SFA[5]	54.70	53.30	52.40	0.15	54.60^{ab}	52.40^{ab}	55.40^{a}	51.60^{b}	0.01	2.65
∑MUFA[5]	20.40^{a}	20.00^{a}	18.40^{b}	0.00	19.60	19.60	20.10	19.80	0.88	1.35
∑PUFA[5]	24.70^{a}	25.40^{a}	29.40^{b}	0.00	25.60	27.20	24.60	27.10	0.27	3.30
∑n-6 PUFA[5]	20.50^{a}	21.60^{a}	25.00^{b}	0.00	21.70	22.90	20.70	22.60	0.37	2.90
∑n-3 PUFA[5]	4.07	3.88	4.22	0.34	4.02^{ab}	4.32^{b}	3.63^{a}	4.17^{ab}	0.03	0.53
∑SFA:PUFA[5]	2.18^{a}	2.06^{ab}	1.72^{b}	0.05	2.16	1.90	2.28	1.75	0.05	0.46
∑n-6:n-3[5]	5.07^{a}	5.62^{b}	5.96^{b}	0.00	5.40	5.33	5.74	5.47	0.55	0.64

$^{a-b}$ Superscripts indicate significant differences at P≤0.05.

[1] Each fatty acid was compared with treatment and incubation day as the main effects.

[2] d11, 11th day of incubation; d13, 13th day of incubation; d15, 15th day of incubation.

[3] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS

and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[4] SD, standard deviation.

[5] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6, omega-6 PUFA; n-3, omega-3 PUFA.

Table 4.6. Fatty acid composition[1] (%) of embryo livers compared by treatment × day.

	TN[2]			TNS[2]			HS[2]			HSS[2]				
CODE	d11[3]	d13[3]	d15[3]	d11	d13	d15	d11	d13	d15	d11	d13	d15	P value	SD[4]
C14:0	0.17	nd[5]	0.06	0.16	nd[5]	0.05	0.17	0.13	0.08	0.15	0.12	0.12	0.6	0.04
C15:0	0.42^{a}	0.36^{ab}	0.25^{bc}	0.39^{a}	0.37^{ab}	0.21^{c}	0.30^{abc}	0.41^{a}	0.26^{bc}	0.41^{a}	0.43^{a}	0.29^{abc}	0.06	0.11
C16:0	19.72^{a}	13.36^{bc}	9.88^{d}	19.80^{a}	14.32^{c}	9.35^{d}	20.78^{a}	15.78^{c}	10.28^{bd}	20.47^{a}	15.94^{c}	9.98^{d}	0.63	4.63
C16:1	0.74^{ab}	0.65^{ab}	0.92^{b}	0.43^{a}	0.49^{a}	0.58^{a}	0.67^{ab}	0.67^{ab}	0.76^{ab}	0.52^{a}	0.73^{ab}	0.73^{ab}	0.38	0.23
C17:0	0.16^{abc}	0.13^{abd}	0.12^{d}	0.18^{ce}	0.17^{bce}	0.12^{ad}	0.20^{e}	0.15^{abcd}	0.14^{abd}	0.19^{ce}	0.17^{bce}	0.14^{abcd}	0.22	0.03
C18:0	19.60^{ab}	22.10^{abcd}	21.90^{abcd}	18.50^{a}	21.00^{abcd}	20.40^{abc}	22.50^{abcd}	23.30^{bcd}	25.70^{d}	19.80^{ab}	24.80^{cd}	21.40^{abcd}	0.10	3.12
C18:1 cis	23.10^{abc}	26.70^{abcd}	28.20^{cd}	22.00^{a}	27.00^{abcd}	30.50^{de}	23.00^{ab}	24.30^{abc}	27.60^{bcd}	22.90^{ab}	24.90^{abcd}	33.90^{e}	0.01	4.28
C18:2 n-6	8.02^{abc}	9.36^{cd}	10.61^{d}	7.51^{ab}	9.93^{d}	9.89^{d}	7.35^{a}	9.06^{bcd}	10.61^{d}	7.10^{a}	9.63^{d}	10.33^{d}	0.18	1.53
C18:3 n-3	1.29	2.81	nd[5]	2.74	2.73	2.72	1.83	2.58	2.74	2.17	1.31	0.15	0.03	1.16
C20:0[6]	0.01^{a}	0.02^{a}	2.86^{b}	0.03^{a}	0.02^{a}	0.14^{ab}	0.07^{ab}	0.05^{ab}	0.32^{ab}	0.03^{a}	0.06^{ab}	0.05^{ab}	0.08	0.84
C20:1 n-9[7]	0.19^{ab}	0.14^{ab}	0.16^{ab}	0.16^{ab}	0.18^{ab}	0.15^{ab}	0.24^{a}	0.18^{ab}	0.12^{b}	0.19^{ab}	0.16^{ab}	0.13^{ab}	0.18	0.15
C20:2 n-6	0.31^{ab}	0.47^{ab}	0.58^{a}	0.35^{ab}	0.33^{ab}	0.46^{ab}	0.29^{ab}	0.28^{ab}	0.41^{ab}	0.35^{ab}	0.27^{b}	0.32^{ab}	0.24	0.48
C20:3 n-6	0.25	0.26	0.26	0.22	0.25	0.24	0.20	0.23	0.23	0.24	0.21	0.21	0.88	0.07
C20:4 n-6	11.14	12.00	10.3	10.48	11.59	10.34	9.62	11.28	10.25	10.15	10.81	9.81	0.88	1.56
C20:5 n-3[6]	0.01^{a}	0.28^{abc}	1.25^{c}	0.07^{abc}	0.15^{abc}	0.54^{bc}	0.14^{abc}	0.06^{abc}	0.19^{abc}	0.12^{abc}	0.01^{ab}	0.07^{abc}	0.01	0.58
C22:0[7]	0.17^{ab}	0.21^{ab}	0.19^{ab}	0.22^{a}	0.17^{ab}	0.20^{ab}	0.16^{ab}	0.11^{bc}	0.15^{ab}	0.19^{ab}	0.12^{bc}	0.09^{c}	0.01	0.14
C22:1	0.63	0.74	0.46	0.73	0.61	0.65	0.67	0.59	0.56	0.54	0.39	0.29	0.75	0.30
C22:2 n-3	0.48	0.55	0.46	0.53	0.55	0.61	0.69	0.66	0.51	0.65	0.57	0.44	0.81	0.27
C22:5n-6	3.33^{a}	2.36^{bcde}	2.51^{bcdf}	2.89^{acdef}	2.33^{bcd}	1.84^{b}	3.25^{af}	3.09^{adef}	2.24^{bc}	3.14^{aef}	2.15^{bc}	2.20^{bc}	0.04	0.65
C22:5 n-3	0.48^{a}	0.31^{abc}	0.30^{abc}	0.44^{ab}	0.34^{abc}	0.24^{c}	0.43^{abc}	0.40^{abc}	0.28^{bc}	0.43^{ab}	0.45^{ab}	0.42^{abc}	0.12	0.13
C22:6 n-3	9.22^{a}	6.54^{bcde}	5.33^{bc}	8.32^{ae}	7.03^{cde}	5.22^{bc}	6.77^{cde}	6.41^{bcde}	5.12^{bc}	7.82^{ade}	5.88^{bcd}	4.67^{b}	0.13	1.77
ΣSFA[8]	40.30^{ab}	33.70^{abcd}	28.90^{acd}	34.80^{abd}	25.70^{cd}	21.00^{c}	44.80^{b}	40.30^{ab}	37.60^{abd}	41.60^{ab}	39.00^{abd}	29.50^{acd}	0.80	10.19
ΣMUFA[8]	24.70	24.80	22.70	17.50	14.80	16.60	24.60	25.80	29.00	24.20	23.10	30.60	0.81	10.25
ΣPUFA[8]	34.70^{a}	34.00^{ab}	31.80^{ab}	33.40^{ab}	35.00^{a}	31.70^{ab}	30.50^{ab}	33.80^{ab}	32.50^{ab}	32.50^{ab}	30.90^{ab}	28.80^{b}	0.19	3.49
Σn-6 PUFA[8]	23.10^{abc}	24.60^{c}	24.60^{c}	21.50^{abc}	24.40^{bc}	23.00^{abc}	20.70^{a}	23.90^{abc}	23.90^{abc}	21.10^{ab}	23.10^{abc}	22.70^{abc}	0.75	2.28
Σn-3 PUFA[8]	2.36	2.85	1.71	3.52	3.55	3.48	3.03	3.44	3.35	3.53	1.96	1.37	0.42	1.83
ΣSFA:ΣPUFA[8]	1.18^{abc}	1.01^{abcd}	0.91^{abd}	1.06^{abcd}	0.73^{ad}	0.67^{d}	1.48^{c}	1.21^{abc}	1.17^{abc}	1.29^{bc}	1.27^{bc}	1.05^{abcd}	0.83	0.35
Σn-6:Σn-3[8]	11.14	10.80	14.04	10.18	12.67	7.60	8.50	9.59	12.81	8.19	15.53	15.98	0.32	8.11

[a-f] superscripts indicate significant differences at P≤0.05

[1] Each fatty acid was compared with treatment, incubation day, and their interaction as main effects.

[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[3] d11, 11th day of incubation; d13, 13th day of incubation; d15, 15th day of incubation.

[4] SD, standard deviation.

[5] nd, not detected.

[6] Transformation of log(x) was used in the analysis but presented means and SD are presented in their original scale.

[7] Transformation of 1/(x) was used in the analysis but presented means and SD are presented in their original scale.

[8] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6, omega-6 PUFA; n-3, omega-3 PUFA.

Table 4.7. Fatty acid composition[1] (%) of embryo livers compared by treatment and day.

	Day[2]				Treatment[3]					
CODE	d11	d13	d15	P value	TN	TNS	HS	HSS	P value	SD[4]
C14:0	0.16[a]	0.08[b]	0.12[b]	<.0001	0.16	0.13	0.15	0.14	0.64	0.04
C15:0	0.38[a]	0.39[a]	0.25[b]	<.0001	0.34	0.32	0.32	0.38	0.23	0.11
C16:0	20.19[a]	14.85[b]	9.87[c]	<.0001	14.30	14.50	15.60	15.50	0.70	4.63
C16:1	0.59[a]	0.64[ab]	0.75[b]	0.02	0.77[a]	0.50[b]	0.70[a]	0.66[a]	0.00	0.23
C17:0	0.18[a]	0.16[b]	0.13[c]	<.0001	0.14[a]	0.16[ab]	0.16[b]	0.16[b]	0.02	0.03
C18:0	20.20[a]	23.00[b]	22.70[b]	0.00	21.10[a]	19.70[a]	23.80[b]	21.90[ab]	0.00	3.12
C18:1 cis	22.80[a]	25.50[b]	30.00[c]	<.0001	25.80	25.80	24.90	27.00	0.44	4.28
C18:2 n-6	7.49[a]	9.49[b]	10.36[c]	<.0001	9.33	9.11	9.01	9.02	0.88	1.53
C18:3 n-3	1.96	2.36	2.36	0.42	1.79[ab]	2.73[b]	2.40[ab]	1.57[a]	0.02	1.16
C20:0	0.39[a]	0.41[a]	1.22[b]	0.00	0.88	0.48	0.79	0.49	0.52	0.84
C20:1 n-9	0.24[a]	0.20[ab]	0.18[b]	0.01	0.18	0.21	0.24	0.20	0.90	0.15
C20:2 n-6	0.43[a]	0.41[a]	0.74[b]	0.02	0.70[a]	0.55[ab]	0.44[ab]	0.41[b]	0.03	0.48
C20:3	0.23	0.23	0.23	0.98	0.26	0.24	0.22	0.22	0.30	0.07
C20:4 n-6	10.30[a]	11.40[b]	10.20[a]	0.00	11.10	10.80	10.40	10.30	0.16	1.56
C20:5 n-3	0.47[a]	0.55[ab]	0.91[b]	0.02	0.65	0.77	0.63	0.51	0.58	0.58
C22:0	0.22[a]	0.19[b]	0.18[ab]	0.03	0.24[a]	0.26[a]	0.16[ab]	0.16[b]	0.00	0.14
C22:1	0.63	0.58	0.50	0.21	0.61[ab]	0.65[b]	0.61[ab]	0.42[a]	0.04	0.30
C22:2	0.59	0.58	0.51	0.43	0.50	0.56	0.62	0.56	0.48	0.27
C22:5n-6	3.15[a]	2.48[b]	2.20[b]	<.0001	2.73[ab]	2.35[a]	2.86[b]	2.51[ab]	0.03	0.65
C22:5 n-3	0.44[a]	0.37[ab]	0.31[b]	0.00	0.36[ab]	0.34[a]	0.37[ab]	0.44[b]	0.06	0.13
C22:6	8.03[a]	6.46[b]	5.09[c]	<.0001	7.03	6.86	6.10	6.12	0.14	1.77
∑SFA[5]	40.40[a]	34.70[b]	29.20[b]	<.0001	34.30[a]	27.20[b]	40.90[a]	36.70[a]	<.0001	10.19
∑MUFA[5]	22.80	22.10	24.70	0.57	24.10[a]	16.30[b]	26.50[a]	26.00[a]	0.00	10.25
∑PUFA[5]	32.80[ab]	33.40[a]	31.20[b]	0.02	33.50[a]	33.40[a]	32.30[ab]	30.70[b]	0.01	3.49
∑n-6 PUFA[5]	21.60[a]	24.00[b]	23.50[b]	<.0001	24.10[a]	23.00[ab]	22.90[ab]	22.30[b]	0.04	2.28
∑n-3 PUFA[5]	3.11	2.95	2.48	0.35	2.31	3.52	3.27	2.29	0.02	1.83
∑SFA:PUFA[5]	1.25[a]	1.06[ab]	0.95[b]	0.00	1.03[ab]	0.82[a]	1.29[c]	1.20[bc]	<.0001	0.35
∑n-6:n-3[5]	9.50	12.30	12.50	0.33	10.30	13.00	12.00	10.30	0.17	8.11

[a-c] Superscripts indicate significant differences at P≤0.05.

[1] Each fatty acid was compared by treatment and incubation day.

[2] d11, 11th day of incubation; d13, 13th day of incubation; d15, 15th day of incubation.

[3] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers

at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[4] SD, standard deviation.

[5] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; n-6, omega-6 PUFA; n-3, omega-3 PUFA.

CHAPTER 5
Lipid oxidation and antioxidant capacity in multigenerational heat stressed Japanese quail (*Coturnix coturnix japonica*)

Abstract: In some areas of the world, climate-controlled poultry houses are not possible and could result in poorer quality poultry products due to deterioration of lipids in tissues. Lipid oxidation occurs during oxidative stress caused by high environmental temperatures. In Japanese quail, heat stress can occur starting at 30˚C; however, as climate change becomes more severe, temperatures above 30˚C may become more frequent. When animals experience heat stress, more oxygen is consumed and metabolism changes. These changes can incur more oxidative damage to lipids, however, antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) can prevent further oxidation. The goal of this study was to determine if selection for low feed conversion ratio (FCR) in Japanese quail at 31.1˚C would result in lower lipid oxidation and more antioxidant activity. It was hypothesized that quail selected for low FCR and not exposed to heat stress would have the lowest amount of lipid oxidation. It was also hypothesized that quail selected for low FCR and exposed to heat stress would have the highest SOD and CAT activities. To test these hypotheses, TBARS analysis was performed on male and female brain, liver, kidney, thigh, and yolk. SOD and CAT activities were determined in male and female brain, liver, kidney, and thigh. Significance was determined at $P \leq 0.05$. Results showed that heat stress did not influence lipid oxidation in any of the tissues; however, brain had the most oxidation, followed by liver> kidney>thigh ($P < 0.0004$) There was also no significant treatment effect on SOD and CAT activities, but kidneys had significantly more CAT activity than brain, liver, and thigh. Brain and thigh had similar CAT activities. Results suggested that heat stress at 31.1˚C did not significantly affect lipid oxidation and antioxidant enzyme activities. Thus, poultry products from quail raised at this temperature may have similar quality to those that are raised within their thermoneutral zone (18-30˚C). Future directions could include heat stress at 31.1˚C and a higher temperature to

determine if total antioxidant capacity is lower and lipid oxidation is higher in eggs laid in the first week than all subsequent weeks of lay.

1. Introduction

Heat stress is becoming a major threat to poultry health and production. High environmental temperatures can increase the concentration of free radicals, leading to an increase in oxidation in the plasma, liver, and heart (Hosseini-Vashan and Raei-Moghadam, 2019). In a homeostatic state, the body has a natural antioxidant defense system which can trap free radicals and prevent oxidative damage. However, there may not be enough circulating antioxidants in the system to control the free radicals when an animal is experiencing heat stress. This is especially true for females that need to trade off antioxidants for the yolk or for herself as was indicated when antioxidants injected into the egg and the dam increased hatchability and growth of the chicks (Tsunekage, 2015).

Antioxidant capacity such as superoxide dismutase (SOD) and catalase (CAT) can be quantified in the body. SOD has compartmentalized enzymes occurring in the mitochondria of liver and heart (MnSOD) and in the cytoplasm, nucleus, peroxisomes, lysosomes, and intermembrane space of mitochondria of lung, yolk sac membrane, thigh muscle, and brain (CuZnSOD), accounting for 90% of the total SOD activity in cells (Weydert and Cullen, 2010; Surai, et al., 2016). There are also equal activities of MnSOD and CuZn-SOD in the kidneys (Surai, et al., 2016). SOD enzymes convert superoxide radicals to hydrogen peroxide and oxygen. However, hydrogen peroxide can also cause oxidative damage; thus, CAT converts it to water and oxygen. In chicks subjected to heat stress, researchers found a decrease in SOD and total antioxidant capacity (Hosseini-Vashan and Raei-Moghadam, 2019). Tissue-dependent SOD

activity was highest in the heart and decreased in the following order: muscle, yolk sac membrane, kidney, lung, then liver (Surai, et al.; 1999; Surai, et al., 2016).

Lipids are a class of nutrients that are highly susceptible to oxidation. An indicator of lipid oxidation is malondialdehyde (MDA). Others found that heat stressed (32˚C) Japanese quail have lower levels of antioxidants and higher levels of MDA than those housed in thermoneutral temperatures (Sahin, et al., 2004). This finding supports another result in which energy demands and corticosterone increased triacylglyceride (TAG) and non-esterified fatty acids (NEFA) in the plasma of heat-stressed broiler chicken (Sahin, et al., 2004). The reaction occurred within mintues when temperatures increased from 25 to 35˚C (Slimen, et al., 2016). TAG and NEFA contribute to energy production, but are also involved in the change of membrane structure and fluidity affecting its potential during heat stress (Slimen, et al., 2016). It is important to study heat stress over a wide range of temperatures to understand its effect on lipid oxidation. Egbuniwe, et al. (2021) reported effects of heat stress in quail exposed to environmental conditions with no artificial simulation in Nigeria where temperatures ranged from 30.2˚C to 32.1˚C.

Previous research revealed that multiple generations of stress is capable of incrementally altering response to stress over time (Ambeskovic, et al., 2019). During embryogenesis, yolk antioxidants are differentially transferred and partitioned among the tissues. Some researchers found that the liver has the highest concentration of antioxidants compared to that of other tissues (Tsunekage and Ricklefs, 2015). Conversely, others reported that the brain has the highest concentration of MDA, followed by the liver, then the heart (Tsunekage and Ricklefs, 2015). The brain contains a high concentration of polyunsaturated fatty acids (PUFA), explaining the high concentration of MDA found in the brain by other researchers (Tsunekage and Ricklefs, 2015).

Mild heat stress in early life of mice, humans, fruit flies, and zebra finch produced adults that were less affected by oxidative stress (Costantini, et al., 2012; Laviola and Macrì, 2013). This was considered a form of priming which prepared the organism to withstand greater oxidative stress (Costantini, et al., 2012). Similarly, male fruit flies were exposed to short term anoxia before emergence and at sexual maturity, they had higher concentrations of SOD and lower lipid and protein damage (López-Martínez and Hahn, 2012; Laviola and Macrì, 2013). In birds, if there is an increase in total antioxidant capacity of egg yolk, there is a decrease in concentration of MDA in egg yolk (Surai, et al., 2016).

Little research has been performed on multigenerational heat stress and antioxidant capacity in Japanese quail. Of particular interest was to discern if selection for high performance in heat stress affected the antioxidant capacity and extent of lipid oxidation. Therefore, the present study was performed with Japanese quail that were selected for a low feed conversion ratio (FCR). It was hypothesized that antioxidant capacity would be higher in quail that were selected for low FCR after being subjected to 10 generations of heat stress when compared to those that were randomly bred in heat stress. It was also hypothesized that quail selected for low FCR and not exposed to heat stress would have the lowest amount of lipid oxidation.

2. Materials and Methods

2.1 Experimental design

The detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Birds were transferred from brooders to cages at 3.5 weeks old; they were placed into cages for mating at 7.43 weeks old and egg collection began when females were 7.86 weeks old. The four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings

(22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS). TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers with 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that were determined as having high fitness (FCR) in HSS were mated.

2.2 Sample collection

Animal care and use was approved by the Institutional Animal Care and Use Committee at the University of California Davis (Protocols #19473 and 21370; Davis, CA). All eggs were collected from each treatment within 24 hours after lay. Eggs were collected for 2 weeks from 20 TN females, 14 TNS females, 20 HS females, and 17 HSS females. Eggs were also collected from 18 TN females, 10 TNS females, 18 HS females, and 16 HSS females at 16 to 17 weeks old. These eggs were for analysis within 24 hours of lay according to lipid oxidation procedures mentioned below.

Adult birds were euthanized using cervical dislocation and dissected for the brain, liver, kidneys, and thighs. The tissues were placed into Whirl-Pak® bags and immediately submerged into liquid nitrogen after collection. Samples were then transferred to a -80˚C freezer until further analysis.

2.3 Lipid oxidation

A total of 30 eggs per treatment were analyzed for lipid oxidation within 24 hours after lay. Egg totals included 100 TN, 97 TNS, 99 HS, and 98 HSS that were analyzed for lipid oxidation after 13.36 weeks of storage at 4˚C. For TN, HS, and HSS, 15 male and 15 female brains, kidneys, and livers were analyzed for lipid oxidation. For TNS, 15 male and 12 female brains, kidneys, and livers were analyzed for lipid oxidation. Determination of lipid oxidation for both thighs included

the following: TN (8 males and 4 females), TNS (6 males and 5 females), HS (8 males and 6 females), and HSS (8 males and 11 females).

A modified thiobarbituric acid reactive substances (TBARS) method was used to measure malondialdehyde (MDA) in samples (Ruberto and Baratta, 2000). Briefly, homogenates of 10% tissue were made with deionized distilled water and 5% butylated hydroxytoluene; 50 µL of the homogenate was vortexed and heated with 112.5 µL of 20% acetic acid (pH 2.0 for maximum color change), 168.75 µL of 0.8% thiobarbituric acid, and 168.75 µL of 2% sodium dodecyl sulfate. The mixture was heated in a 95°C dry bath for 60 minutes, followed by adding 1.0 mL of butanol, mixing, and centrifuging at 1200 × g for 10 minutes. The extracted organic upper layer was vortexed and measured for absorbance at 532 nm (band width 0.5 nm) on a Synergy HT spectrophotometer (BioTek Instruments, Winooski, Vermont, USA).

A standard calibration curve was developed using 1mM of 1,1,3,3-tetramethoxypropane (TMP) to determine concentration of MDA (nmol/mg wet weight) in adult tissues. Egg yolks were not analyzed with a standard calibration curve; therefore, the quantity of MDA in egg yolks were reported as absorbance (nm).

2.4 Catalase and superoxide dismutase activity

Catalase activity (BioVision, Catalog #K773-100) and superoxide dismutase (BioVision, Catalog #K335-100) activity were analyzed using commercially available kits and in accordance with the instructions of the manufacturer. For catalase activity, quantities of homogenate used were as follows: 50 µL of 1:2 for brain and thigh, 5 µL of 1:2 for liver, and 5 µL of 1:1 for kidney. For superoxide dismutase activity, quantities of homogenate were 10 µL of 1:2 thigh, brain, and liver and 5 µL of 1:2 for kidney.

2.5 Statistical analysis

Analyses of data were performed in R 4.0.0 (R Core Team, 2020; RStudio Team, 2022) to test significance ($P \leq 0.05$). The Shapiro-Wilk test was used to determine normality of residuals of models and data were considered normal at $W \geq 0.95$ or $P \geq 0.05$. Levene's test and Q-Q plots were used to determine homogeneity of variances and variances were considered equal at $P \geq 0.05$. Lipid oxidation of egg yolks collected over 2 weeks were analyzed using one-way repeated measures ANOVA to determine significant differences among treatment, week of lay, and treatment × week of lay. Due to multiple eggs collected from each female over the course of a week, females were included in the model as a random effect. Lipid oxidation of adult tissues was analyzed using one-way repeated measures ANOVA to determine significant differences among treatments, tissue types, sex, and their interactions. For SOD activity and CAT activity, one-way repeated measures ANOVA was used to determine significant differences among treatments, tissue types, and their interactions. Sex was not determined as significant and was not included in the SOD activity and CAT activity models. Due to multiple tissue collection from each quail, bird was included as a random effect for lipid oxidation, SOD activity, and CAT models. Tukey's method for comparing estimates was used if the interaction was significant at $P \leq 0.05$.

3. Results

3.1 Lipid oxidation of egg yolks

Egg yolks compared by treatment within 24 hours after lay had no significant difference among treatments (Figure 1). However, when egg yolks were compared by treatment × week of lay (including 24 hours after lay at week 16), HS week 1 had significantly more lipid oxidation than all weeks of TN and TNS and weeks 2 and 16 for HS and HSS ($P < 0.008$). HS week 2 had significantly less lipid oxidation than TN week 1 and 2 and HSS week 1 ($P < 0.01$). HSS week 1

had significantly more lipid oxidation than TNS week 1 and week 16 (p=0.00059; Figure 1). TNS 24 hours after lay also had significantly less lipid oxidation than TN week 1 (p=0.30). When treatments were compared without the week effect, TNS had significantly less lipid oxidation than all other treatments (P<0.03). When weeks were compared without the treatment effect, yolks from week 1 had significantly more lipid oxidation than yolks analyzed within 24 hours of lay and week 2 eggs (P<0.0001).

3.2 Lipid oxidation of adult tissues

The interaction between treatment, tissue, and sex were significant at P<0.0001 (Table 1). When compared by treatment × tissue × sex, all adult brains had significantly higher concentrations of MDA than all other tissues (P<0.05) except (1) TN male brains shared significance with TNS and HSS male livers and TNS male thighs and (2) TNS female brains and HSS female brains shared significance with TNS and HSS male livers (P>0.05). There were no significant treatment or sex effects within brain.

Kidneys

HS male and female kidneys had significantly lower concentrations of MDA than TNS male livers but significantly higher concentrations of MDA than HSS female thighs (Table 1). HS female kidneys also had significantly lower concentrations of MDA than HSS male livers and TN male livers (P<0.02). HSS male and female kidneys had significantly lower concentrations of MDA than HSS and TNS male livers but significantly higher concentrations of MDA than HSS female thighs (P<0.003).

TN male and female kidneys had significantly lower concentrations of MDA than TNS male livers but significantly higher concentrations of MDA than HSS female thighs (P<0.04). TN female kidneys also had significantly lower concentrations of MDA than HSS and TN male livers

(P<0.05). TN male kidneys had significantly higher concentrations of MDA than HS and TN male thighs (P<0.02). TNS male and female kidneys had significantly lower concentrations of MDA than TNS male livers and had significantly higher concentrations of MDA than HSS female thighs (P<0.004).

Livers

HS male and female livers had significantly lower concentrations of MDA than HSS and TNS male livers and had significantly higher concentrations of MDA than HSS female thighs (P<0.002). HS female livers also had significantly lower concentrations of MDA than TN male livers (p=0.031). TN male and female livers had significantly higher concentrations of MDA than TN and HS male thighs (P<0.02). TN female livers also had significantly higher concentrations of MDA than HSS female thighs and significantly lower concentrations of MDA than TNS male livers (P<0.04). TN male livers had significantly higher concentrations of MDA than HS female thighs (p=0.016).

TNS female livers had significantly lower concentrations of MDA than HSS and TNS male livers and significantly higher concentrations of MDA than HSS female thighs (P<0.05). TNS male livers had significantly higher concentrations of MDA than TN and HS male thighs; TN, TNS, and HS female thighs; and HSS female livers (P<0.002). HSS female livers had significantly lower concentrations of MDA than HSS male livers and significantly higher concentrations of MDA than HSS female thighs (P<0.02). HSS male livers had significantly higher concentrations of MDA than TN and HS male thighs and TN, TNS, and HS female thighs (P<0.05).

Thighs

HSS female thighs had significantly lower concentrations of MDA than TNS and HSS male thighs ($P<0.0001$). TNS male thighs had significantly higher concentrations of MDA than TN and HS male thighs ($P<0.04$).

Treatment x tissue

When sex was removed from the model, the interaction between treatment and tissue was significant at $P<0.0001$ (data not shown). Brain from all treatments had significantly higher concentrations of MDA than all other tissues ($P<0.0001$). TN, TNS and HSS livers and TNS kidneys had significantly higher concentrations of MDA than TN, HS, and HSS thighs ($P<0.02$). TN and TNS livers also had significantly higher concentrations of MDA than HS and HSS kidneys and HS livers ($P<0.05$). HSS livers had significantly higher concentrations of MDA than HS livers and HSS kidneys ($P<0.03$). TN kidneys had significantly higher concentrations of MDA than HSS thighs ($p=0.010$).

Tissue and sex

When treatment was removed from the model, tissue had a significant effect on MDA concentration with brain>liver>kidney>thigh at $P<0.0004$ (data not shown). When MDA concentration was compared by sex alone, females had significantly less than males ($P<0.0001$).

3.3 SOD and CAT activities

For SOD activity, there were no significant differences when compared by treatment, tissue, sex, and their interaction ($P>0.05$; Table 2). However, when SOD activity was compared by treatment, tissue and their interaction, tissue had a significant effect at $p=0.0001$. TNS livers had significantly less SOD activity than HSS kidneys and HSS thighs ($P<0.005$). When SOD activity was compared by tissue, liver had 10.26%, 9.09%, and 12.08% less activity than brain, kidney, and thigh, respectively ($P<0.005$).

For CAT activity, there was no sex effect (p=0.63), but there were treatment, tissue, and treatment × tissue effects (P<0.0001; Figure 2). Across all treatments, brains and thighs had significantly less CAT activity than kidneys and livers (P<0.0001). HS kidneys had significantly more CAT activity than livers from all treatments and HSS kidneys (P<0.0001). HSS kidneys had significantly less CAT activity than TNS and HSS livers and TN and TNS kidneys (P<0.01). TN and TNS kidneys had significantly more CAT activity than livers from all treatments (P<0.0001). HS livers had significantly less CAT activity than livers from all other treatments (P<0.05). HSS brains had significantly more CAT activity than TN and HSS thighs (P<0.05). TNS livers had significantly more CAT activity than TN and HSS livers (P<0.0001).

4. Discussion

4.1 Lipid oxidation of egg yolks

The significant lipid oxidation observed in egg yolks stored for 13.36 weeks were likely due to the extended storage of the eggs. While Jones and Musgrove (2015) noted that extending egg storage 30 days past their shelf-life or sell-by date would not compromise their physical-chemical quality, they noted that Haugh units and albumen height decreased after 10 weeks of cold storage. Additionally, in another study, Jones et al. (2018), demonstrated that the quality of eggs stored at 4˚C for 15 weeks had little change in yolk shape characteristics and maintained Grade A quality. Thus, information from the current study seemed to support previous findings that quality may be conserved up to week 15 of storage as there were no significant indications of lipid oxidation between egg yolks analyzed at 24 hours after lay and analyzed after 13.36 weeks of storage.

Contrarily, week 1 yolks had significantly more lipid oxidation than those analyzed at week 2 and within 24 hours of lay, indicating that there may not be enough antioxidants transferred to

the egg during the first week of lay. Alternatively, stressful events increase glucocorticoids such as corticosterone which can divert energy away from reproduction; thus, decreasing synthesis of yolk precursors (Wang, et al., 2017). Because quail in the present study were transferred to their respective mating cages 3 days before egg collection began, the stress of moving could have impacted the quality of egg yolk deposited and caused the increased lipid oxidation seen in week 1.

As observed in Chapter 4, compared to HSS, TNS had significantly higher SFA in the yolk than HSS, likely contributing to significantly less lipid oxidation for TNS yolk in the current study. Others also showed that a lower degree of unsaturation in particular organs of Japanese quail is a protective adaptation that can prevent high levels of lipid oxidation (Gutiérrez, et al., 2006). Findings from Chapter 2, the current study, and that of Gutiérrez, et al. (2006) may indicate that there is a survival adaptation to decrease lipid oxidation when birds are chosen for low FCR in a heat stress environment, but never exposed to heat stress.

4.2 Lipid oxidation of adult tissues

Precursors of MDA and TBARS are derivatives of peroxyl radicals from PUFA with 3 or more double bonds (Frankel, 2005). Particularly, under acidic conditions as in the TBARS analysis, peroxyl radicals of α-linolenic acid is the most significant precursor of MDA (Frankel, 2005). With high mobilization of lipids and the vulnerability of PUFA to oxidation, results from the present study support the hypothesis that tissues with higher degrees of unsaturation would increase the amount of lipid oxidation.

Brain tissue of adult quail had significantly higher amounts of lipid oxidation which agreed with the findings of others (Gutiérrez, et al., 2006; Tsunekage, 2015). As reported in Chapter 3 (above), the adult brain is comprised of approximately 11 to 14% DHA, a long chain PUFA,

whereas the kidney had ~2%, the thigh had ~1-2%, and the liver had ~1-4%. Thus, 14% DHA was likely the predominant reason for the high level of oxidation found in the adult brain. There are also less antioxidants present in the brain when compared to other tissues (Gutiérrez, et al., 2006; Yigit, et al., 2014; Surai, et al., 2016).

It is known that females invest more energy in egg production, egg laying, and brooding (Hiyama, et al., 2018). As well, studies in mice treated with estradiol, the form of estrogen that is highest in circulation, had higher levels of fatty acid oxidation in the liver due to increased oxygen consumption and liver ATP production (Palmisano, et al., 2017). Thus, it was hypothesized that females would have more lipid oxidation as oxygen consumption increased to meet reproductive demands. However, results of other investigators and those of the current work did not support the hypothesis and indicated that when lipid oxidation was compared by sex alone, females had less lipid oxidation than males (Hamano, 2014; Gomes, et al., 2019).

Males had higher MDA than females. Testosterone administration in rabbits increased MDA levels in plasma (Aydilek and Aksakal, 2005). Authors suggested that testosterone decreased high-density lipoproteins and cholesterol, which can act as an antioxidant; thus, increasing lipid oxidation (Aydilek and Aksakal, 2005). Although quail in the present study were not administered testosterone, researchers reported that sexually mature male quail tended to have higher concentrations of circulating testosterone than females (Hiyama, et al., 2018). Testosterone is also known to increase metabolic rate and subsequently increase oxidative stress in male zebra finches (Alonso-Alvarez, et al., 2007). As well, males in the present work may have experienced more lipid oxidation due to energy spent on reproductive behaviors such as aggression, courtship songs, and territory establishment (Hiyama, et al., 2018; Gomes, et al., 2019).

Others have reported that acutely heat stressed broilers (40˚C for 5 hours) had a 4-fold increase in MDA as opposed to a 1.2- to 1.5-fold increase in those that were chronically heat stressed at 32˚C for 14 d (Azad, et al., 2010; Akbarian, et al., 2016). Therefore, the insignificance of treatment effect, regardless of tissue type or sex, indicated that heat stress at 31.1˚C did not affect lipid oxidation.

4.3 SOD and CAT activities

When stress-sensitive rats were subjected to chronic mild stress there was an increase in lipid peroxidation and CAT activity, but decreased SOD activity in certain parts of the brain (Laviola and Macrì, 2013). Similar to another report, catalase activity in the brain of quail in the present study was extremely low (Nazıroğlu, 2012). In 10-day-old quail that were heat stressed at 34˚C for 8 hours/day there was a significant increase in MDA in the serum, muscle, and liver; however, there was a significant decrease in SOD, CAT, and GPx activities for these quail as noted by others (Sahin, et al., 2012). Although not signifiantly different, livers in the present study had numerically lower SOD activity than all other tissues which is opposite to expectations due to the liver's important role in lipid metabolism in avian species. However, in another study, SOD activity in embryo liver samples was lowest when compared to the heart, muscle, yolk sac membrane, kidney, and lung (Surai, et al., 2016).

Compared to all tissues evaluated, the kidneys had the most CAT activity, particularly for TN, TNS, and HS, indicating that more hydrogen peroxide was decomposed in the kidneys in these treatments. For the HS treatment, heat stress causes an increase in respiration which triggers the kidneys to retain hydrogen and results in compensatory metabolic acidosis (Sejian, et al., 2015). This increased activity in the kidneys may cause more recruitment of CAT enzymes; however, it does not explain why HSS did not have more CAT activity. Others have found that male Japanese

quail exposed to heat stress at 34˚C for 8 hours a day had significantly higher MDA, but no change in CAT, GSH, and GPx (Türk, et al., 2016). Therefore, upregulation of CAT activity may not be a priority during heat stress.

As noted by other investigators, SOD activity in the current study did not significantly change with heat stress at 31.1˚C (Azad, et al., 2010). Investigators reported that acute heat stress at 40˚C caused an increase in SOD but did not affect other antioxidant enzymes like GPx (Ramnath, et al., 2008). Broilers that were exposed to heat stress at 38˚C for 3 hours showed an increase in lipid oxidation in the blood and an increase in CAT, SOD, and GSH reductase (Altan, et al., 2003). For this reason, it was expected that SOD activity of HS or HSS would reflect their environmental temperature; but the temperature may not have been high enough to cause such a physiological reaction or the change may not have been great enough to be significant.

5. Conclusions

Future directions could include heat stress (30 to 35˚C) to determine if total antioxidant capacity is lower and lipid oxidation is higher in eggs laid in the first week than all subsequent weeks of lay. Findings could help producers adjust their feeding programs to reflect the demands of the females during critical times such as onset of lay. The higher level of CAT activity in the kidneys is also another point of interest and should be further investigated. There were no overall differences between heat stress at 31.1˚C and no heat stress. However, the key findings from this study were:

1. Quail eggs stored at 4˚C for 13.35 weeks did not have significantly different MDA levels than those that were analyzed within 24 hours after lay.
2. CAT activity was highest in kidneys, followed by liver, then thigh and brain.
3. MDA concentration was as follows: brain>liver>kidney>thigh.

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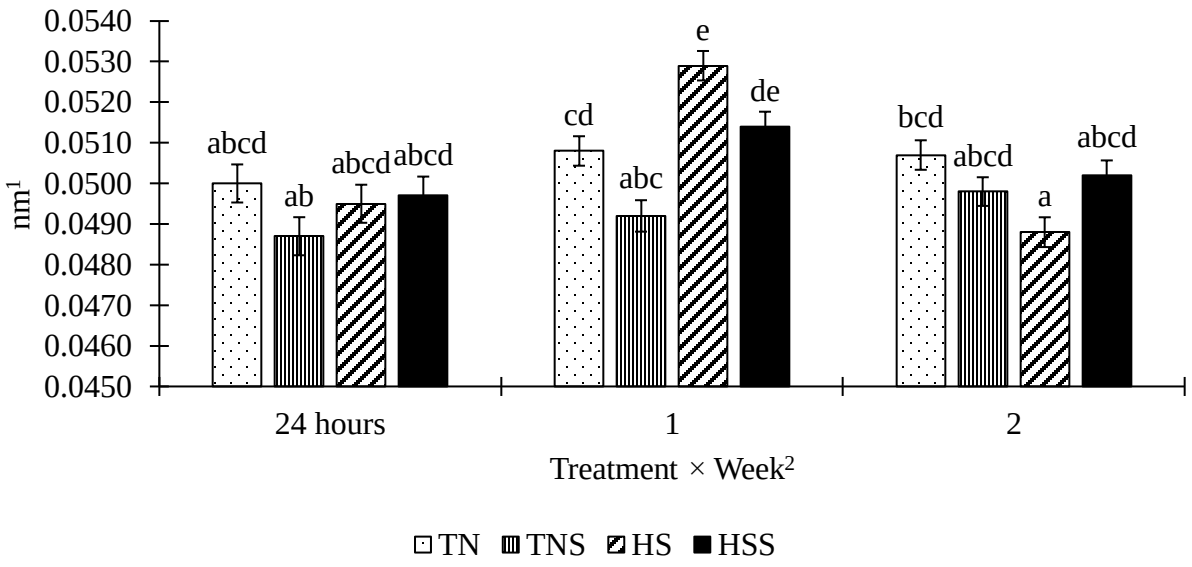

Figure 5.1. Lipid oxidation of egg yolks isolated within 24 hours after lay and 13.36 weeks after storage at 4˚C.

[a-e] Superscripts indicate significant differences at P≤0.05. Values were compared by treatment × week.

[1] Absorbance was measured at 532 nm.

[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

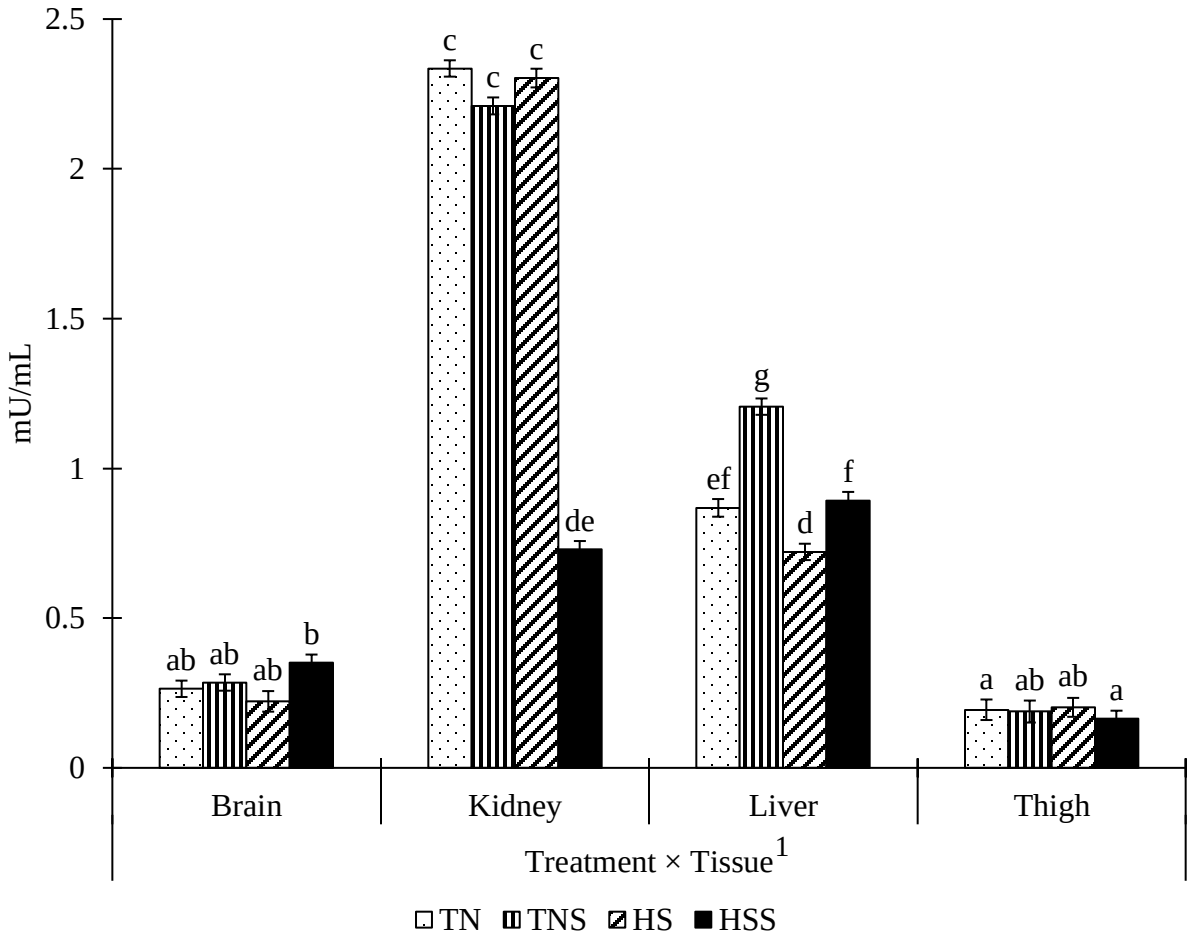

Figure 5.2. Catalase activity (nmol/min/mL) is presented in adult brain, kidney, liver, and thighs.

[a-f] Superscripts indicate significant differences at P≤0.05. Values were compared on treatment × tissue.

[1] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

Table 5.1. Lipid oxidation[1] of adult brain, kidney, liver, and thigh.

Tissue	Sex	Treatment[2]	MDA[3] (nmol/mg wet weight)
Brain	M	TN	8.84±0.26^{abcd}
		TNS	9.31±0.26^{cd}
		HS	10.01±0.26^{d}
		HSS	9.60±0.26^{cd}
	F	TN	9.87±0.26^{d}
		TNS	9.18±0.29^{bcd}
		HS	9.83±0.26^{d}
		HSS	9.08±0.26^{bcd}
Kidney	M	TN	6.89±0.26^{efg}
		TNS	6.52±0.26^{efgh}
		HS	6.43±0.26^{efgh}
		HSS	5.99±0.26^{efh}
	F	TN	5.91±0.26^{eh}
		TNS	6.58±0.29^{efgh}
		HS	5.77±0.26^{eh}
		HSS	6.11±0.26^{efh}
Liver	M	TN	7.32±0.26^{fgi}
		TNS	8.32±0.26^{abci}
		HS	6.06±0.26^{efh}
		HSS	7.81±0.26^{abgi}
	F	TN	6.89±0.26^{efg}
		TNS	6.31±0.29^{efh}
		HS	5.86±0.26^{eh}
		HSS	6.31±0.26^{efh}
Thigh	M	TN	5.02±0.35^{hj}
		TNS	7.18±0.41^{aefgi}
		HS	5.03±0.35^{hj}
		HSS	6.86±0.35^{efghi}
	F	TN	5.21±0.50^{efhj}
		TNS	5.80±0.45^{efhj}
		HS	5.30±0.41^{ehj}
		HSS	3.83±0.30^{j}

[a-j] Superscripts indicate significant differences at P≤0.05. Values were compared on treatment × tissue × sex.

[1] Absorbance was measured at 532 nm.

[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C,

TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[3] Malondialdehyde, MDA (nmol/mg wet weight). Results are presented as mean ± SE.

Table 5.2. Superoxide dismutase activity[1] in the adult brain, kidney, liver, and thigh.

Tissue	Treatment[2]	SOD activity (% inhibition)[3]
Brain	TN	84.30±3.70^{ab}
	TNS	83.60±3.82^{ab}
	HS	81.60±3.70^{ab}
	HSS	82.90±3.70^{ab}
Kidney	TN	74.40±3.70^{ab}
	TNS	81.10±3.70^{ab}
	HS	81.60±3.70^{ab}
	HSS	90.70±3.70^{b}
Liver	TN	75.00±3.70^{ab}
	TNS	67.30±3.70^{a}
	HS	76.30±3.70^{ab}
	HSS	72.90±3.70^{ab}
Thigh	TN	83.40±4.27^{ab}
	TNS	84.90±4.46^{ab}
	HS	81.00±3.96^{ab}
	HSS	88.90±3.40^{b}

$^{a-j}$ Superscripts indicate significant differences at $P \leq 0.05$. Values were compared using treatment × tissue interactions.

[1] Adult tissue were analyzed for SOD activity using a commercial kit to detect % inhibition of superoxide anions.

[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[3] Superoxide dismutase, SOD. Results are presented as mean ± SE.

CHAPTER 6
Proteomics of adult female Japanese quail (*Coturnix coturnix japonica)* livers subjected to multigenerational heat stress

Abstract: Heat stress can alter protein abundance in various pathways. However, the length of exposure, severity of heat, and demands of the animal can influence the extent of protein abundance alteration. Therefore, the objective of the current study was to determine the effects of initial heat stress at 31.1˚C on overall protein abundance in livers of actively laying female Japanese quail. It was hypothesized that quail selected for high performance (low FCR) in a heat stress environment would upregulate proteins involved in β-oxidation and the TCA cycle to meet increased energy demands. To test this hypothesis, 4 treatment groups were formed: (1) thermoneutral (TN, 22.2˚C), (2) thermoneutral siblings (TNS, 22.2˚C), (3) heat stress (HS, 31.1˚C), and (4) heat stress siblings (HSS, 31.1˚C). TN and HS were random bred in their respective temperatures. TNS and HSS were siblings reared at the two temperatures. HSS quail were used to determine low FCR at 31.1˚C and their corresponding TNS were mated to create the next generation's TNS and HSS. This was repeated for 10 generations. Proteins of livers from sexually mature females were quantified on Skyline. Significant differences were determined at $P \leq 0.05$. Results showed that when compared to TN, (1) HSS had 118 significantly down-regulated proteins and 56 significantly upregulated proteins and (2) HS had 75 significantly down-regulated proteins and 2 significantly upregulated proteins. Differences in protein abundance among all other comparisons were minimal (≤5) or insignificant. TN and/or TNS had significantly less antioxidants (SOD Cu/Zn and CAT) and lipoprotein transport from liver to egg than those in a heat stress temperature. However, TN had significantly more proteins involved in adipogenesis, lipogenesis, and fatty acid oxidation than HSS. The lower abundance of β-oxidation enzymes for HS and HSS could indicate that heat stressed quail decreased energy production to prevent further oxidative damage as hypothesized. However, results from this study disproved the hypothesis because birds in thermoneutral temperatures had more proteins involved in energy expenditure than those in

chronic heat stress. Future studies should include proteomic analysis of acute heat stress in quail between 30˚C and 34˚C. Studies, especially at the lower temperatures, could provide information on the initial measurable effects of heat stress on quail and other avian species.

1. Introduction

Heat stress can increase energy production to decrease heat increment and body temperature (Hosseini-Vashan and Raei-Moghadam, 2019). During oxidative stress, there are three levels of defense against free radicals. The first line of defense includes certain antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase, (CAT), and metal-binding proteins. These biochemicals will prevent free radical formation by removing precursors of free radicals or inactivating free radicals directly. The second line of defense includes chain-breaking antioxidants such as vitamins (A, C, and E), coenzyme Q, carotenoids, and glutathione (GSH). These molecules can inhibit peroxidation by keeping chain reactions low and can prevent propagation of lipid peroxidation by scavenging peroxyl and other intermediates of chain reactions. The third line of defense includes lipases, ligase, polymerases, phospholipases, and heat shock proteins (HSP). These molecules can eliminate or repair damaged molecules (Surai, et al., 2016). HSP have been such an integral part of the heat stress response that there have been discussions on genetically selecting birds with polymorphisms in HSP genes to increase heat tolerance (Pawar, et al., 2016).

With the different levels of defense against free radicals, researchers reported that there is a rapid increase of SOD, CAT, and GPx immediately after heat stress (Akbarian, et al., 2016). Within the first 6 hours of heat stress, there is also an increase in cellular demand which leads to mitochondrial transport and β-oxidation of fatty acids for ATP synthesis (Mujahid, et al., 2007). The enhanced activity in the mitochondria increases production of reactive oxygen species, thereby

requiring more electron carriers to mitigate the high levels of oxygen (Akbarian, et al., 2016). However, there is evidence that heat stress causes oxidative damage to enzymes, cellular lipids, and the mitochondrial membrane, leading to lower abundance of these proteins (Emami, et al., 2021).

Poultry species have been reported to exhibit different physiological changes during chronic heat stress. Broilers experiencing chronic heat stress (34˚C for 41 days) beginning at 1-day-old had decreased GPx activity in their blood, liver, and kidney; however, CAT increased in the blood, liver, kidney, and heart (Seven, et al., 2009). Others found that (1) β-oxidation activity increased in broilers after 9 days of heat exposure but decreased activity after 14 days and (2) TCA cycle activity decreased steadily after 9 days of heat stress (Azad, et al., 2010). Fewer studies have been performed on chronic heat stress proteomics in Japanese quail (Kang and Shim, 2020).

The liver is a key organ for regulation of lipid metabolism and accounts for up to 90% in vivo synthesis of fatty acids in avian species (Emami, et al., 2021). The importance of the liver in nutrient metabolism makes it highly susceptible to oxidative stress. Lipogenesis is known to be intensified by estrogen due to increased demand of vitellogenin for egg yolk deposition (Alvarenga, et al., 2011). However, during heat stress, glucocorticoids such as corticosterone are increased, having inhibitory actions on synthesis of sex steroid hormones such as estrogen. Therefore, understanding the effects of heat stress in sexually mature female birds is important as there may be tradeoffs in energy distribution.

When animals are chosen for production, high performance phenotypes such as a low FCR can be used for selective breeding. However, elevated performance during heat stress was shown to exacerbate the stress effects on the animal and cause compromises in performance and productivity (Orhan, et al., 2013). Therefore, rather than choosing high performance in

thermoneutral temperatures (18-30˚C for Japanese quail) it may be advantageous to select for high performance in heat stress temperatures (>30˚C). By choosing for high performance at an elevated temperature, physiological adaptation may occur and compromises may be lessened.

In previous chapters, it was determined that heat stress (31.1˚C) for quail did not produce clear significant differences when compared to the thermoneutral temperature (22.2˚C). Thus, the goal of the present study was to determine if the abundance of lipid metabolism, energy metabolism, and egg production proteins were significantly altered during initial heat stress (31.1˚C) in livers of actively laying female Japanese quail. Particularly, it was of interest to investigate the effects of selection for low FCR during heat stress. It was hypothesized that quail subjected to heat stress at 31.1˚C will upregulate proteins involved in lipid metabolism, energy metabolism, and HSP to meet demands of heat stress and egg production.

2. Materials and Methods

2.1 Experimental design (Chapter 2, Figure 1)

The detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. The four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

2.2 Sample collection

Animal care and use was approved by the Institutional Animal Care and Use Committee at the University of California Davis (Protocols #19473 and 21370; Davis, CA). Female quail (16.86-weeks-old) were euthanized using cervical dislocation. Livers were harvested, placed into WhirlPak® bags, and immediately submerged into liquid nitrogen after collection. Samples were then transferred to a -80˚C freezer until further analysis.

2.3 Protein extraction and in-solution digestion

Protein extraction was performed as previously reported (Kültz, et al., 2013; Root, et al., 2021). Briefly, liver samples were cooled in liquid nitrogen and ground into a powder in a Protein LoBind Eppendorf tube (LR-MCF). Then, 8M urea (1.5x sample wt/vol) was added to denature proteins and inhibit proteases and phosphatases. To reduce proteins, 10mM dithiothreitol (DTT) was added. The solution was incubated at 37˚C for 30 minutes.

To alkylate the proteins, 30 mM iodoacetamide was added and incubated in the dark at room temperature for 30 min. To remove nucleic acids and contaminants, proteins were precipitated in an ice-cold solution of 10% trichloroacetic acid/90% acetone/0.15% DTT (5x the total volume). This solution was incubated at -30˚C for 30 minutes, then centrifuged at 4°C at 19,000 x g for 5 min. The supernatant containing acetone-soluble non-protein contaminants was discarded and the precipitated protein pellet underwent one wash with ice-cold 100% acetone/0.15% DTT. The supernatant was discarded once again, and 8M urea (5x the original sample wt/vol) was added to dissolve the protein pellet. This solution was incubated on a rotator for 30 min at room temperature.

The dissolved protein pellet was centrifuged at 19,000 x g for 5 minutes at room temperature to pellet any remaining insoluble material. The supernatant was transferred to a clean

LR-MCF tube and stored at -80°C until trypsin digestion. To determine protein concentration, a Pierce™ bicinchoninic acid (BCA) protein assay compatible with diluted urea (Thermo Scientific™, Catalog # 23225) was performed. As determined by the BCA protein assay, relative volumes (µL) of sample containing 50 µg of protein were added to 0.5 ml LR-MCF tubes. LCMS grade water (4x volume of sample) and 1M ammonium bicarbonate buffer (pH 8.5, final concentration 100 mM) were added to the sample to achieve a 150ng/100µL total protein concentration.

To cleave proteins from the carboxyl side of lysine or arginine, immobilized trypsin (Promega, Catalog # V9012) was added at a 1:25 ratio relative to the amount of protein in solution. Samples were incubated on a rotator at 35°C for exactly 16 hours. Immobilized trypsin and insoluble contaminants were centrifuged out of solution at 500 x g for 2 minutes at room temperature. The supernatants were transferred to clean LR-MCF tubes and further removal of insoluble material was performed by centrifugation at 19,000 x g for 5 minutes at room temperature. The supernatants were transferred to clean LR-MCF tubes.

Samples were dried using a SpeedVac (Thermo-Savant, ISS-110) until urea precipitates began forming. Peptide pellets were resuspended in LCMS grade water containing 0.1% MS-grade formic acid and incubated for 15 minutes at room temperature to reconstitute samples at a total peptide concentration of 100 ng/µL. This solution was transferred to total recovery glass vials (Waters 600000669CV) for sample injection.

2.4 Peptide separation and mass spectrometry

Peptide separation was performed as previously reported (Kültz, et al., 2013; Root, et al., 2021). To briefly summarize, 2µL of peptide samples were injected with a nanoAcquity sample manager (Waters, Milford, MA). A Symmetry trap column (Waters, Catalog # 186003514) was

used to trap the samples for 1 minute at 15 µL/minute and a nanoAcquity binary solvent manager (Waters) for reversed phase liquid chromatography was used to separate peptides on a 1.7 µm particle size BEH C18 column (250mm x 75µm, Waters, Catalog # 186003545). To elute peptides, a dual pico-emitter tip (New Objective FS360–20-10-D-20, Woburn, MA) was used with a 125 min linear gradient (3% to 35%) acetonitrile directly into a UHR-qTOF mass spectrometer (Impact II, Bruker). Samples were processed in batches using Hystar 4.1 (Bruker). A 68 fmol BSA peptide mix was used at least once a week as a quality control standard to monitor instrument performance.

2.5 Data-dependent acquisition (DDA) and data-independent acquisition (DIA) library construction

DIA assay library was constructed as previously described using the quail proteome as a reference (Root, et al., 2021). All samples were loaded onto Skyline to be quantified.

2.6 Statistical analysis of protein abundance

Protein abundance values were exported from Skyline and analyzed in R 4.0.0 (Pino, et al., 2020; R Core Team, 2020; RStudio Team, 2022;). % Total protein abundance (% TPA) was calculated as 100 × (individual protein abundance/total protein abundance) and used for statistical analysis. One-way ANOVA was used to determine significant differences among treatments ($P \leq 0.05$). The Shapiro-Wilk test was used to determine normality of residuals of models and data were considered normal at $W \geq 0.95$ or $P \geq 0.05$. Levene's test and Q-Q plots were used to determine homogeneity of variances and variances were considered equal at $P \geq 0.05$. If there were significant differences, pairwise contrasts were made with confidence levels of 0.95 using the Tukey method for comparing estimates. Values are reported as averages with standard errors. The summation of peptides, transitions, and isoform abundances introduces errors to the values used for statistical analysis and results were interpreted accordingly.

To determine significance of up and downregulation of proteins, fold change (FC) and adjusted p-values were calculated from MSstats, a R package that is incorporated into Skyline (Choi, et al., 2014; Pino, et al., 2020). Significant downregulation of proteins was determined at FC≤0.5 and significant upregulation of proteins was determined at FC≥2. Adjusted p-values cutoff was set at 0.05. Fold change was log2 transformed and adjusted p-values were -log10 transformed, then plotted in volcano plots.

3. Results

3.1 Antioxidant proteins

There were no significant differences in % TPA among treatments in GSH proteins (Figure 1). However, GPx-1 had the highest % TPA of all the glutathione proteins (Figure 1). HS had significantly higher CAT % TPA than TN (p=0.049); however, they were both not significantly different from TNS and HSS (Figure 2). There were no significant differences in % TPA of MnSOD, mitochondrial (p=0.31); however, TNS had significantly lower Cu/Zn SOD % TPA when compared to HS (p=0.0082; Figure 3).

3.2 HSP

There were no significant differences in % TPA in any of the HSP except for HSP70, mitochondrial and HSP70, protein 4 (Figure 4). HSS had significantly less HSP 70, mitochondrial % TPA than TNS (p=0.044). HSS had significantly less HSP 70, protein 4 % TPA than TN (p=0.027).

3.3 Fatty acid binding proteins and lipid transportation proteins

Only FABP, brain had significant differences with TN having significantly higher % TPA than all other treatments (p=0.0043; Table 2). Both vitellogenin-1 (p=0.0053) and vitellogenin-2 (p=0.040) had significantly higher % TPA in HS than TNS (Table 3). HS also had significantly

higher % TPA of vitellogenin-1 than TN (p=0.017). TNS had significantly higher apolipoprotein A-IV % TPA than both HS and HSS, but TN was not significantly different from any of the other treatments (p=0.019; Table 3).

3.4 Fatty acid breakdown proteins

Of all proteins identified in fatty acid breakdown, fatty acyl-CoA hydrolase precursor, medium-chain like isoforms 1 and 2, long-chain fatty acid-CoA ligase 5, and long-chain fatty acid-CoA ligase ACSBG2 isoforms 1 to 4 had significantly higher % TPA in TN than HS and HSS, but TNS was not significantly different from any of the treatments (Table 4). Monoglyceride lipase isoforms 1 to 3 had significantly higher % TPA in TN when compared to HS; however, TNS and HSS were not significantly different from any of the other treatments. HS had significantly higher % TPA of monoacylglycerol lipase ABHD6 when compared to HSS; however, both HS and HSS were not significantly different from TN and TNS (Table 4).

3.5 β-oxidation proteins

TN had significantly higher % TPA than HS and HSS in mitochondrial carnitine/acylcarnitine carrier protein, probable acyl-CoA dehydrogenase 6 isoform 1, and acyl-CoA dehydrogenase family member 11- like; however, TNS was not significantly different from any of the other treatments (Table 5). TNS also had significantly higher acyl-CoA dehydrogenase family member 11- like % TPA than HS and HSS. For the low-quality protein: carnitine O-acetyltransferase, TNS had significantly higher quantities than both HS and HSS; but TN was not significantly different from all other treatments (Table 5). However, for carnitine O-acetyltransferase 1, liver isoform, isoform 1, and isoform 2, TN was significantly different from HSS, but HS and TNS were not significantly different from TN or HSS (Table 5). For all 4 isoforms of short/branched chain specific acyl-CoA dehydrogenase, mitochondrial, TN was

significantly lower in % TPA than TNS; but both were not significantly different from HS and HSS (Table 5).

3.6 Fatty acid synthesis proteins

Three proteins, low-quality protein: acetyl-CoA carboxylase 2 and constitutive coactivator of peroxisome proliferator-activated receptor gamma (PPAR-γ) -like protein 1 isoforms 1 and 2, were determined as important to fatty acid synthesis. For these, TN had significantly higher % TPA when compared to HSS; however, both TN and HSS were not significantly different from TNS and HS (Table 6).

3.7 Protein fold changes

The comparison between TNS and TN had one significantly down-regulated protein (acyl-CoA 6-desaturase) and five significantly upregulated proteins (Figure 4a). The comparison between HSS and TN had the highest number of significant fold-changes with HSS having 118 significantly down-regulated proteins and 56 significantly upregulated proteins (Figure 4c).

The comparison between HS and TN delineated 75 significantly down-regulated proteins and 2 significantly upregulated proteins (fructose-bisphosphate aldolase C-like and mid1-interacting protein 1; Figure 4b). Protein 4.1 isoforms 1-23 and isoforms 26-32 accounted for 30 of the 75 significantly down-regulated proteins in the HS and TN comparison (Table S1). There were also 8 isoforms of calcium-binding mitochondrial carrier protein and 6 isoforms of 6-phosphofructo-2-kinase (Table S1). There were 39 significantly down-regulated proteins shared between TN versus HS and TN versus HSS.

The comparison between HSS and HS revealed one significantly down-regulated protein (putative dimethylaniline monooxygenase [N-oxide-forming] 6 isoform 2) and no significantly upregulated proteins (Figure 4d). The comparison between TNS and HS indicated 2 significantly

down-regulated proteins (thyroid hormone-inducible hepatic protein and mid1-interacting protein 1) and no significantly upregulated proteins (Figure 4e). The comparison between HSS and TNS did not have any significantly downregulated or upregulated proteins (Figure 4f).

4. Discussion

4.1 Antioxidant proteins

Others have reported that high environmental temperatures increase oxidation in the liver of poultry species (Hosseini-Vashan and Raei-Moghadam, 2019). However, in the current study, there were no significant treatment effects on GPx, an important antioxidant enzyme. Regardless, there was a relatively high % TPA of GPx-1 in the female quail liver as also reported by others (Wilaison and Mori, 2009).

With increased demand on antioxidants in heat stressed animals, studies showed that heat stress decreased SOD concentration in the blood (Hosseini-Vashan and Raei-Moghadam, 2019; Surai, et al., 2019). However, in the present study, there was significantly less SOD Cu/Zn protein in female TNS livers when compared to that in HS and significantly less % TPA of CAT enzymes in TN compared to HS, thus, indicating that HS quail had more circulating antioxidant enzymes than those at the thermoneutral temperature. Others have found that antioxidant enzyme activities are upregulated during acute heat stress; however, chronic heat stress leads to a depletion of antioxidant reserves (Akbarian, et al., 2016). Thus, as not previously reported to the author's knowledge, quail at 31.1˚C were experiencing mild heat stress that caused an upregulation of antioxidant proteins, but they were not experiencing enough heat stress to cause significant oxidation.

4.2 HSP

HSP are involved in chaperone protein translocation across membranes, protein folding, and disassembling of proteins complexes (Craig, 2018). However, several HSP have different roles, depending on their location in the body (Craig, 2018). HSP protein 4 (HSP4) is a key protein that increases during endoplasmic reticulum stress (Kapulkin, et al., 2005). Additionally, HSP4 is upregulated in the necrosis pathways and cell cycle checkpoint/arrest pathways (Park, et al., 2015). The results from the present study agree with that of other investigators because a significant increase of HSP4 % TPA in HSS compared to TN could indicate that there was an environmental stressor present.

Contrarily, TNS had a higher HSP70 mitochondria (mtHSP) % TPA than HSS. mtHSP70 is a critical molecular chaperone through the mitochondrial membrane and mitochondrial biogenesis (Craig, 2018; Havalová, et al., 2021). In human mitochondria, it has been shown to act as a stress-survival factor by assisting in refolding or degrading non-functional proteins (Havalová, et al., 2021). Contrary to the results of the present study, others found that Japanese quail (35-day-old) exposed to cyclic heat stress at 34˚C for 8h/d had higher levels of HSP60, HSP70, and HSP90 (Orhan, et al., 2013). Therefore, TNS having a higher mtHSP70 was not expected. This finding indicated that 31.1˚C did not incur a stress response in HSS; but it does not explain why TNS had higher mtHSP70.

4.3 Fatty acid binding proteins and lipid transportation proteins

Vitellogenin is a crucial protein made in the avian liver for egg production because it provides phosvitin containing 80% of the phosphorus in eggs (Cherian, 2005). Estradiol stimulates production of yolk proteins such as vitellogenin II and apolipoprotein by the liver (Elnagar and Abd-Elhady, 2009). This relationship is so closely associated that vitellogenin has been used as a

biomarker for environmental estradiol pollution (Elnagar and Abd-Elhady, 2009). Stemming from this association, results in the present study showed that HS had significantly higher % TPA of vitellogenin I and vitellogenin II than TNS, which likely indicated more circulating estradiol in HS than TNS. However, from Chapter 7 (Table 5) there was no significant difference in estradiol between the two treatments. The significantly higher % TPA of vitellogenin-1 and vitellogenin-2 in HS than TNS female livers is opposite to findings indicating that oxidative damage on hepatic cell membranes decreased vitellogenin and triacylglycerides (Sahin, et al., 2002).

Apolipoprotein A-IV (Apo A-IV) participates in lipid metabolism, glucose homeostasis, and food intake (Qu, et al., 2019). Others have found that Apo A-IV was upregulated in heat stressed mice which could be a reason for decreased feed intake during heat stress (Memon, et al., 2016). FABP7 facilitates transport and uptake of docosahexaenoic acid and arachidonic acid (Gerstner, et al., 2008). Possibly, TN having significantly more FABP and brain (FABP7) and TNS having significantly more Apo A-IV than HS and HSS indicated that there was more nutrient transfer for TN and TNS than those in a heat stress environment. This possibility was not expected because it was hypothesized that HS and HSS would have more energy expenditure and therefore, lipid transporters in circulation.

4.4 Fatty acid breakdown proteins

Monoglyceride lipase (MAGL) isoforms hydrolyze monoglycerides to glycerol and non-esterified fatty acids for fuel or lipid synthesis (Savinaineen, et al., 2012; Grabner, et al., 2017). MAGL also hydrolyze the endocannabinoid 2-arachidonoylglycerol (2-AG) to proinflammatory derivatives of arachidonic acid (Nomura, et al., 2011). Researchers have found that sulfenylation of MAGL in the brain during periods of high H_2O_2 will deactivate MAGL, which is protective against oxidative stress effects such as neurodegeneration or chronic neuropathic pain (Dotsey, et

al., 2015). Little information is known about the physiological function of monoacylglycerol lipase ABHD6 (ABHD6); although, it has also been linked to 2-AG (Savinaineen, et al., 2012). ABHD6 has been located in proximity to 2-AG which could indicate that it regulates 2-AG levels at the site of its generation (Savinaineen, et al., 2012). In the current study, TN had higher % TPA of MAGL isoforms 1-3 than HS; however, HS had higher levels of ABHD6 than HSS, indicating that there may have been significantly more lipid utilization as fuel and/or downstream inflammation in TN compared to HS. Additionally, HS having more ABHD6 than HSS suggested that HS had more control over inflammatory events than HSS and TN.

Fatty acyl-CoAs are used in β-oxidation and synthesis of fatty acids (Waku, 1992). Fatty acyl-CoA hydrolase is responsible for hydrolyzing fatty acyl-CoA thioesters to CoA and fatty acids (Waku, 1992). It was also suggested to protect the β-oxidation process in peroxisomes (Waku, 1992). Long-chain fatty acid-CoA ligase 5 (ACSL5) and long-chain fatty acid-CoA ligase bubblegum 2 (ASCBG2) catalyze the conversion of long-chain fatty acids to their active acyl-CoA forms (Ohkuni, et al., 2013). In the current study, TN had significantly higher % TPA of fatty acyl-CoA hydrolase precursor, ACSL5, and ACSBG2 than HS and HSS, thus, indicating that TN had more energy expenditure than HS and HSS. As previously mentioned, this does not agree with the original hypothesis that HS and HSS would increase energy use during heat stress.

4.5 β-oxidation proteins

In broilers that were subjected to acute heat stress at 34˚C for 6 hours, there was an increase in activity and expression of enzymes associated with β-oxidation, TCA cycle, and fatty acid transport (Mujahid, et al., 2007). Heat stress at 41˚C in human cell cultures also decrease long-chain β-oxidation enzymes present on mitochondrial membranes (Li, et al., 2010). Important enzymes involved in β-oxidation are long chain fatty acid oxidation enzymes (carnitine O-

palitoyltransferase) and short-chain fatty acid oxidation enzymes (acyl-CoA dehydrogenase 6, short/branched chain specific acyl-CoA dehydrogenase, and carnitine O-acetyltransferase) (Govindasamy, et al., 2004; Li, et al., 2010). As such, TNS and/or TN had significantly more % TPA long chain and short chain fatty acid oxidation enzymes than HS and/or HSS. Thus, there was more β-oxidation occurring in TN and TNS than in HS and HSS.

Results of the current study did not agree with others who found that six hours after heat stress at 34˚C, broiler chicken increased key enzymes of β-oxidation such as carnitine-palmitoyl-transferase I and II, 3-hydroxyacyl CoA dehydrogenase, as well as long-chain acyl CoA dehydrogenase gene expression and enzyme activity (Mujahid, et al., 2007). However, these researchers found that after 18 hours of heat stress, the gene expression of enzymes were significantly lower than those of chicken housed at a thermoneutral temperature (25˚C) (Mujahid, et al., 2007). Therefore, it could be possible that at the time of analysis for the present study, HS and HSS experienced chronic heat stress and decreased energy production to prevent further oxidative damage.

4.6 Fatty acid synthesis proteins

Acetyl-CoA carboxylase 2 is located in the outer mitochondrial membrane and is essential to catalyze the rate-limiting ATP-dependent carboxylation of acetyl-CoA to malonyl-CoA (Hunkeler, et al., 2018). This is the first step in fatty acid synthesis or lipogenesis. PPAR-γ plays a key role in storage and mobilization of lipids. In particular, it is critical in adipogenesis and insulin sensitivity (Medina-Gomez, et al., 2007). In the current study, TN had significantly more % TPA of acetyl-CoA carboxylase 2 and constitutive coactivator of PPAR-γ-like protein 1 isoforms 1 and 2 than HSS. This suggested that TN had significantly more adipogenesis and lipogenesis than HSS. Lipogenesis and adipogenesis was hypothesized to be higher in TN than all

other treatment groups due to decreased stress and lower mobilization of lipids for energy. Taken together with other significant differences in protein abundance, TN and TNS had more energy production from lipids as well as more lipid and adipose synthesis.

4.7 Protein fold changes

4.7.1 TN versus TNS

There was one protein (acyl-CoA 6-desaturase) that was significantly downregulated in TNS as compared to TN. This enzyme is involved in fatty acid metabolism, particularly the biosynthesis of unsaturated fatty acids from α -linolenic acid (Ge, et al., 2003). The five proteins that were significantly upregulated in TNS as compared to TN were predominantly involved in amino acid metabolism and carbohydrate metabolism (GenomeNet, Kyoto, Japan). Previous results, as shown above, indicated that lipogenesis was significantly higher in TN.

Relative to other comparisons, TN versus TNS did not have many significant up or downregulated proteins, indicating that they had similar metabolism. Therefore, selectively breeding for low FCR in heat stress without further exposure to heat stress did not incur any significant metabolic differences compared to random bred in a thermoneutral temperature.

4.7.2 TN versus HS

The proteins that were significantly downregulated in HS as compared to TN were predominantly involved in cell structure, fatty acid degradation, glycolysis, the TCA cycle, molecular transport, and steroid and thyroid hormone transport. Glycerol-3-phosphate acyltransferase 1, mitochondrial isoform 1 and 2, long chain fatty acid ligase 5, and ATP/ADP translocase 1 were among the downregulated proteins. Glycerol-3-phosphate acyltransferase 1, mitochondrial isoform 1 and 2, and long chain fatty acid ligase 5 are both essential to the triacylglycerol synthesis and fatty acid chain elongation (Kohlmeier, 2015). ATP/ADP translocase

1 is involved in the exchange of ADP and ATP across the mitochondrial inner membrane and is essential in regulating the ADP/ATP ratio in mitochondrial oxidative phosphorylation (Chevrollier, et al., 2011). However, the protein that was significantly upregulated in HS as compared to TN was fructose-bisphosphate aldolase C-like, an important enzyme in glycolysis and gluconeogenesis, and mid1-interacting protein 1 (MID1IP1) (Ziveri, et al., 2017; Meng, et al., 2020). MID1IP1 regulates and binds to acetyl-CoA carboxylase which is an essential enzyme in fatty acid synthesis (Meng, et al., 2020).

Overall, there was more downregulation of lipid synthesis in HS when compared to TN. Additionally, the downregulation of ATP/ADP translocase 1 indicated that there was also a decrease in energy metabolism in HS when compared to TN. This finding is similar to that of other investigators for birds experiencing chronic heat stress (Mujahid, et al., 2007).

4.7.3 TN versus HSS

The comparison of TN to HSS resulted in the most significantly up and downregulated proteins, suggesting that there were clear metabolic differences between the two treatments. The predominate functions of the downregulated proteins were involvement in the TCA cycle, protein or molecular transport, bile synthesis, cell structure, fatty acid synthesis, cell-cell signaling, and thyroid hormones (T3 and T4) transport. The predominant functions of upregulated proteins were antioxidant (GPx), autophagy, fatty acid breakdown, immunity, iron delivery, nucleotide synthesis, RNA synthesis, and protein transport to the mitochondria.

3β-hydroxysteroid dehydrogenase type 7-like isoform 2 was downregulated and is a key enzyme in biosynthesis of most steroid hormones (Abbaszade, et al., 1995). Possibly, there was a downregulation of corticosterone in HSS as compared to TN. In conjunction with the downregulation of various enzymes of the TCA cycle, it appears that quail in HSS downregulated

pathways that would lead to an increase in oxidation. Contrarily, others have found that acute heat stress increases the TCA cycle because there is an increase in skeletal muscle abundance of pyruvate dehydrogenase kinase 4 and lactate dehydrogenase mRNA (Slimen, et al., 2016).

The upregulation of iron delivery indicated an increase in oxygen consumption in HSS. The upregulation of antioxidant proteins such as GPx and glutathione S-transferase indicated HSS was experiencing more oxidation than TN. Taken together, there were downregulations of pro-oxidant proteins and an upregulation of antioxidant and oxygen carrying proteins. Thus, HSS had a physiological response to heat stress; however, it was not evident in lipid oxidation of various tissues as presented in Chapter 5.

Although acute heat stress was not studied in the present study, others found that during the initial phase of heat stress, there is an increase in β-oxidation which feeds into the TCA cycle in mitochondria (Mujahid, et al., 2007). However, during the end of the acute heat stress, there is a downregulation of avian uncoupling protein (avUCP) leading to an increase in oxidative stress (Mujahid, et al., 2007). Chronic heat stress causes a decrease in mitochondrial metabolic oxidative capacity; however, there are no physiological changes when birds are acclimated to high temperatures (Azad, et al., 2010; Akbarian, et al., 2016). Therefore, results from the current study agreed with several findings of others on effects of chronic heat stress on metabolism.

4.7.4 TNS versus HS

The two significantly downregulated proteins in TNS when compared to HS were thyroid hormone-inducible hepatic protein and MID1IP1, which are both important for fatty acid synthesis (Aipoalani, et al., 2010; Meng, et al., 2020). Although these two proteins were significantly downregulated, there was no clear evidence of significant metabolomic changes in TNS compared to HS, indicating that selection for low FCR in heat stress and not being exposed to heat stress still

incurs a fitness advantage. TNS quail could, therefore, be better prepared to be in hotter temperatures due to being primed through selective breeding.

4.7.5 TNS versus HSS and HS versus HSS

There were no significant fold changes in protein abundance between TNS and HSS, indicating that environmental changes did not significantly overcome the genetic similarities between the two treatment groups. Thus, poultry that are selectively bred for low FCR in heat stress can adapt to a wider range of temperatures without significant changes in their metabolism.

In the present work, there were no significantly upregulated proteins and only one significantly downregulated protein (putative dimethylaniline monooxygenase [N-oxide-forming] 6 isoform 2) which is involved in the detoxification process (Tarchini, et al., 2000). Overall, this finding suggested that quail selectively bred to have higher performance in heat stress were not metabolizing nutrients differently than those that were random bred. Perhaps selection for low FCR in heat stress at 31.1˚C did not result in physiologically different quail than those that were not selectively bred at 31.1˚C.

5. Conclusions

Future studies should include proteomic analysis of acute heat stress quail between 30˚C and >34˚C. There are currently numerous studies on poultry and heat stress at 34˚C; however, there are not many studies using temperatures immediately outside the thermoneutral zone of quail (Mujahid, et al., 2007; Seven, et al., 2009; Azad, et al., 2010; Kang and Shim, 2020; Egbuniwe, et al., 2021). Together with the information obtained in the current study, analysis of the protein abundance during acute heat stress could help determine nutritional requirements for different periods of heat stress in poultry. The most notable findings from this study were:

1. Quail in a thermoneutral temperature had significantly less % TPA of antioxidants (SOD Cu/Zn and CAT), lipoprotein transport from liver to egg, and HSP4 than those in a heat stress temperature. However, TN had significantly more % TPA involved in adipogenesis and lipogenesis than HSS.
2. Chronic heat stress in both HS and HSS decreased % TPA involved in TCA cycle activity. TNS and/or TN had significantly more β-oxidation enzymes than HS and/or HSS. This downregulation of proteins involved in β-oxidation and the TCA cycle in HS and HSS could have been an effort to decrease energy production and prevent further oxidative damage.
3. Differences in protein abundance between HSS and HS, TNS and HS, and TNS and HSS were minimal or insignificant.

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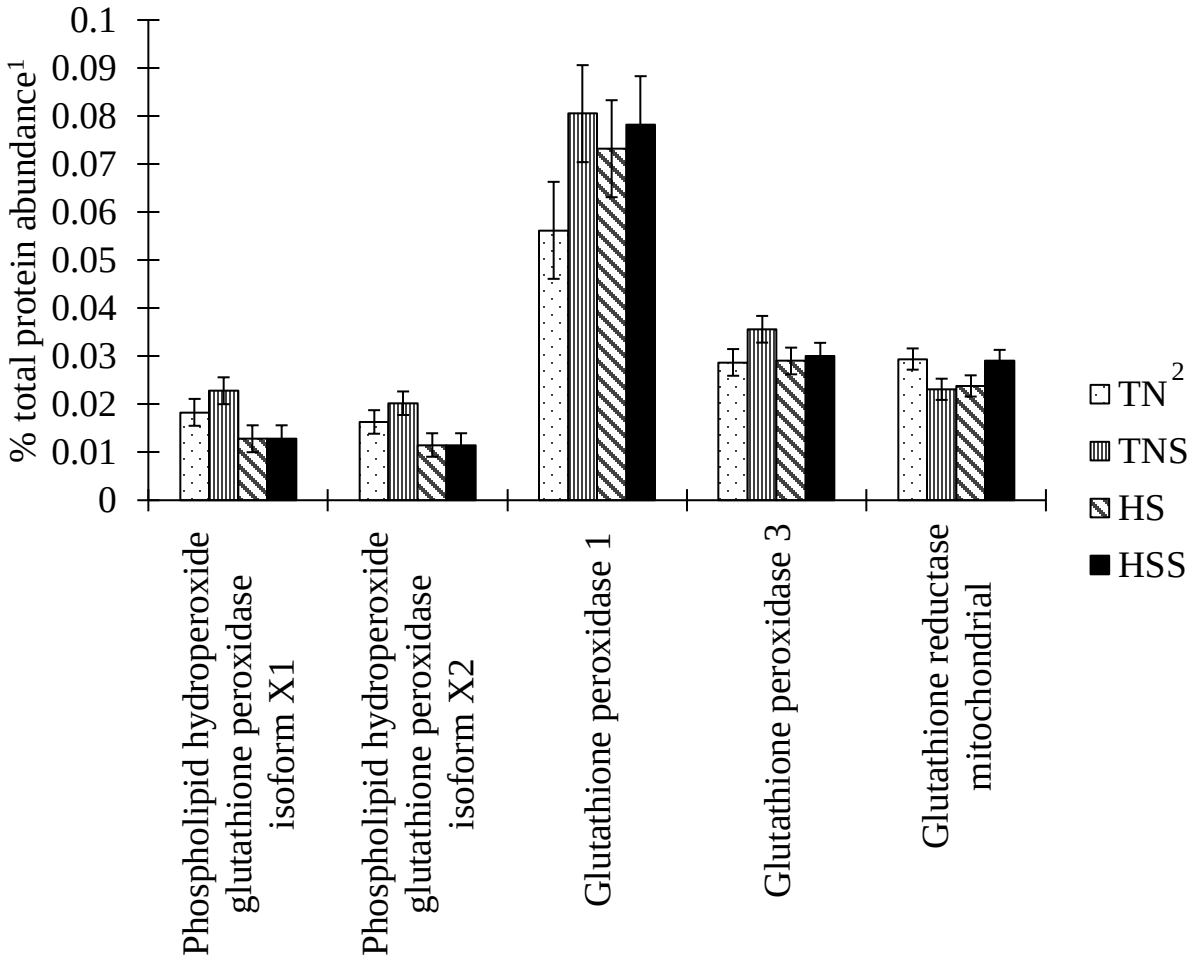


Figure 6.1. Glutathione peroxidase proteins of adult female Japanese quail livers.
[1] % total protein abundance = 100*(individual protein abundance/total protein abundance).
[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.
Superscripts indicate significant differences among treatments at $P \leq 0.05$.

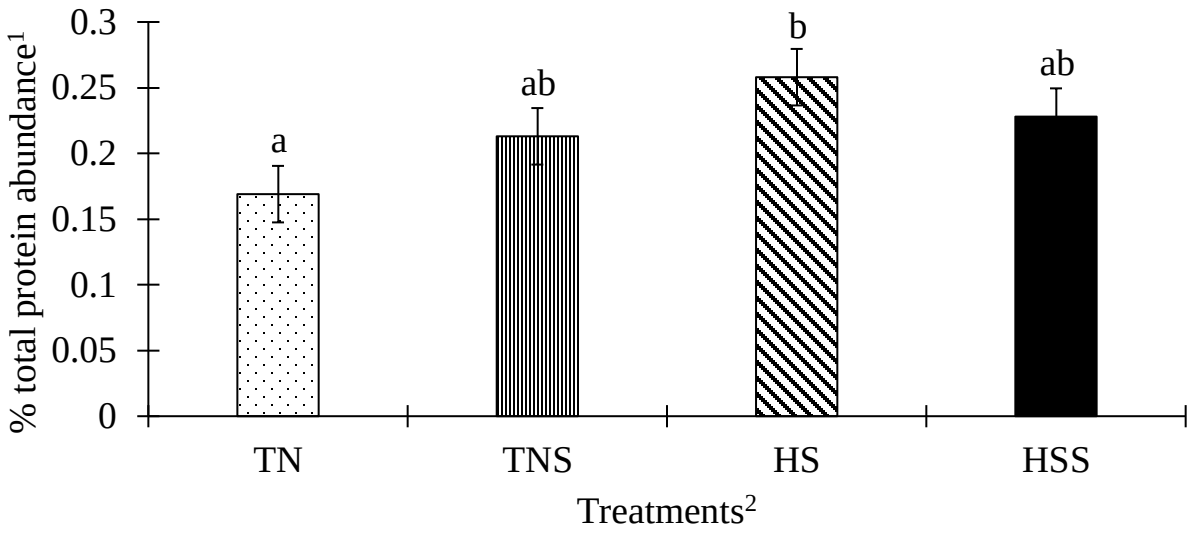


Figure 6.2. Catalase proteins of adult female Japanese quail livers.
[1] % total protein abundance = 100*(individual protein abundance/total protein abundance).
[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.
[a-b] Superscripts indicate significant differences among treatments at $P \leq 0.05$.

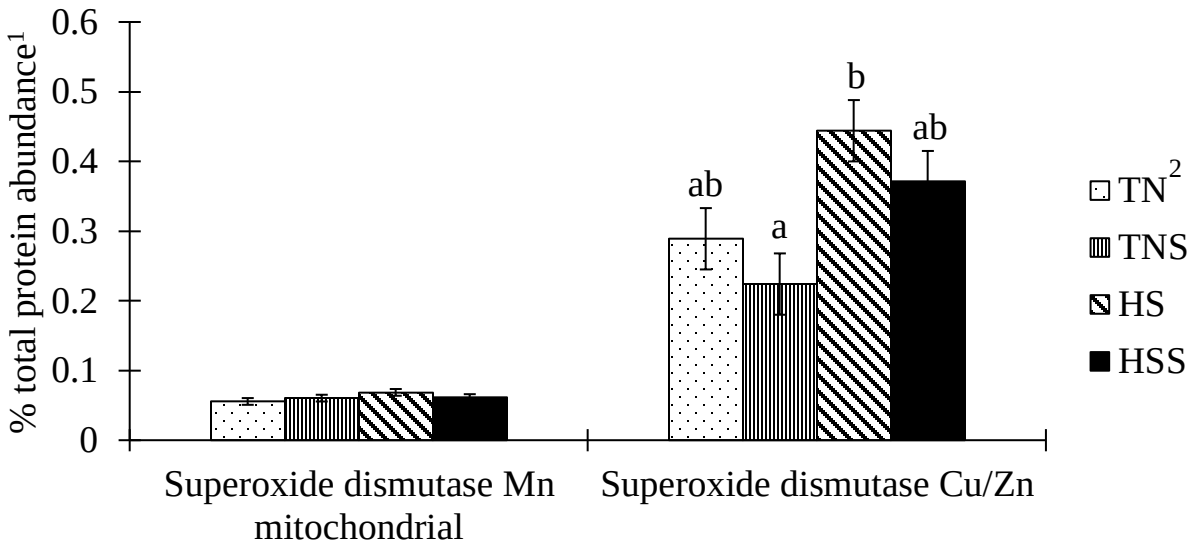
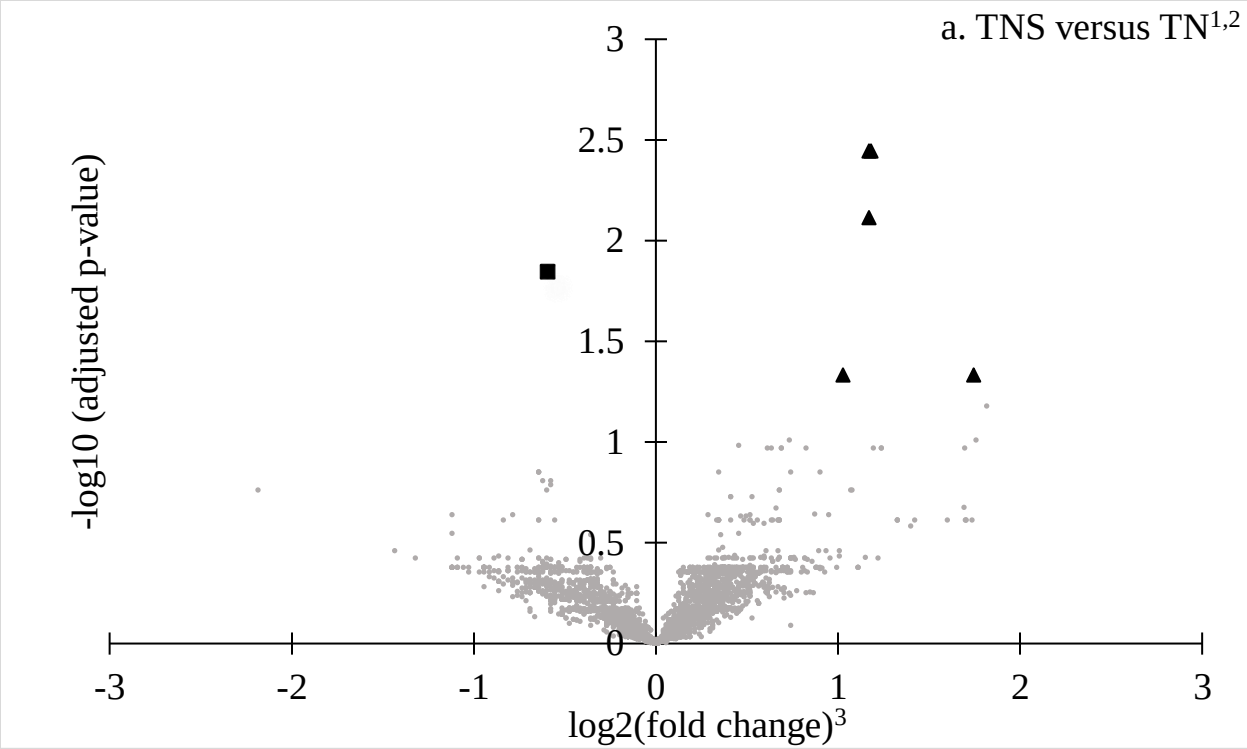
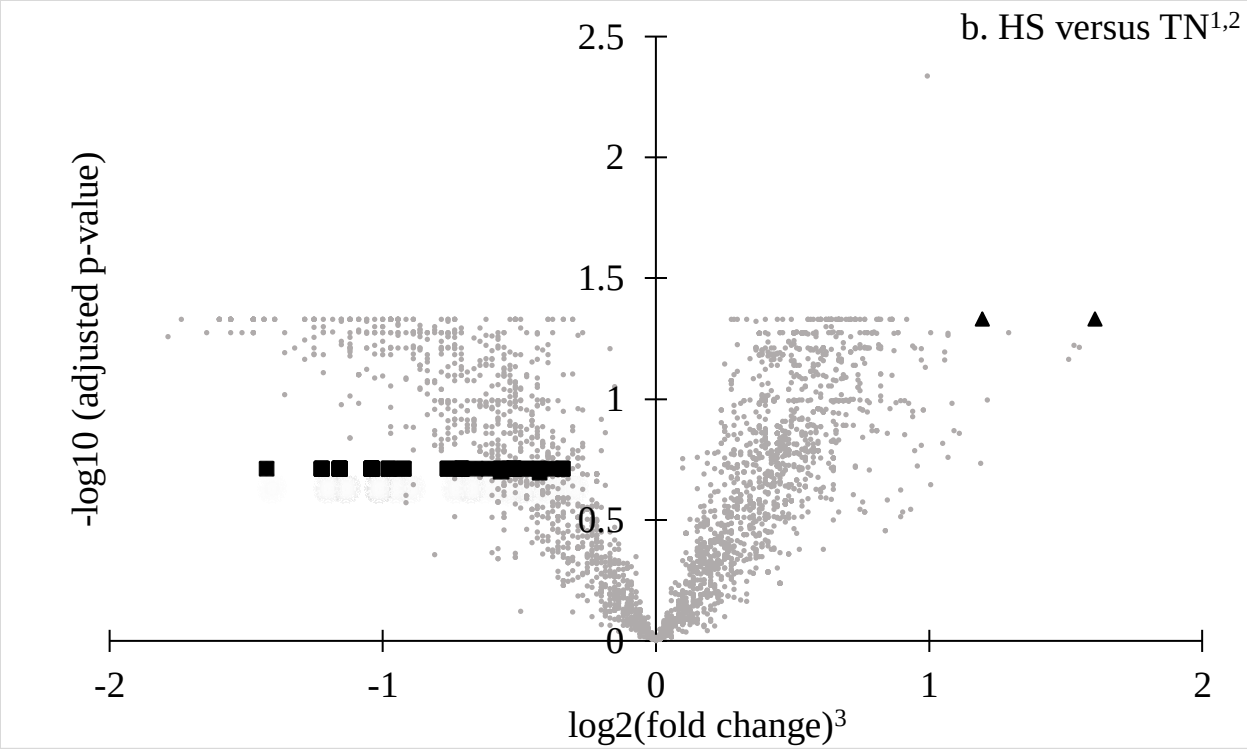
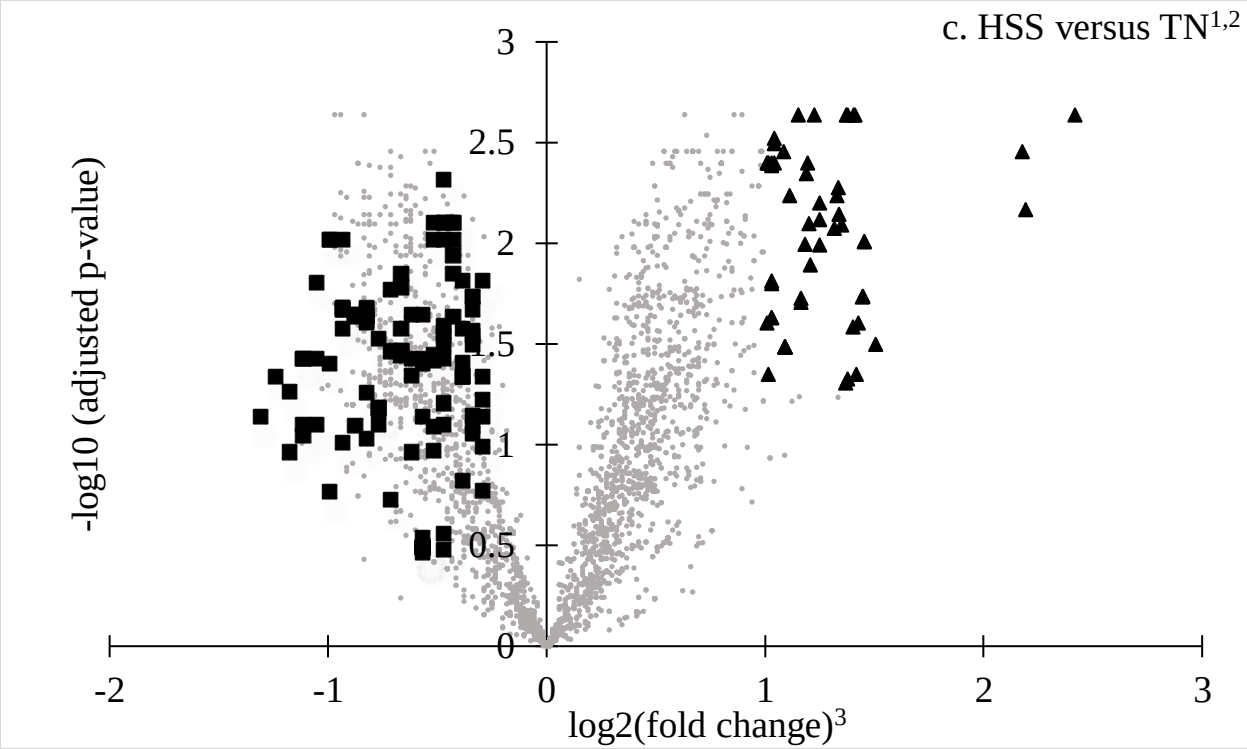
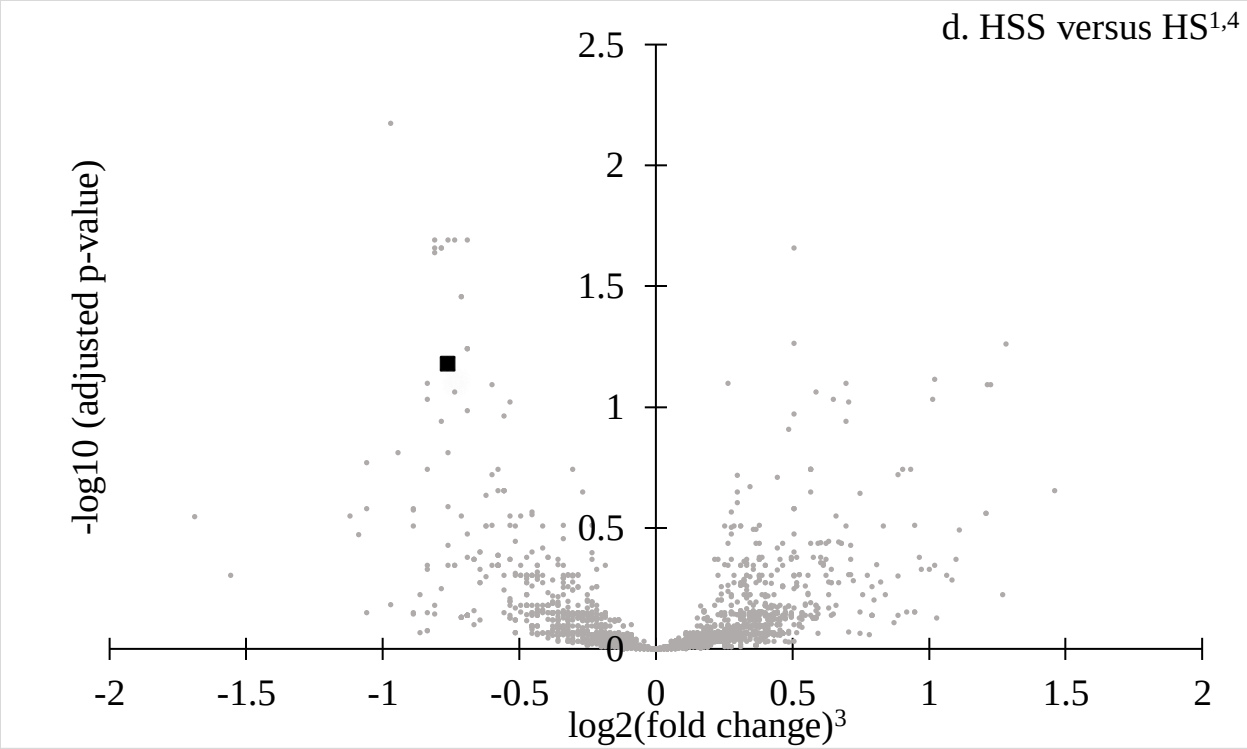


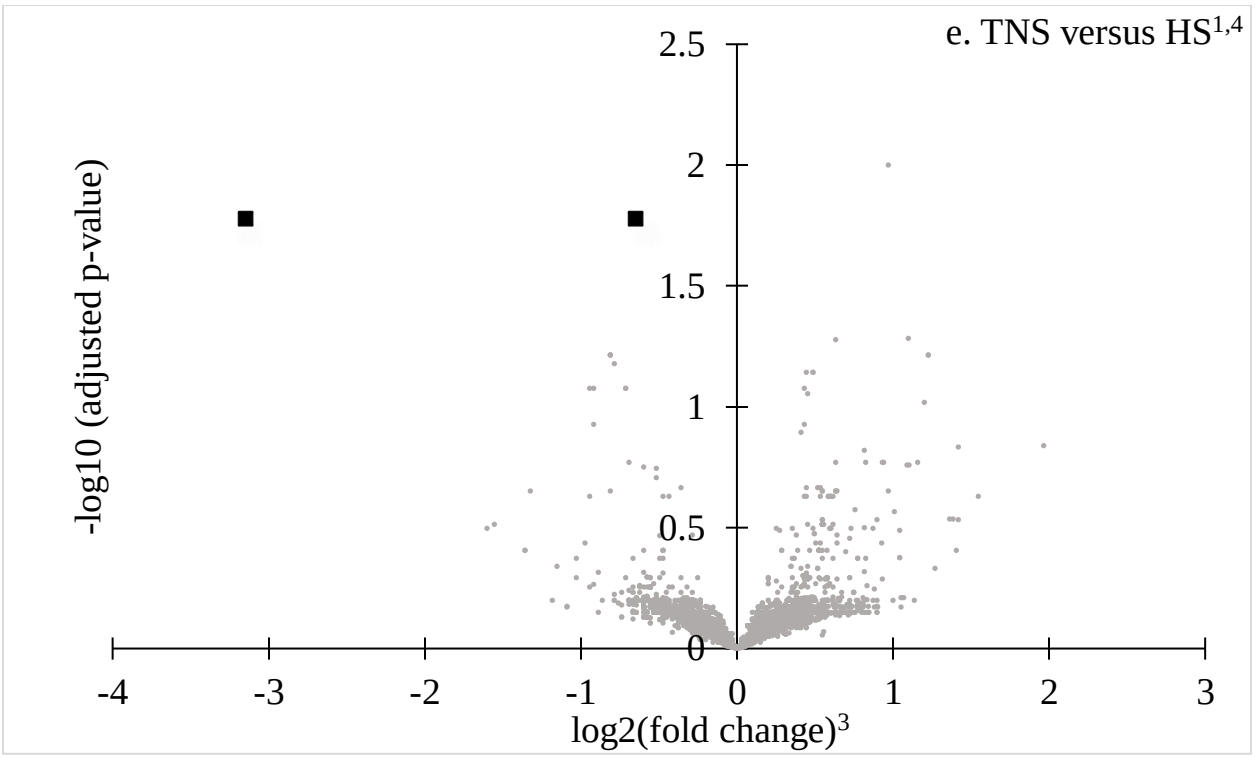
Figure 6.3. Superoxide dismutase proteins of adult female Japanese quail livers.
[1] % total protein abundance = 100*(individual protein abundance/total protein abundance).
[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.
[a-b] Superscripts indicate significant differences among treatments at $P \leq 0.05$.


a. TNS versus TN[1,2]
-log10 (adjusted p-value)
3
2.5
2
1.5
1
0.5
0
-3
-2
-1
0
1
2
3
log2(fold change)[3]


b. HS versus TN[1,2]
-log10 (adjusted p-value)
2.5
2
1.5
1
0.5
0
-2
-1
0
1
2
log2(fold change)[3]


c. HSS versus TN[1,2]
-log10 (adjusted p-value)
log2(fold change)[3]
3
2.5
2
1.5
1
0.5
0
-2
-1
0
1
2
3


d. HSS versus HS[1,4]
-log10 (adjusted p-value)
log2(fold change)[3]
2.5
2
1.5
1
0.5
0
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2



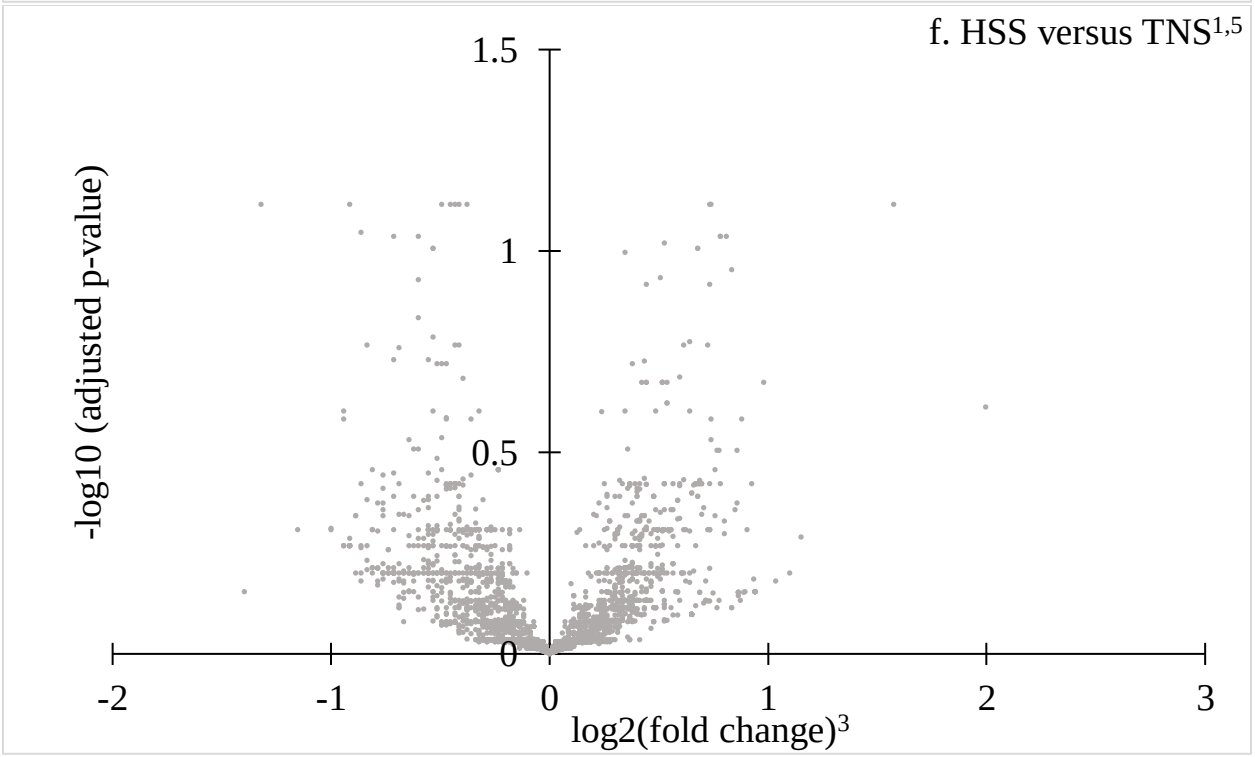


Figure 6.4a-f. Volcano plots of fold change in protein abundance and PEAKS significance score for all proteins with ≥2 unique peptides found in adult female Japanese quail livers.

■ Black squares are proteins with log2(0.5)-fold-change and significance threshold P>-log10(0.05).

▲ Black triangles are proteins with log2(2)-fold-change and significance threshold P>-log10(0.05).

▲■ Proteins satisfying fold change ≥2 or ≤0.5 and significance P≥-log10(0.05) were selected and detailed in Table S1.

[1] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[2] Figures 4a-c are compared to TN as the control (e.g., positive fold changes represent higher abundances in TNS versus TN).

[3] Fold changes are relative to the control.

[4] Figures 4d and e are compared to HS as the control (e.g., positive fold changes represent higher abundance in HSS versus HS).

[5] Figure 4f is compared to TNS as the control (e.g., positive fold changes represent higher abundance in HSS versus TNS).

Table 6.1. Heat shock proteins (HSP) of adult female Japanese quail livers.

	% Total protein abundance[1]					
Protein	TN[2]	TNS[2]	HS[2]	HSS[2]	SE	P-value
HSP10 mitochondrial	0.366	0.440	0.532	0.453	0.053	0.196
HSP60 mitochondrial	0.082	0.068	0.084	0.074	0.008	0.455
HSP70 protein 4L	0.008	0.007	0.008	0.008	0.000	0.360
HSP70 protein 4	0.005[a]	0.005[ab]	0.006[ab]	0.007[b]	0.000	0.027
HSP70 protein 13	0.013	0.013	0.012	0.012	0.001	0.563
HSP70 mitochondrial	0.038[ab]	0.043[a]	0.032[ab]	0.027[b]	0.004	0.044
Heat shock related 70 protein 2	0.078	0.062	0.060	0.054	0.007	0.161
Heat shock cognate 71	0.076	0.064	0.064	0.055	0.009	0.488
HSP75 mitochondrial	0.033	0.034	0.019	0.020	0.006	0.122
HSP75 mitochondrial isoform 1	0.033	0.034	0.019	0.020	0.006	0.122
HSP90 α	0.039	0.035	0.024	0.025	0.005	0.132
HSP90 β	0.066	0.052	0.035	0.036	0.010	0.099
HSP90 β isoform 1	0.078	0.062	0.041	0.043	0.012	0.099
HSP90 activator ATPase homolog 1	0.015	0.015	0.016	0.016	0.001	0.961
HSP105 isoform 1	0.006	0.006	0.007	0.005	0.001	0.150
HSP105 isoform 2	0.006	0.006	0.007	0.005	0.001	0.150

[1] % total protein abundance = 100*(individual protein abundance/total protein abundance).

[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[a-b] Superscripts indicate significant differences among treatments at $P \leq 0.05$.

Table 6.2. Fatty acid-binding proteins of adult female Japanese quail livers.

	% Total protein abundance[1]					
Protein	TN[2]	TNS[2]	HS[2]	HSS[2]	SE	P-value
fatty acid-binding protein 5	0.009	0.006	0.007	0.005	0.001	0.060
fatty acid-binding protein, extracellular	0.002	0.002	0.002	0.002	0.000	0.271
fatty acid-binding protein, adipocyte	0.072	0.061	0.052	0.048	0.011	0.434
fatty acid-binding protein, brain	0.117[a]	0.062[b]	0.072[b]	0.065[b]	0.011	0.004
fatty acid-binding protein 1, liver	0.338	0.354	0.475	0.418	0.044	0.135
fatty acid-binding protein 2, liver	0.851	0.754	1.054	0.830	0.084	0.095
fatty acid-binding protein, heart	0.073	0.058	0.056	0.049	0.009	0.362

[1] % total protein abundance = 100*(individual protein abundance/total protein abundance).
[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.
[a-b] Superscripts indicate significant differences among treatments at P≤0.05.

Table 6.3. Lipid transport proteins of adult female Japanese quail livers.

	% Total protein abundance[1]					
Protein	TN[2]	TNS[2]	HS[2]	HSS[2]	SE	P-value
vitellogenin-1	0.029[a]	0.028[a]	0.041[b]	0.034[ab]	0.003	0.005
vitellogenin-2	0.030[ab]	0.025[a]	0.034[b]	0.032[ab]	0.002	0.051
apolipoprotein A-IV	0.005[ab]	0.007[b]	0.004[a]	0.004[a]	0.001	0.019

[1] % total protein abundance = 100*(individual protein abundance/total protein abundance).
[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.
[a-b] Superscripts indicate significant differences among treatments at P≤0.05.

Table 6.4. Fatty acid breakdown proteins of adult female Japanese quail livers.

	% Total protein abundance[1]					
Protein	TN[2]	TNS[2]	HS[2]	HSS[2]	SE	P-value
monoglyceride lipase isoform 1	0.007[a]	0.005[ab]	0.004[b]	0.005[ab]	0.001	0.061
monoglyceride lipase isoform 2	0.007[a]	0.005[ab]	0.004[b]	0.005[ab]	0.001	0.059
monoglyceride lipase isoform 3	0.007[a]	0.005[ab]	0.004[b]	0.005[ab]	0.001	0.060
monoacylglycerol lipase ABHD6	0.005[ab]	0.004[ab]	0.006[a]	0.003[b]	0.001	0.005
fatty acyl-CoA hydrolase precursor, medium chain-like	0.027[a]	0.021[ab]	0.013[b]	0.013[b]	0.003	0.006
fatty acyl-CoA hydrolase precursor, medium chain-like isoform 1a	0.022[a]	0.018[ab]	0.010[b]	0.010[b]	0.003	0.008
fatty acyl-CoA hydrolase precursor, medium chain-like isoform 2	0.022[a]	0.016[ab]	0.010[b]	0.010[b]	0.002	0.001
long-chain fatty acid-CoA ligase 5	0.011[a]	0.007[ab]	0.005[b]	0.004[b]	0.001	0.003
long-chain fatty acid-CoA ligase ACSBG2 isoform 1	0.011[a]	0.008[ab]	0.007[b]	0.007[b]	0.001	0.020
long-chain fatty-acid-CoA ligase ACSBG2 isoform 2	0.011[a]	0.008[ab]	0.007[b]	0.006[b]	0.001	0.012
long-chain fatty acid-CoA ligase ACSBG2 isoform 3	0.011[a]	0.008[ab]	0.007[b]	0.007[b]	0.001	0.019
long-chain fatty acid-CoA ligase ACSBG2 isoform 4	0.011[a]	0.008[ab]	0.007[b]	0.007[b]	0.001	0.019

[1] % total protein abundance = 100*(individual protein abundance/total protein abundance).

[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[a-b] Superscripts indicate significant differences among treatments at $P \leq 0.05$.

Table 6.5. β-oxidation proteins of adult female Japanese quail livers.

	% Total protein abundance[1]					
Protein	TN[2]	TNS[2]	HS[2]	HSS[2]	SE	P-value
LOW QUALITY PROTEIN: carnitine O-acetyltransferase	0.012^{ab}	0.014^{a}	0.008^{b}	0.009^{b}	0.001	0.002
carnitine O-palmitoyltransferase 1, liver isoform, isoform 1	0.006^{a}	0.005^{ab}	0.004^{ab}	0.003^{b}	0.001	0.028
carnitine O-palmitoyltransferase 1, liver isoform, isoform 2	0.006^{a}	0.005^{ab}	0.004^{ab}	0.003^{b}	0.001	0.028
mitochondrial carnitine/acylcarnitine carrier protein	0.035^{a}	0.024^{ab}	0.016^{b}	0.016^{b}	0.004	0.006
probable acyl-CoA dehydrogenase 6 isoform 1	0.005^{a}	0.003^{ab}	0.002^{b}	0.002^{b}	0.000	0.004
acyl-CoA dehydrogenase family member 11-like	0.009^{a}	0.010^{a}	0.005^{b}	0.005^{b}	0.001	0.001
short/branched chain specific acyl-CoA dehydrogenase, mitochondrial isoform 1	0.020^{a}	0.055^{b}	0.031^{ab}	0.048^{ab}	0.008	0.021
short/branched chain specific acyl-CoA dehydrogenase, mitochondrial isoform 2	0.020^{a}	0.055^{b}	0.031^{ab}	0.048^{ab}	0.008	0.022
short/branched chain specific acyl-CoA dehydrogenase, mitochondrial isoform 3	0.020^{a}	0.055^{b}	0.033^{ab}	0.048^{ab}	0.008	0.018
short/branched chain specific acyl-CoA dehydrogenase, mitochondrial isoform 4	0.025^{a}	0.068^{b}	0.039^{ab}	0.052^{ab}	0.008	0.014

[1] % total protein abundance = 100*(individual protein abundance/total protein abundance).

[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[a-b] Superscripts indicate significant differences among treatments at $P \leq 0.05$.

Table 6.6. Fatty acid synthesis proteins of adult female Japanese quail livers.

	% Total protein abundance[1]					
Protein	TN[2]	TNS[2]	HS[2]	HSS[2]	SE	P-value
LOW QUALITY PROTEIN: acetyl-CoA carboxylase 2	0.016[a]	0.012[ab]	0.015[ab]	0.010[b]	0.001	0.019
constitutive coactivator of PPAR-γ-like protein 1 isoform 1	0.008[a]	0.007[ab]	0.007[ab]	0.007[b]	0.000	0.023
constitutive coactivator of PPAR-γ-like protein 1 isoform 2	0.008[a]	0.007[ab]	0.007[ab]	0.007[b]	0.000	0.023

[1] % total protein abundance = 100*(individual protein abundance/total protein abundance).

[2] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[a-b] Superscripts indicate significant differences among treatments at $P \leq 0.05$.

Table S6.1. Down- and upregulated proteins in pair-wise comparisons.

Treatment comparison	Downregulation		Upregulation	
	Accession ID	Protein name	Accession ID	Protein name
TN versus TNS	XP_015719125.1	acyl-CoA 6-desaturase	XP_015705590.1	hemoglobin subunit rho
			XP_015727989.1	isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial isoform X2
			XP_015717621.1	cytochrome P450 4V2
			XP_015727988.2	isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial isoform X1
			XP_015722733.1	glutathione S-transferase omega-1-like
TN versus HS	XP_015707652.1	lambda-crystallin homolog	XP_015706229.1	fructose-bisphosphate aldolase C-like
	XP_015716291.1	ADP/ATP translocase 2	XP_015740114.1	mid1-interacting protein 1
	XP_032301695.1	histamine N-methyltransferase-like isoform X2		
	XP_015711230.1	alpha-tocopherol transfer protein isoform X2		
	XP_015722805.1	long-chain-fatty-acid--CoA ligase 5		
	XP_015724693.1	sterol 26-hydroxylase, mitochondrial		
	XP_015735950.1	clathrin heavy chain 1 isoform X1		
	XP_015708416.1	FAST kinase domain-containing protein 4		
	XP_015719934.1	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial		
	XP_015712582.1	adipocyte plasma membrane-associated protein [Coturnix japonica]		
	XP_015729966.1	6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 isoform X1 [Coturnix japonica]		
	XP_015729967.1	6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 isoform X2 [Coturnix japonica]		
	XP_015729968.1	6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 isoform X3 [Coturnix japonica]		
	XP_015729969.1	6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 isoform X4 [Coturnix japonica]		
	XP_032303169.1	6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 isoform X6 [Coturnix japonica]		

	XP_032303170.1	6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 isoform X5 [Coturnix japonica]		
	XP_015710223.1	thiopurine S-methyltransferase [Coturnix japonica]		
	XP_015716053.1	monocarboxylate transporter 8 [Coturnix japonica]		
	XP_015724632.1	histamine N-methyltransferase-like isoform X1 [Coturnix japonica]		
	XP_015739658.2	amine oxidase [flavin-containing] A [Coturnix japonica]		
	XP_015723210.1	UDP-glucuronosyltransferase 1-9-like isoform X15 [Coturnix japonica]		
	XP_015723200.1	UDP-glucuronosyltransferase 1-6-like isoform X3 [Coturnix japonica]		
	XP_015723205.1	UDP-glucuronosyltransferase 1-1-like isoform X8 [Coturnix japonica]		
	XP_015738957.1	protein 4.1 isoform X30 [Coturnix japonica]		
	XP_032297695.1	very long-chain acyl-CoA synthetase-like isoform X2 [Coturnix japonica]		
	XP_032301645.1	UDP-glucuronosyltransferase 1-1-like isoform X11 [Coturnix japonica]		
	XP_032301646.1	UDP-glucuronosyltransferase 1-1-like isoform X13 [Coturnix japonica]		
	XP_032304903.1	protein 4.1 isoform X15 [Coturnix japonica]		
	XP_032304905.1	protein 4.1 isoform X18 [Coturnix japonica]		
	XP_015706398.1	very long-chain acyl-CoA synthetase-like isoform X1 [Coturnix japonica]		
	XP_015706766.1	NADH dehydrogenase [ubiquinone] iron-sulfur protein 2, mitochondrial [Coturnix japonica]		
	XP_015725513.1	sorting and assembly machinery component 50 homolog [Coturnix japonica]		
	NP_572017.1	cytochrome c oxidase subunit II (mitochondrion) [Coturnix japonica]		
	XP_015706162.1	LOW QUALITY PROTEIN: 3-beta-hydroxysteroid-Delta(8),Delta(7)-isomerase [Coturnix japonica]		

	XP_015714456.1	phosphate carrier protein, mitochondrial isoform X1 [Coturnix japonica]		
	XP_015709084.1	calcium-binding mitochondrial carrier protein Aralar2 isoform X1 [Coturnix japonica]		
	XP_015709086.1	calcium-binding mitochondrial carrier protein Aralar2 isoform X4 [Coturnix japonica]		
	XP_015714463.1	phosphate carrier protein, mitochondrial isoform X2 [Coturnix japonica]		
	XP_015718985.1	eukaryotic translation initiation factor 3 subunit M [Coturnix japonica]		
	XP_015709085.1	calcium-binding mitochondrial carrier protein Aralar2 isoform X3 [Coturnix japonica]		
	XP_015738927.2	protein 4.1 isoform X2 [Coturnix japonica]		
	XP_015738932.1	protein 4.1 isoform X13 [Coturnix japonica]		
	XP_015738935.2	protein 4.1 isoform X9 [Coturnix japonica]		
	XP_015738942.2	protein 4.1 isoform X17 [Coturnix japonica]		
	XP_015738949.2	protein 4.1 isoform X1 [Coturnix japonica]		
	XP_032299036.1	calcium-binding mitochondrial carrier protein Aralar2 isoform X2 [Coturnix japonica]		
	XP_032304893.1	protein 4.1 isoform X3 [Coturnix japonica]		
	XP_032304894.1	protein 4.1 isoform X4 [Coturnix japonica]		
	XP_032304895.1	protein 4.1 isoform X5 [Coturnix japonica]		
	XP_032304896.1	protein 4.1 isoform X6 [Coturnix japonica]		
	XP_032304897.1	protein 4.1 isoform X7 [Coturnix japonica]		
	XP_032304898.1	protein 4.1 isoform X8 [Coturnix japonica]		
	XP_032304899.1	protein 4.1 isoform X10 [Coturnix japonica]		
	XP_032304900.1	protein 4.1 isoform X11 [Coturnix japonica]		
	XP_032304901.1	protein 4.1 isoform X12 [Coturnix japonica]		
	XP_032304902.1	protein 4.1 isoform X14 [Coturnix japonica]		
	XP_032304907.1	protein 4.1 isoform X20 [Coturnix japonica]		
	XP_015738946.1	protein 4.1 isoform X23 [Coturnix japonica]		

	XP_015738960.1	protein 4.1 isoform X31 [Coturnix japonica]		
	XP_032304904.1	protein 4.1 isoform X16 [Coturnix japonica]		
	XP_032304906.1	protein 4.1 isoform X19 [Coturnix japonica]		
	XP_032304908.1	protein 4.1 isoform X21 [Coturnix japonica]		
	XP_032304909.1	protein 4.1 isoform X22 [Coturnix japonica]		
	XP_032304910.1	protein 4.1 isoform X26 [Coturnix japonica]		
	XP_032304911.1	protein 4.1 isoform X27 [Coturnix japonica]		
	XP_032304912.1	protein 4.1 isoform X28 [Coturnix japonica]		
	XP_032304913.1	protein 4.1 isoform X29 [Coturnix japonica]		
	XP_032304914.1	protein 4.1 isoform X32 [Coturnix japonica]		
	XP_015722870.1	glycerol-3-phosphate acyltransferase 1, mitochondrial isoform X2 [Coturnix japonica]		
	XP_015724001.1	calcium-binding mitochondrial carrier protein Aralar1 isoform X1 [Coturnix japonica]		
	XP_015724002.1	calcium-binding mitochondrial carrier protein Aralar1 isoform X2 [Coturnix japonica]		
	XP_015724003.1	calcium-binding mitochondrial carrier protein Aralar1 isoform X4 [Coturnix japonica]		
	XP_015724873.1	dimethylaniline monooxygenase [N-oxide-forming] 5-like [Coturnix japonica]		
	XP_032301728.1	calcium-binding mitochondrial carrier protein Aralar1 isoform X3 [Coturnix japonica]		
	XP_032301219.1	glycerol-3-phosphate acyltransferase 1, mitochondrial isoform X1 [Coturnix japonica]		
TN versus HSS	XP_015716053.1	monocarboxylate transporter 8 [Coturnix japonica]	XP_015732923.1	hemoglobin subunit alpha-D [Coturnix japonica]
	XP_015724693.1	sterol 26-hydroxylase, mitochondrial [Coturnix japonica]	XP_015732935.1	hemoglobin subunit pi [Coturnix japonica]
	XP_015733210.1	sarcoplasmic/endoplasmic reticulum calcium ATPase 2 isoform X2 [Coturnix japonica]	XP_015732919.1	hemoglobin subunit alpha-A [Coturnix japonica]
	XP_015741605.1	coatomer subunit epsilon [Coturnix japonica]	XP_015718320.1	histone H5 [Coturnix japonica]
	XP_032298165.1	platelet glycoprotein 4 [Coturnix japonica]	XP_015716836.1	hematopoietic prostaglandin D synthase [Coturnix japonica]

	XP_032304348.1	3 beta-hydroxysteroid dehydrogenase type 7-like isoform X2 [Coturnix japonica]	XP_032297790.1	nicotinate-nucleotide pyrophosphorylase [carboxylating] isoform X2 [Coturnix japonica]
	XP_015706395.2	LOW QUALITY PROTEIN: potassium-transporting ATPase alpha chain 1-like [Coturnix japonica]	XP_032297789.1	nicotinate-nucleotide pyrophosphorylase [carboxylating] isoform X1 [Coturnix japonica]
	XP_015719977.1	LOW QUALITY PROTEIN: very-long-chain 3-oxoacyl-CoA reductase [Coturnix japonica]	XP_015706640.1	nicotinate-nucleotide pyrophosphorylase [carboxylating] isoform X3 [Coturnix japonica]
	XP_015725513.1	sorting and assembly machinery component 50 homolog [Coturnix japonica]	XP_015726098.1	CXXC motif containing zinc binding protein isoform X2 [Coturnix japonica]
	XP_015728942.1	aminopeptidase N [Coturnix japonica]	XP_032301510.1	short/branched chain specific acyl-CoA dehydrogenase, mitochondrial isoform X4 [Coturnix japonica]
	XP_015729475.1	fatty acyl-CoA hydrolase precursor, medium chain-like isoform X1 [Coturnix japonica]	XP_015722733.1	glutathione S-transferase omega-1-like [Coturnix japonica]
	XP_015729476.1	fatty acyl-CoA hydrolase precursor, medium chain-like isoform X2 [Coturnix japonica]	XP_015721861.1	prosaposin isoform X1 [Coturnix japonica]
	XP_015730868.1	dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 [Coturnix japonica]	XP_015726097.1	CXXC motif containing zinc binding protein isoform X1 [Coturnix japonica]
	XP_015733209.1	sarcoplasmic/endoplasmic reticulum calcium ATPase 2 isoform X1 [Coturnix japonica]	XP_015707211.1	protein SGT1 homolog [Coturnix japonica]
	XP_015739658.2	amine oxidase [flavin-containing] A [Coturnix japonica]	XP_015723020.1	short/branched chain specific acyl-CoA dehydrogenase, mitochondrial isoform X3 [Coturnix japonica]
	XP_015716177.1	adenylosuccinate lyase [Coturnix japonica]	XP_015721862.1	prosaposin isoform X2 [Coturnix japonica]
	XP_015719125.1	acyl-CoA 6-desaturase [Coturnix japonica]	XP_015723017.1	short/branched chain specific acyl-CoA dehydrogenase, mitochondrial isoform X1 [Coturnix japonica]
	XP_015721433.1	fermitin family homolog 2 isoform X1 [Coturnix japonica]	XP_015708052.1	hemoglobin subunit beta [Coturnix japonica]
	XP_015721435.2	fermitin family homolog 2 isoform X3 [Coturnix japonica]	XP_015708054.1	hemoglobin subunit beta-like [Coturnix japonica]
	XP_015721436.2	fermitin family homolog 2 isoform X4 [Coturnix japonica]	XP_015711309.1	carbonic anhydrase 2 isoform X1 [Coturnix japonica]
	XP_032297493.1	protochlorophyllide reductase, chloroplastic isoform X2 [Coturnix japonica]	XP_015711310.1	carbonic anhydrase 2 isoform X2 [Coturnix japonica]
	XP_032297494.1	retinol dehydrogenase 12 isoform X3 [Coturnix japonica]	XP_015740140.1	prefoldin subunit 2 [Coturnix japonica]
	XP_015721239.1	protein phosphatase 1A isoform X3 [Coturnix japonica]	XP_015705599.1	hemoglobin subunit epsilon [Coturnix japonica]
	XP_015730976.1	glucosamine-6-phosphate isomerase 1 [Coturnix japonica]	XP_015733530.1	coiled-coil-helix-coiled-coil-helix domain-containing protein 10, mitochondrial [Coturnix japonica]

	XP_015735253.1	myosin-10 isoform X3 [Coturnix japonica]	XP_015735325.1	39S ribosomal protein L12, mitochondrial [Coturnix japonica]
	XP_015735254.1	myosin-10 isoform X4 [Coturnix japonica]	XP_015705965.1	histone H2A, sperm-like [Coturnix japonica]
	XP_032300514.1	protein phosphatase 1A isoform X2 [Coturnix japonica]	XP_015717315.1	vitamin D-binding protein [Coturnix japonica]
	XP_032301695.1	histamine N-methyltransferase-like isoform X2 [Coturnix japonica]	XP_015705590.1	hemoglobin subunit rho [Coturnix japonica]
	XP_015706707.1	hepatic lectin [Coturnix japonica]	XP_015729232.1	glutathione S-transferase kappa 1 isoform X2 [Coturnix japonica]
	XP_015712582.1	adipocyte plasma membrane-associated protein [Coturnix japonica]	XP_015717009.1	serum albumin [Coturnix japonica]
	XP_015712900.1	ribokinase [Coturnix japonica]	XP_015733582.1	D-dopachrome decarboxylase [Coturnix japonica]
	XP_015716917.1	cytochrome P450 2D17 [Coturnix japonica]	XP_015705673.1	isocitrate dehydrogenase [NAD] subunit beta, mitochondrial isoform X1 [Coturnix japonica]
	XP_015721434.2	fermitin family homolog 2 isoform X2 [Coturnix japonica]	XP_032297003.1	soluble scavenger receptor cysteine-rich domain-containing protein SSC5D-like [Coturnix japonica]
	XP_015723197.1	UDP-glucuronosyltransferase 1-9-like isoform X1 [Coturnix japonica]	XP_015739298.1	histone H2A [Coturnix japonica]
	XP_015724322.1	metalloreductase STEAP3 isoform X2 [Coturnix japonica]	XP_015729223.1	glutathione S-transferase kappa 1 isoform X1 [Coturnix japonica]
	XP_015727435.1	neutral cholesterol ester hydrolase 1 [Coturnix japonica]	XP_015733883.1	immunoglobulin lambda-1 light chain isoform X1 [Coturnix japonica]
	XP_015735251.1	myosin-10 isoform X1 [Coturnix japonica]	XP_015733884.1	immunoglobulin lambda-1 light chain isoform X2 [Coturnix japonica]
	XP_015735252.1	myosin-10 isoform X2 [Coturnix japonica]	XP_015733885.1	immunoglobulin lambda-1 light chain isoform X4 [Coturnix japonica]
	XP_015735255.1	myosin-10 isoform X5 [Coturnix japonica]	XP_032303858.1	immunoglobulin lambda-1 light chain isoform X3 [Coturnix japonica]
	XP_032297492.1	retinol dehydrogenase 12 isoform X1 [Coturnix japonica]	XP_032303859.1	immunoglobulin lambda-1 light chain isoform X5 [Coturnix japonica]
	XP_032297495.1	retinol dehydrogenase 13 isoform X4 [Coturnix japonica]	XP_032303860.1	immunoglobulin lambda-1 light chain isoform X6 [Coturnix japonica]
	XP_015705475.1	transmembrane emp24 domain-containing protein 7 [Coturnix japonica]	XP_015718294.1	grpE protein homolog 1, mitochondrial [Coturnix japonica]
	XP_015709095.1	serum paraoxonase/arylesterase 2 isoform X1 [Coturnix japonica]	XP_015715221.1	histone H2B 1/2/3/4/6-like [Coturnix japonica]
	XP_015709096.1	serum paraoxonase/arylesterase 2 isoform X2 [Coturnix japonica]	XP_015730523.1	inosine-5'-monophosphate dehydrogenase 2 isoform X2 [Coturnix japonica]

	XP_015711782.1	ras-related protein Rap-1b [Coturnix japonica]	XP_015730524.2	inosine-5'-monophosphate dehydrogenase 2 isoform X3 [Coturnix japonica]
	XP_015732149.1	nodal modulator 3 [Coturnix japonica]	XP_015719573.1	proteasome subunit alpha type-1 [Coturnix japonica]
	XP_032302044.1	putative dimethylaniline monooxygenase [N-oxide-forming] 6 isoform X2 [Coturnix japonica]	XP_015731156.1	sequestosome-1 isoform X1 [Coturnix japonica]
	XP_015707652.1	lambda-crystallin homolog [Coturnix japonica]	XP_015731157.1	sequestosome-1 isoform X2 [Coturnix japonica]
	XP_015718985.1	eukaryotic translation initiation factor 3 subunit M [Coturnix japonica]	XP_015734940.1	actin-related protein 2/3 complex subunit 5-like protein [Coturnix japonica]
	XP_015724323.1	metalloreductase STEAP3 isoform X3 [Coturnix japonica]	XP_015735089.1	tripartite motif-containing protein 65 isoform X1 [Coturnix japonica]
	XP_015724632.1	histamine N-methyltransferase-like isoform X1 [Coturnix japonica]	XP_015735090.1	tripartite motif-containing protein 65 isoform X2 [Coturnix japonica]
	XP_015735313.1	kynurenine formamidase [Coturnix japonica]	XP_015708284.1	late histone H2B.L4 [Coturnix japonica]
	XP_015738946.1	protein 4.1 isoform X23 [Coturnix japonica]	XP_015719004.1	reticulocalbin-1 [Coturnix japonica]
	XP_015738960.1	protein 4.1 isoform X31 [Coturnix japonica]	XP_015730074.1	glutathione peroxidase 1 [Coturnix japonica]
	XP_015740850.1	ras-related protein Rap-1A [Coturnix japonica]	XP_015719168.1	ferritin heavy chain [Coturnix japonica]
	XP_032304904.1	protein 4.1 isoform X16 [Coturnix japonica]	XP_015730522.1	inosine-5'-monophosphate dehydrogenase 2 isoform X1 [Coturnix japonica]
	XP_032304906.1	protein 4.1 isoform X19 [Coturnix japonica]		
	XP_032304908.1	protein 4.1 isoform X21 [Coturnix japonica]		
	XP_032304909.1	protein 4.1 isoform X22 [Coturnix japonica]		
	XP_032304910.1	protein 4.1 isoform X26 [Coturnix japonica]		
	XP_032304911.1	protein 4.1 isoform X27 [Coturnix japonica]		
	XP_032304912.1	protein 4.1 isoform X28 [Coturnix japonica]		
	XP_032304913.1	protein 4.1 isoform X29 [Coturnix japonica]		
	XP_032304914.1	protein 4.1 isoform X32 [Coturnix japonica]		
	XP_015706162.1	LOW QUALITY PROTEIN: 3-beta-hydroxysteroid-Delta(8),Delta(7)-isomerase [Coturnix japonica]		
	XP_015711639.1	carboxypeptidase Q [Coturnix japonica]		
	XP_015723212.1	UDP-glucuronosyltransferase 1-1-like isoform X18 [Coturnix japonica]		

	XP_015739441.1	LOW QUALITY PROTEIN: potassium-transporting ATPase alpha chain 2 [Coturnix japonica]		
	XP_015706543.1	eukaryotic translation initiation factor 3 subunit K [Coturnix japonica]		
	XP_015723199.1	UDP-glucuronosyltransferase 1-1-like isoform X2 [Coturnix japonica]		
	XP_015723206.1	UDP-glucuronosyltransferase 1-9-like isoform X14 [Coturnix japonica]		
	XP_015723211.1	UDP-glucuronosyltransferase 1-1-like isoform X17 [Coturnix japonica]		
	XP_015724873.1	dimethylaniline monooxygenase [N-oxide-forming] 5-like [Coturnix japonica]		
	XP_015736385.1	sarcoplasmic/endoplasmic reticulum calcium ATPase 3 isoform X1 [Coturnix japonica]		
	XP_015723208.1	UDP-glucuronosyltransferase 1-1-like isoform X16 [Coturnix japonica]		
	XP_015736386.1	sarcoplasmic/endoplasmic reticulum calcium ATPase 3 isoform X2 [Coturnix japonica]		
	XP_015742571.1	retinol dehydrogenase 7-like [Coturnix japonica]		
	XP_015722805.1	long-chain-fatty-acid--CoA ligase 5 [Coturnix japonica]		
	XP_015724001.1	calcium-binding mitochondrial carrier protein Aralar1 isoform X1 [Coturnix japonica]		
	XP_015724002.1	calcium-binding mitochondrial carrier protein Aralar1 isoform X2 [Coturnix japonica]		
	XP_015724003.1	calcium-binding mitochondrial carrier protein Aralar1 isoform X4 [Coturnix japonica]		
	XP_015738251.1	dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit [Coturnix japonica]		
	XP_032301728.1	calcium-binding mitochondrial carrier protein Aralar1 isoform X3 [Coturnix japonica]		
	XP_015706398.1	very long-chain acyl-CoA synthetase-like isoform X1 [Coturnix japonica]		
	XP_015722870.1	glycerol-3-phosphate acyltransferase 1, mitochondrial isoform X2 [Coturnix japonica]		

	XP_015723200.1	UDP-glucuronosyltransferase 1-6-like isoform X3 [Coturnix japonica]		
	XP_015723205.1	UDP-glucuronosyltransferase 1-1-like isoform X8 [Coturnix japonica]		
	XP_015723207.1	UDP-glucuronosyltransferase 1-1-like isoform X10 [Coturnix japonica]		
	XP_015723210.1	UDP-glucuronosyltransferase 1-9-like isoform X15 [Coturnix japonica]		
	XP_015730883.1	protein transport protein Sec61 subunit alpha [Coturnix japonica]		
	XP_032297695.1	very long-chain acyl-CoA synthetase-like isoform X2 [Coturnix japonica]		
	XP_032301643.1	UDP-glucuronosyltransferase 1-1-like isoform X7 [Coturnix japonica]		
	XP_032301219.1	glycerol-3-phosphate acyltransferase 1, mitochondrial isoform X1 [Coturnix japonica]		
	XP_032301644.1	UDP-glucuronosyltransferase 1-2-like isoform X9 [Coturnix japonica]		
	XP_032301645.1	UDP-glucuronosyltransferase 1-1-like isoform X11 [Coturnix japonica]		
	XP_032301646.1	UDP-glucuronosyltransferase 1-1-like isoform X13 [Coturnix japonica]		
	XP_015704933.1	alpha-mannosidase 2 [Coturnix japonica]		
	XP_015709084.1	calcium-binding mitochondrial carrier protein Aralar2 isoform X1 [Coturnix japonica]		
	XP_015709085.1	calcium-binding mitochondrial carrier protein Aralar2 isoform X3 [Coturnix japonica]		
	XP_015709086.1	calcium-binding mitochondrial carrier protein Aralar2 isoform X4 [Coturnix japonica]		
	XP_015724718.1	GTPase KRas isoform X2 [Coturnix japonica]		
	XP_015733755.1	glutathione hydrolase 5 proenzyme [Coturnix japonica]		
	XP_032299036.1	calcium-binding mitochondrial carrier protein Aralar2 isoform X2 [Coturnix japonica]		
	XP_015709386.1	dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit STT3B [Coturnix japonica]		

	XP_015714456.1	phosphate carrier protein, mitochondrial isoform X1 [Coturnix japonica]		
	XP_015714463.1	phosphate carrier protein, mitochondrial isoform X2 [Coturnix japonica]		
	XP_015736929.1	dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2 isoform X4 [Coturnix japonica]		
	NP_572017.1	cytochrome c oxidase subunit II (mitochondrion) [Coturnix japonica]		
	XP_015725475.1	protein transport protein Sec61 subunit alpha isoform X2 [Coturnix japonica]		
	XP_015736927.1	dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2 isoform X3 [Coturnix japonica]		
	XP_015724708.1	GTPase KRas isoform X1 [Coturnix japonica]		
	XP_015724726.1	GTPase KRas isoform X3 [Coturnix japonica]		
	XP_015736924.1	dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2 isoform X1 [Coturnix japonica]		
	XP_015736925.1	dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2 isoform X2 [Coturnix japonica]		
	XP_015740675.1	GTPase NRas [Coturnix japonica]		
	XP_032300922.1	GTPase HRas isoform X2 [Coturnix japonica]		
	XP_015725551.1	protein transport protein Sec61 subunit alpha isoform X1 [Coturnix japonica]		
	XP_015739536.1	dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit STT3A [Coturnix japonica]		
HS versus HSS	XP_032302044.1	putative dimethylaniline monooxygenase [N-oxide-forming] 6 isoform X2		
HS versus TNS	XP_015708008.1	thyroid hormone-inducible hepatic protein		
	XP_015740114.1	mid1-interacting protein 1		

CHAPTER 7
Steroid hormones of multigenerational heat stressed Japanese quail (*Coturnix coturnix japonica*)

Abstract: Steroid hormones play a seminal role in the animal's response to heat stress and can be used to determine the extent of stress an animal is experiencing. Glucocorticoids are a class of steroid hormones responsible for the stress response such as increased vasodilation, panting, decreased reproduction, and mobilization of energy. By investigating the transition from a thermoneutral zone to heat stress, procedures can be implemented to mitigate heat stress at an earlier stage. Poultry chosen for high performance, such as low feed conversion ratio (FCR), can have negative metabolic consequences when first encountering heat stress. Possibly, by choosing for high performance in heat stress, animals in future generations may have a decreased heat stress response. The goal of this study was to determine if selection for low FCR in heat stress (31.1˚C) decreased glucocorticoid levels and influenced hormones involved in reproduction in Japanese quail sera. Thus, 4 treatment groups were formed and repeated for 10 generations: (1) thermoneutral (TN, 22.2˚C), (2) thermoneutral siblings (TNS, 22.2˚C), (3) heat stress (HS, 31.1˚C), and (4) heat stress siblings (HSS, 31.1˚C). TN and HS were random bred in their respective temperatures. TNS and HSS were siblings reared at the two temperatures. HSS quail were used to determine low FCR at 31.1˚C and their corresponding TNS were mated to create the next generation's TNS and HSS. Sera were collected across treatments at 4 hours (acute) and 3 weeks (chronic) post-heat stress and analyzed for steroid hormones using a LC-MS. Significance was determined at $P \leq 0.05$. The most notable findings from this study were: (1) Chronic males, particularly from TN, had significantly more glucocorticoids, progestogens, androgens than other treatments and (2) of the 29 steroid hormones analyzed, 13 were significantly higher in chronic than acute. Findings could indicate that selective breeding for higher performance at 31.1˚C does not change the quail's susceptibility to stress and that hormones shift in concentration when quails

undergo sexual maturity. Overall, heat stress at 31.1˚C did not illicit a significant hormonal stress response in Japanese quail after 10 generations of selective breeding for low FCR.

1. Introduction

Hormones associated with stress play an important role in lipid metabolism. Some important hormones are corticosterone and estradiol. Birds decrease feed intake during heat stress, which results in decreased metabolic rate and, subsequently, metabolic heat production (Renaudeau, et al., 2012). Corticosterone levels in plasma increase in response to stress and decrease secretion of follicle stimulating hormone, luteinizing hormone, and subsequently, estradiol. Estradiol has a role in calcium homeostasis, oocyte formation, and lipoprotein production (Palmisano, et al., 2017). Estrogen can regulate feed intake and impact phospholipid transfer in the chicken liver (Taouis, et al., 2001). Also, estrogen can regulate liver metabolism and serum lipoprotein levels (Palmisano, et al., 2017).

Changes in estrogen, androgens, and progesterone trigger the change in concentration and metabolism of lipids in the liver (Noble and Cocchi, 1990). While estrogen is responsible for the enhancement of hepatic lipogenesis for yolk deposition, other hormones influence avian responses to stress (Cherian, 2015). For instance, in response to high levels of nest predation, females will increase androgens in the embryos to increase growth rates and thereby, elevate the concentration of reactive oxygen species and oxidative stress (Tsunekage, 2015).

Heat stress in laying females may influence circulating hormones which can be deposited into eggs, thus preparing the next generation for environmental change (Laviola and Macrì, 2013; Tsunekage, 2015). For instance, high corticosterone injected into fertilized Japanese quail eggs shortened telomeres, increased acute stress response, and decreased total antioxidant capacity compared to those not injected with corticosterone for which the range of corticosterone/g in yolk

is 0.8 to 27.3 ng (Haussmann, et al., 2012). There was also a decrease in HPA responsiveness and higher reactive oxygen molecules in plasma of offspring from high corticosterone injected eggs (Haussmann, et al., 2012). This finding demonstrated that if female stress hormones can enter the egg at high enough amounts, there may be detrimental effects related to oxidation.

The inoculation hypothesis states that the extent of stressful exposure in utero can affect the offspring into adulthood. Responses vary depending on the severity of the exposure: (1) Severe or intense early stress can cause serious maladaptation, (2) Moderate stress exposure may be ideal to illicit adaptive physiological and behavioral mechanisms to cope with future stress, and (3) Too little exposure to stress in early life may cause the individual to be unprepared for future stress (Laviola and Macrì, 2013). Thus, deposition of stress hormones into eggs could incur beneficial survival mechanisms in offspring.

Evidence from avian and human species suggests that stress can alter development of the hypothalamus-pituitary-gonad (HPG) axis and suppress ovarian function in mature females (Rozenboim, et al., 2007; Laviola and Macrì, 2013). Stress many also modulate risky behavior in chicken through the interaction between the stress response system (SRS), sex hormones (testosterone, dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulfate (DHEA-S), serotonin, and dopamine. The level of stress and species will dictate the response of the HPG axis, hypothalamus-pituitary-adrenal (HPA) axis, and the SRS (Fallahsharoudi, et al., 2015).

In low stress environments, there is little activation of the HPA axis and SRS with little disruption in immunity, reproduction, energy, and fitness. In moderate stress environments, there is increased activation of the HPA axis and SRS with trade-offs occurring. Immediate benefits take priority over long-term goals (Laviola and Macrì, 2013).

In unpredictable stress and dangerous environments, animals can become either agonistic or withdrawn, both experiencing high HPA and sympathetic nervous system responsiveness (Laviola and Macrì, 2013). In severe or traumatic stress environment, the animal may become unresponsive to threats, danger, social feedback, or social context.

There are also sex differences to stress response. Males in short-lived species will invest more in mating and not rearing offspring, whereas long-lived species will have higher parental investment (Laviola and Macrì, 2013). In both cases, females have more fixed costs because they are typically responsible for bearing the offspring (Laviola and Macrì, 2013). Studies showed that testosterone induces oxidative stress while antioxidants mitigate it; thus, creating a negative correlation between testosterone and antioxidants. Estrogen induces increment in body fat and there is typically higher fat content in females (Leclercq, 1984). Estrogen receptor-α transcriptionally regulates 43 lipid genes, leading to genetic sexual dimorphisms of triacylglyceride metabolism and fatty acid oxidation (Palmisano, et al., 2017). Deficiencies of estrogen also lead to liver fat accumulation (Palmisano, et al., 2017).

According to Renaudeau, et al (2012), chicken cannot survive when body temperatures are above 4˚C beyond their thermoneutral zone in acute heat stress, but they can acclimate to reach a new steady state if the temperature change is gradual. Acute, chronic, and sex differences in thermotolerance may be reflected in steroid hormone differences between the first exposure and 4 weeks after exposure to high ambient temperature (Renaudeau, et al., 2012).

The objectives of this study were to determine if there was acclimation after 3 weeks or adaptation after 10 generations to heat stress as indicated by steroid hormone regulation. Additionally, this study aimed to determine if selection for high performance in a heat stress environment could lower stress hormones; thus, allowing for unaltered lipid metabolism as

compared to quails that were not selected for high performance in heat stress. Corticosterone was expected to be higher in treatments in heat stress and estradiol and testosterone were expected to be negatively correlated with corticosterone levels. Although heat stress was expected to increase for quail in heat stress, it was expected to be lower for birds selected for high performance in heat stress.

2. Materials and Methods

2.1 Experimental design (Chapter 2, Figure 1)

The detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. The four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

2.2 Sera collection

Animal care and use was approved by the Institutional Animal Care and Use Committee at the University of California Davis (Protocols #19473 and 21370; Davis, CA). After an initial 4 hours of heat exposure and fasting for 1 hour, 1 mL of whole blood was collected from the right or left jugular vein from each of n=15 male/female pairs in each treatment. To test for chronic effects, after an additional 3 weeks of heat exposure, the same 15 pairs in each treatment were fasted for 1 hour before 1 mL of whole blood was collected from the right or left jugular vein.

Birds were handled no more than 2 times to reduce stress. Whole blood (0.9 mL) was centrifuged to separate serum and stored at -80˚C until analysis.

2.3 Steroid hormone analysis

Steroid hormones were analyzed by the Metabolomics Laboratory at the University of CA UC Davis (Davis, CA) using high performance liquid chromatography and tandem mass spectroscopy. Briefly, samples were thawed on ice and 50 µl were pipetted in polypropylene Eppendorf tube/plate. Surrogate standards, CUDA + PHAU, antioxidants, and ACN/MeOH were added. The mixture was vortexed and centrifuged at 6˚C for 5 min at 15,000 x g. The supernatant was transferred to another Eppendorf tube, spin filtered (0.1 µm) for 5 min at 12,000 x g, aliquoted, and stored in -20˚C until analysis.

2.4 Statistical analysis

Analyses of data were performed in R 4.0.0 (R Core Team, 2020; RStudio Team, 2022) to test significance ($P \leq 0.05$). The Shapiro-Wilk test was used to determine normality of residuals of models and data were considered normal at $W \geq 0.95$ or $P \geq 0.05$. Levene's test and Q-Q plots were used to determine homogeneity of variances and variances were considered equal at $P \geq 0.05$. The differences among treatments were determined using a one-way repeated measures ANOVA for the main effects and their interactions. The main effects were treatment, sex, length of exposure, and their interactions. Tukey method for comparison was used for analysis of significant pairwise differences of means. All data were reported as means ± SE where appropriate.

3. Results

3.1 Glucocorticoids

ANOVA results for treatment × sex × exposure effects were for corticosterone, cortisol, or cortexolone ($P > 0.05$; Table 1). There were no other significant effects for cortisol and

cortexolone. However, when post-hoc analysis was performed for corticosterone, TN acute females had significantly more corticosterone than TNS acute males ($p=0.0086$). There were also significant treatment × sex, sex × exposure ($p=0.040$), and treatment ($p=0.0050$) effects on corticosterone. TN females and chronic males had significantly more corticosterone than all other treatments (Table 2). TN had significantly more corticosterone than TNS and HS ($p=0.01$).

ANOVA results showed that exposure ($p=0.045$), sex × exposure ($p=0.049$), and treatment × sex × exposure ($p=0.021$) effects were significant for cortisone. TN chronic males had significantly more cortisone than TN acute males, TNS acute males, TNS chronic females, HS across both sexes and exposures, and HSS acute males and females. When exposure and sex × exposure were compared in separate models, there were no significant differences. However, when pairwise comparisons were made for treatment × exposure, TN chronic had significantly more cortisone than HS acute (Table 2).

3.2 Progestogens

There were no significant ANOVA results for progesterone. However, treatment × exposure, sex × exposure, and exposure had significant effects for dihydroprogesterone (DHP). HS chronic and HSS acute and chronic had significantly more DHP than TN acute. Chronic males and females had significantly more DHP than acute males and females. When grouped over sex and treatment, acute had less DHP than chronic ($P<0.0001$; Table 4).

ANOVA results showed that treatment × sex × exposure did not significantly affect 17α-hydroxyprogesterone (17α-OHP); however, there were significant pairwise comparisons when post-hoc analysis was performed. TN chronic males had significantly higher 17α-OHP than TN acute females ($p=0.045$), TNS acute males ($p=0.027$), HS acute females ($p=0.019$), and HSS acute females ($p=0.023$; Table 3). There were also significant treatment × sex ($p=0.0046$), sex

(p=0.0042), and exposure (p=0.0039) effects on 17α-OHP concentration in the sera. When grouped over exposure, TN males had significantly more 17α-OHP than females from TN, HS, and HSS and TNS males (p=0.0057). When grouped over treatment and exposure, males had significantly more 17α-OHP than females (p=0.012) and when grouped over treatment and sex, chronic had significantly more 17α-OHP than acute (p=0.0015; Table 4).

Although the ANOVA did not determine that there was a significant treatment × sex × exposure effect on 20α-hydroxyprogesterone (20α-OHP), there were significant pairwise comparisons in the post hoc analysis. TN chronic males had significantly more 20α-OHP than all other treatments except TN acute females, TNS acute females, HS chronic males, HSS chronic males, and HSS chronic females (Table 3). Sex (p=0.038), exposure (p=0.0031), treatment × exposure (p=0.050), and sex × exposure (p=0.0031) significantly affected 20α-OHP concentrations; however, when post-hoc analysis was performed on sex alone, there were no significant pairwise comparisons. For treatment × exposure, TN chronic had significantly more 20α-OHP than HS acute. For sex × exposure, chronic males had significantly more 20α-OHP than all other treatments (p=0.0064). For exposure, chronic had significantly more 20α-OHP than acute (p=0.0034; Table 4).

3.3 Neurosteroids

There were no significant effects on 17-OH pregnenolone, but there was a significant exposure effect on pregnenolone, β-pregnanolone, and etiocholanolone with chronic having significantly higher concentration of those three hormones than acute (P<0.03; Table 6). When compared over sex and exposure, β-pregnanolone concentration was significantly lower in TNS than HS and HSS (p=0.020; Table 6). β-pregnanolone also had significant pairwise comparisons for treatment × sex, treatment × exposure, and sex × exposure. TNS females had significantly

less β-pregnanolone than HS males. TNS acute had significantly less β-pregnanolone than HS and HSS chronic. Acute females had significantly less β-pregnanolone than chronic males (Table 6).

The ANOVA with treatment × sex × exposure in the model showed that allo-pregnanolone (Allo) concentration was only significantly affected by sex. However, in the pairwise comparisons, TN chronic males had significantly higher concentrations of Allo than HSS acute females, TN acute females, and TN chronic females ($P<0.05$; Table 5). When sex was the only main effect in the model, males had a significantly higher concentration of Allo than females ($p=0.018$). There were also significant pairwise comparisons of Allo for treatment × sex and sex × exposure with HSS females and TN females having significantly less than TN males and chronic females having significantly less than chronic males (Table 6).

Etiocholanolone also had significant pairwise comparisons for treatment × exposure and sex × exposure (Table 6). Acute TNS had significantly less etiocholanolone than HS chronic. Acute males had significantly less etiocholanolone than chronic males.

3.4 Mineralocorticoids

There were no significant differences for aldosterone (Table 7 and 8). ANOVA results with treatment × sex × exposure in the model showed that there was a significant exposure effect on cortexone. When exposure was the only main effect in the model, chronic had significantly more cortexone than acute ($p=0.0061$). When pairwise comparisons were made for cortexone, sex × exposure had a significant effect with acute females having significantly less cortexone than chronic males (Table 8).

3.5 Estrogens

There were no significant differences for estradiol and 2-methoxyestradiol (Table 9). ANOVA results showed that treatment × sex and treatment × sex × exposure had significant

effects on estrone concentrations; however, there were no significant differences in the post-hoc analysis.

Exposure and treatment × sex × exposure had significant effects on estriol concentration. Pairwise comparisons showed that TN chronic males had significantly higher concentrations of estriol than all other treatments except HS and HSS chronic males and HSS chronic females ($P<0.05$). When pairwise comparisons were made, sex × exposure had a significant effect on estriol with chronic males having significantly more estriol than acute males (Table 10). When grouped over treatment and sex, chronic has significantly more estriol than acute ($p=0.0025$).

3.6 Androgens

There were no significant effects on testosterone glucuronide, dihydrotestosterone, and androstenedione. Trans-androsterone had significant treatment × sex × exposure results in the ANOVA; however, there were no significant results in the post hoc analysis (Table 11). Grouped over treatment and sex, chronic had significantly more testosterone ($p=0.018$), dihydrotestosterone (DHT), cis-androsterone ($p=0.0015$), androstenediol ($p=0.035$), and dehydroepiandrosterone sulfate (DHEA-S; $p=0.026$) than acute (Table 15). When grouped over treatment, acute males had significantly less cis-androsterone and androstanediol than chronic males ($p=0.011$; Table 14). When androstenediol was compared by treatment × sex × exposure, TN chronic males had significantly more androstenediol than all other treatments except TN chronic females, TNS chronic males, HS chronic males, HS acute and chronic females, and HSS chronic males ($P<0.04$; Table 11).

When DHEA was compared by treatment × sex × exposure, HSS acute males had significantly more DHEA than TN chronic males, TNS acute females, HS acute females, and HS acute males ($P<0.04$). HSS chronic females had significantly more DHEA than TN chronic males

(p=0.034; Table 11). When DHEA was only compared by treatment, TN had significantly less than HSS (p=0.014; Table 15).

DHEA-S was also significantly affected by treatment × sex × exposure (p=0.024), treatment × exposure (p=0.0059), sex × exposure, and treatment (p=0.018). For the interaction of treatment × sex × exposure, HS acute females had significantly less DHEA-S than HS chronic females, HSS acute males, and HSS chronic females. TN chronic females had significantly less DHEA-S than HS chronic females. HS acute males had significantly less DHEA-S than HSS acute males and HSS chronic females (Table 11). When grouped over sex, TN chronic and HS acute had significantly less DHEA-S than HS and HSS chronic (Table 13). When grouped over treatment, acute females had significantly less DHEA-S than chronic females (Table 14). When compared by treatment only, TN had significantly less DHEA-S than HSS (Table 15).

4. Discussion

4.1 Glucocorticoids

In the current study TN had significantly more corticosterone than all other treatments which is opposite findings of others (Imik, et al., 2009; Pu, et al., 2020). In the study by Imik, et al., (2009), heat stressed Japanese quail (34˚C for 9 hours a day) had higher levels of corticosterone and catecholamines, which increased lipid oxidation, free radicals in cells, and cholesterol, but decreased triacylglycerides. Alternatively, others reported that corticosterone and cortisol increased plasma and liver triacylglycerides in broilers and quails (Sahin, et al., 2004; Pu, et al., 2020). Regardless, corticosterone changes lipid metabolism through the inhibition of insulin release; thereby, increasing blood glucose and triacylglycerides (Imik, et al., 2009).

Corticosterone was also significantly higher in TN acute females than TNS acute males; however, overall, chronic males had significantly more corticosterone than all other treatments.

Cortisone was significantly higher in TN chronic males than quails across all treatments, sexes, and lengths of exposure. When considering these findings collectively, it appeared that TN was experiencing higher stress than those that were in the heat stress environment. This finding could indicate that selective breeding for higher performance at 31.1˚C does not change the quail's susceptibility to stress and is in opposition to findings of others indicating that commercial broilers had higher susceptibility to stress when compared to their ancestral, non-selected counterparts (Soleimani, et al., 2011). Importantly, results reported herein indicated that repeated selection at 31.1˚C does not increase stress hormones when compared to a thermoneutral temperature.

Corticosterone is of particular interest because of its ability to influence offspring success. Others have found that elevated plasma glucocorticoids could be deposited into eggs during lay and increase survival behaviors such as aggressive begging for food (Hayward and Wingfield, 2004). Corticosterone implanted into actively laying female Japanese quail also resulted in offspring with less mass and slower growth (Hayward and Wingfield, 2004). In relation to the current study, it appears that differences observed among treatments may not have been due to differences in stress response as measured by corticosterone.

4.2 Progestogens

Progestogens such as DHP, 17α-OHP, and 20α-OHP were higher in chronic than acute. A particular trend in the current study was that TN chronic males had more 17α-OHP and 20α-OHP than many of the other treatment groups. There have been several studies on females and progestogens, especially progesterone; however, there have not been many studies on males and progestogens (Oettel and Mukhopadhyay, 2004; Rozenboim, et al., 2007; Xiong, et al., 2021). The numerous studies on progesterone and female poultry are due to its importance as a regulator of

lay; however, studies show that it also has an important role as a precursor to neurosteroids (Oettel and Mukhopadhyay, 2004).

Many studies relate progesterone to females, but metabolites of progesterone such as DHP, 17α-OHP, and 20α-OHP are important to the conversion of other hormones such as testosterone (Oettel and Mukhopadhyay, 2004). Although not significantly different, findings in the present study indicated that chronic males and females had higher levels of testosterone. Additionally, other studies showed that heat stress at high temperatures (42˚C for 12 hours) decreased progesterone and testosterone in laying hens (Rozenboim, et al., 2007). This association between stress and decreased progesterone and testosterone supports the findings that TN males had higher levels of progesterone metabolites than other treatments.

4.3 Neurosteroids

Pregnenolone is the precursor of progesterone, which in turn metabolizes into 17α-OHP, deoxycorticosterone, and pregnanediol (Oettel and Mukhopadhyay, 2004). Pregnenolone, β-pregnanolone, and etiocholanolone were significantly higher in chronic than acute, which was expected due to the high concentration of 17α-OHP.

Additionally, allo-pregnanolone (Allo) was significantly higher in males than females, particularly in TN. Allo is a neuroactive metabolite of progesterone and has been studied for its effect in modulating stress (Locci and Pinna, 2017; Locci and Pinna, 2019). This hormone is biosynthesized in the brain and its deficiency in the brain modulates the action of γ-amino butyric acid (GABA) at the $GABA_A$ receptor. This modulation was determined to be the cause of increased aggressive behavior, and increased levels of anxiety in stressed mice (Pinna, et al., 2003). In rodents, stress can be a cause for Allo deficiency (Pinna, et al. 2003; Locci and Pinna, 2019). This

strong association between Allo and modulation of stress indicates that, in the present study, TN males were significantly less stressed than all other treatments.

4.4 Mineralocorticoids

17α-OHP is converted to cortexone, also known as 11-deoxycorticosterone, by the enzyme CYP21A2 (Loke, et al., 2021). Current study findings indicated that chronic quail had a significantly higher concentration of 11-deoxycorticosterone than acute, which would confirm the presence of high levels of 17α-OHP. Additionally, 11-deoxycorticosterone serves as a precursor for corticosterone and subsequently aldosterone.

Aldosterone is an essential mineralocorticoid involved in sodium and water homeostasis in the body (Freitas, et al., 2022). Aldosterone was not significantly different among treatments in the current study, indicating that there was no significant difference in renal sodium reabsorption and potassium excretion (Jaisser and Farman, 2016). Confirmation was noted by the insignificant results on potassium found in Chapter 2. However, in Chapter 2, there was a significant length of exposure effect on sodium with chronic having more than acute. Taken together, it appears that aldosterone levels remain unchanged in quail that are subjected to chronic heat stress at 31.1˚C although others have noted that aldosterone increased during times of water loss (possible during heat stress) to improve fluid retention (Schleh, et al., 2018).

4.5 Estrogens

Estradiol stimulates vitellogenesis in the female liver, feed intake, and deposition of calcium in the medullary part of long bones and eggshells (Elnagar and Abd-Elhady, 2009). Results of the current study showed that there were no significant differences in estradiol among all treatments. This finding does not agree with that of others where 20 days in heat stress increased 17β-estradiol in sexually mature female quail (Pu, et al., 2020). However, the same researchers

found that a 10-day heat stress challenge decreased serum 17β-estradiol (Pu, et al., 2020). Others found that a 5-day heat stress period in laying hens decreased estradiol and progesterone levels in serum (Li, et al., 2020). This association indicates that the duration of heat stress can influence the change in sex hormones. In the current study, samples were analyzed after 4 hours and after 3 weeks of heat stress; however, there were no significant differences in estradiol when compared by length of exposure.

While estradiol levels were not significantly different among treatments, sex, and length of exposure, there was a significantly higher concentration of estriol in chronic birds, particularly in TN males. From a study examining estrogens and androgens in developing chicks, researchers reported that estriol was not affected by sex (Wang, et al., 2019). Estriol has also been used as a treatment of diseases such as multiple sclerosis in both male and female humans (Palaszynski, et al., 2004). Administering estriol to male and female mice decreased inflammatory cytokines (Palaszynski, et al., 2004). Similar findings reported here could indicate that chronic TN males had significantly less inflammatory events than all other treatments.

4.6 Androgens

Across both male and females, testosterone, cis-androsterone, androstenediol, and DHEA-S were higher in chronic than acute. Androstanediol is derived from testosterone; therefore, as expected, there was more androstanediol in chronic males than acute. DHEA is the predominant precursor to sex steroid hormones such as estradiol and testosterone (Oberbeck and Kobbe, 2010). Both DHEA and DHEA-S are the most abundant circulating steroid hormones; however, during times of stress, metabolism and concentration of DHEA is decreased (Wang, et al., 2009; Oberbeck and Kobbe, 2010). Additionally, DHEA and DHEA-S increased with puberty (Oberbeck and Kobbe, 2010). Therefore, due to the sexual maturity of chronic quail, DHEA and DHEA-S were

significantly higher in chronic than acute. Contrarily, as others have noted, DHEA and DHEA-S decreased during stress, but in the present study, it increased in HSS as compared to TN (Wang, et al., 2009; Oberbeck and Kobbe, 2010).

Decreases in lipogenesis and fat cell growth were noted in DHEA-administered rats (Mohan and Cleary, 1988). Another study reported that rats fed high-fat diets and treated with DHEA decreased triacylglyceride levels in female livers and had no effect on male livers (Cecconello, et al., 2015). The current study also did not find any significant differences in DHEA or DHEA-S levels between males and females. The proposed mechanism for this decrease in lipogenesis involves a DHEA-induced inhibition of glucose-6-phosphate dehydrogenase activity and an increase in long-chain fatty acyl-CoA hydrolase (Mohan and Cleary, 1988).

As reported by other investigators, the significantly higher levels of DHEA for HSS compared to TN should have also increased fatty acyl-CoA hydrolase (Mohan and Cleary, 1988). Instead, in the current study there was a significant decrease in medium-chain-like fatty acyl-CoA hydrolase precursor in HSS compared to TN (Chapter 6 above). The proteomics analysis of female quail livers did not detect long-chain fatty acyl-CoA hydrolase (Chapter 6). However, the proteomics analysis showed that TN had significantly more proteins involved in adipogenesis and lipogenesis than HSS, further corroborating the findings that DHEA decreased lipogenesis (Chapter 6).

5. Conclusions

The most notable findings from the current study were:

1. Chronic males, particularly from TN, had significantly more glucocorticoids (corticosterone and cortisone), progestogens (DHP, 17α-OHP, and 20α-OHP), neurosteroids (β-pregnanolone, Allo, and etiocholanolone), estrogens (estriol), and

androgens (cis-androsterone, androstanediol, and androstenediol) than other acute males and females, especially from HSS. Thus, measurements for glucocorticoids did not support the expectation that selective breeding for higher performance at 31.1˚C for 10 generations improved the quail's susceptibility to stress.

2. Of the 29 steroid hormones analyzed, 13 were significantly higher in chronic than acute quail. As expected, this result demonstrated that hormones shift in concentration when quails undergo sexual maturity.

Future studies should further investigate steroid hormones at temperatures immediately outside the thermoneutral zone of quail and other poultry species. Although it is important to understand hormonal response to more extreme temperature differences, it is also of value to accurately pinpoint when responses begin to occur. By investigating the transition from a thermoneutral zone to heat stress, procedures can be implemented to mitigate heat stress at an earlier stage.

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Table 7.1. Concentrations of glucocorticoids in sera compared by treatment × sex × exposure (ng/mL).

Trt[1]	Sex	Exposure[2]	Corticosterone[3]	Cortisol[3]	Cortisone	Cortexolone[3]
TN	Male	Acute	0.38±0.25^{ab}	0.04±0.01	0.01±0.00^{a}	0.01±0.01
		Chronic	2.07±0.29^{ab}	0.06±0.02	0.03±0.00^{b}	0.01±0.01
	Female	Acute	1.62±0.21^{b}	0.07±0.01	0.01±0.00^{ab}	0.01±0.01
		Chronic	0.54±0.19^{ab}	0.04±0.01	0.01±0.00^{ab}	0.02±0.01
TNS	Male	Acute	0.11±0.19^{a}	0.03±0.01	0.01±0.00^{a}	0.01±0.01
		Chronic	0.28±0.25^{ab}	0.02±0.01	0.01±0.00^{ab}	0.01±0.01
	Female	Acute	0.32±0.21^{ab}	0.04±0.01	0.01±0.00^{ab}	0.01±0.01
		Chronic	0.51±0.21^{ab}	0.03±0.01	0.01±0.00^{a}	0.02±0.01
HS	Male	Acute	0.11±0.23^{ab}	0.03±0.01	0.01±0.00^{a}	0.02±0.01
		Chronic	0.67±0.19^{ab}	0.03±0.01	0.01±0.00^{a}	0.02±0.01
	Female	Acute	0.17±0.17^{ab}	0.04±0.01	0.01±0.00^{a}	0.02±0.01
		Chronic	0.21±0.21^{ab}	0.03±0.01	0.01±0.00^{a}	0.01±0.01
HSS	Male	Acute	0.46±0.25^{ab}	0.04±0.01	0.01±0.00^{a}	0.03±0.01
		Chronic	0.42±0.18^{ab}	0.04±0.01	0.01±0.00^{ab}	0.01±0.01
	Female	Acute	0.10±0.17^{ab}	0.03±0.01	0.01±0.00^{a}	0.03±0.01
		Chronic	0.36±0.23^{ab}	0.03±0.01	0.01±0.00^{ab}	0.03±0.01
P-value			0.11	0.72	0.02	0.71

$^{a-b}$ Superscripts indicate significant differences at P≤0.05.

[1] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[2] Acute, exposure to respective temperature for 4 hours; chronic, exposure to respective temperatures for 3 weeks.

[3] Transformation of log(x) was used in the analysis but presented mean ± SE are presented in their original scale.

Table 7.2. Concentrations of glucocorticoids in sera compared by treatment × sex, treatment × exposure, sex × exposure, treatment, sex, and exposure (ng/mL).

Treatment × Sex						
	Trt[1]	Sex	Corticosterone[3]	Cortisol[3]	Cortisone[3]	Cortexolone[3]
	TN	Male	1.10±0.22[ab]	0.04±0.01	0.01±0.00	0.01±0.01
		Female	1.04±0.16[b]	0.05±0.01	0.01±0.00	0.02±0.01
	TNS	Male	0.17±0.18[a]	0.03±0.01	0.01±0.00	0.01±0.01
		Female	0.42±0.17[ab]	0.04±0.01	0.01±0.00	0.02±0.01
	HS	Male	0.44±0.17[ab]	0.03±0.01	0.01±0.00	0.02±0.01
		Female	0.19±0.15[ab]	0.04±0.01	0.01±0.00	0.02±0.00
	HSS	Male	0.43±0.17[ab]	0.04±0.01	0.01±0.00	0.02±0.01
		Female	0.20±0.16[ab]	0.03±0.01	0.01±0.00	0.03±0.01
P-value			0.16	0.70	0.97	0.50
Treatment × Exposure						
	Trt[1]	Exposure[2]	Corticosterone[3]	Cortisol[3]	Cortisone[3]	Cortexolone[3]
	TN	Acute	1.12±0.19	0.05±0.01	0.01±0.00[ab]	0.01±0.01
		Chronic	1.00±0.19	0.04±0.01	0.02±0.00[b]	0.02±0.01
	TNS	Acute	0.21±0.16	0.03±0.01	0.01±0.00[ab]	0.01±0.01
		Chronic	0.42±0.19	0.03±0.01	0.01±0.00[ab]	0.02±0.01
	HS	Acute	0.15±0.16	0.04±0.01	0.01±0.00[a]	0.02±0.01
		Chronic	0.46±0.16	0.03±0.01	0.01±0.00[ab]	0.02±0.01
	HSS	Acute	0.21±0.16	0.03±0.01	0.01±0.00[ab]	0.03±0.01
		Chronic	0.40±0.16	0.04±0.01	0.01±0.00[ab]	0.02±0.01
P-value			0.71	0.88	0.24	0.50
Sex × Exposure						
	Sex	Exposure[2]	Corticosterone[3]	Cortisol[3]	Cortisone[3]	Cortexolone[3]
	Male	Acute	0.23±0.15[a]	0.03±0.01	0.01±0.00	0.02±0.00
		Chronic	0.70±0.14[b]	0.04±0.01	0.01±0.00	0.02±0.00
	Female	Acute	0.47±0.12[ab]	0.04±0.01	0.01±0.00	0.02±0.00
		Chronic	0.42±0.13[ab]	0.03±0.01	0.01±0.00	0.02±0.00
P-value			0.05	0.44	0.16	0.60
Treatment, Sex, and Exposure						
			Corticosterone[3]	Cortisol[3]	Cortisone[3]	Cortexolone[3]
Trt[1]		TN	1.06±0.13[b]	0.05±0.01	0.01±0.00	0.02±0.00
		TNS	0.30±0.12[a]	0.03±0.01	0.01±0.00	0.01±0.00
		HS	0.30±0.11[a]	0.03±0.01	0.01±0.00	0.02±0.00
		HSS	0.30±0.12[ab]	0.03±0.01	0.01±0.00	0.02±0.00
		P-value	0.01	0.21	0.35	0.33
Sex		Male	0.48±0.10	0.03±0.00	0.01±0.00	0.02±0.00
		Female	0.45±0.09	0.04±0.00	0.01±0.00	0.02±0.00
		P-value	0.90	0.90	0.89	0.57
Exposure[2]		Chronic	0.38±0.09	0.04±0.00	0.01±0.00	0.02±0.00
		Acute	0.55±0.10	0.03±0.00	0.01±0.00	0.02±0.00
		P-value	0.08	0.77	0.16	0.69

[a-b] Superscripts indicate significant differences at P≤0.05.

[1] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[2] Acute, exposure to respective temperature for 4 hours; chronic, exposure to respective temperatures for 3 weeks.

[3] Transformation of log(x) was used in the analysis but presented mean ± SE are presented in their original scale.

Table 7.3. Concentrations of progestogens in sera compared by treatment × sex × exposure (ng/mL).

Trt[1]	Sex	Exposure[2]	Progesterone[3]	DHP[3,4]	17α-OHP[4]	20α-OHP[3,4]
TN	Male	Acute	0.01±0.01	0.24±0.20	0.05±0.01^{ab}	0.10±0.09^{a}
		Chronic	0.02±0.01	1.22±0.26	0.07±0.01^{b}	0.76±0.12^{b}
	Female	Acute	0.00±0.01	0.32±0.18	0.02±0.01^{a}	0.21±0.08^{ab}
		Chronic	0.03±0.01	0.65±0.17	0.03±0.01^{ab}	0.22±0.08^{a}
TNS	Male	Acute	0.01±0.01	0.17±0.17	0.02±0.01^{a}	0.12±0.08^{a}
		Chronic	0.01±0.01	0.39±0.22	0.03±0.01^{ab}	0.10±0.10^{a}
	Female	Acute	0.01±0.01	0.30±0.18	0.04±0.01^{ab}	0.17±0.09^{ab}
		Chronic	0.01±0.01	0.45±0.18	0.03±0.01^{ab}	0.12±0.09^{a}
HS	Male	Acute	0.02±0.01	0.22±0.20	0.02±0.01^{ab}	0.16±0.09^{a}
		Chronic	0.03±0.01	0.83±0.17	0.05±0.01^{ab}	0.33±0.08^{ab}
	Female	Acute	0.01±0.01	0.29±0.15	0.02±0.01^{a}	0.10±0.07^{a}
		Chronic	0.01±0.01	0.51±0.18	0.03±0.01^{ab}	0.16±0.09^{a}
HSS	Male	Acute	0.01±0.01	0.29±0.22	0.03±0.01^{ab}	0.12±0.10^{a}
		Chronic	0.01±0.01	0.77±0.16	0.03±0.01^{ab}	0.42±0.08^{ab}
	Female	Acute	0.01±0.01	0.31±0.15	0.02±0.01^{a}	0.11±0.07^{a}
		Chronic	0.03±0.01	0.70±0.20	0.03±0.01^{ab}	0.22±0.10^{ab}
P-value			0.66	0.83	0.43	0.12

[a-b] Superscripts indicate significant differences at P≤0.05.

[1] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[2] Acute, exposure to respective temperature for 4 hours; chronic, exposure to respective temperatures for 3 weeks.

[3] Transformation of log(x) was used in the analysis but presented mean ± SE are presented in their original scale.

[4] 17α-Hydroxyprogesterone, 17α-OHP; Dihydroprogesterone, DHP; 20α-Dihydroprogesterone, 20α-DHP.

Table 7.4. Concentrations of progestogens in sera compared by treatment × sex, treatment × exposure, sex × exposure, treatment, sex, and exposure (ng/mL).

Treatment × Sex						
	Trt[1]	Sex	Progesterone[3]	DHP[3,4]	17α-OHP[3,4]	20α-DHP[3,4]
	TN	Male	0.01±0.01	0.61±0.17	0.06±0.01[b]	0.35±0.08
		Female	0.01±0.01	0.48±0.14	0.02±0.01[a]	0.19±0.07
	TNS	Male	0.01±0.01	0.25±0.15	0.02±0.01[a]	0.12±0.07
		Female	0.01±0.01	0.37±0.14	0.03±0.01[ab]	0.15±0.07
	HS	Male	0.03±0.01	0.57±0.14	0.04±0.01[ab]	0.24±0.07
		Female	0.01±0.01	0.37±0.13	0.02±0.00[a]	0.12±0.06
	HSS	Male	0.01±0.01	0.61±0.14	0.03±0.01[ab]	0.31±0.07
		Female	0.02±0.01	0.46±0.13	0.02±0.01[a]	0.14±0.07
P-value			0.54	0.48	0.01	0.28
Treatment × Exposure						
	Trt	Exposure[2]	Progesterone[3]	DHP[3,4]	17α-OHP[3,4]	20α-DHP[3,4]
	TN	Acute	0.00±0.01	0.28±0.13[a]	0.03±0.01	0.17±0.07[ab]
		Chronic	0.02±0.01	0.82±0.14[ab]	0.04±0.01	0.37±0.07[b]
	TNS	Acute	0.01±0.01	0.23±0.12[ab]	0.03±0.01	0.14±0.06[ab]
		Chronic	0.01±0.01	0.43±0.14[ab]	0.03±0.01	0.11±0.07[ab]
	HS	Acute	0.02±0.01	0.26±0.12[ab]	0.02±0	0.12±0.06[a]
		Chronic	0.02±0.01	0.68±0.12[b]	0.04±0.01	0.26±0.06[ab]
	HSS	Acute	0.01±0.01	0.31±0.12[b]	0.02±0.01	0.12±0.06[ab]
		Chronic	0.02±0.01	0.74±0.12[b]	0.03±0.01	0.34±0.07[ab]
P-value			0.29	0.87	0.45	0.07
Sex × Exposure						
	Sex	Exposure	Progesterone[3]	DHP[3,4]	17α-OHP[3,4]	20α-DHP[3,4]
	Male	Acute	0.01±0.01	0.22±0.10[a]	0.03±0.00[a]	0.12±0.05[a]
		Chronic	0.02±0.01	0.78±0.09[b]	0.04±0.00[b]	0.38±0.05[b]
	Female	Acute	0.01±0.00	0.31±0.08[a]	0.02±0.00[a]	0.14±0.04[a]
		Chronic	0.02±0.00	0.58±0.09[b]	0.03±0.00[ab]	0.17±0.05[a]
P-value			0.61	0.06	0.36	0.01
Treatment, Sex, and Exposure						
			Progesterone[3]	DHP[3,4]	17α-OHP[3,4]	20α-DHP[3,4]
Trt		TN	0.01±0.01	0.53±0.11	0.04±0.00	0.25±0.05
		TNS	0.01±0.01	0.31±0.10	0.03±0.00	0.13±0.05
		HS	0.02±0.01	0.46±0.09	0.03±0.00	0.17±0.05
		HSS	0.01±0.01	0.53±0.09	0.03±0.00	0.22±0.05
		P-value	0.68	0.31	0.43	0.34
Sex		Male	0.01±0.00	0.51±0.07	0.04±0.00[a]	0.25±0.04
		Female	0.01±0.00	0.42±0.07	0.02±0.00[b]	0.15±0.03
		P-value	0.61	0.73	0.01	0.10
Exposure		Chronic	0.01±0.00	0.27±0.06[a]	0.02±0.00[a]	0.12±0.03[a]
		Acute	0.02±0.00	0.67±0.06[b]	0.04±0.00[b]	0.28±0.04[b]
		P-value	0.06	<0.0001	0.00	0.00

[a-b] Superscripts indicate significant differences at P≤0.05.

[1] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[2] Acute, exposure to respective temperature for 4 hours; chronic, exposure to respective temperatures for 3 weeks.

[3] Transformation of log(x) was used in the analysis but presented mean ± SE are presented in their original scale.

[4] 17α-Hydroxyprogesterone, 17α-OHP; Dihydroprogesterone, DHP; 20α-Dihydroprogesterone, 20α-DHP.

Table 7.5. Concentrations of neurosteroids in sera compared by treatment × sex × exposure (ng/mL).

	Trt[1]	Sex	Exposure[2]	Pregnenolone[3]	Allo pregnanolone	β-pregnanolone[3]	17-OH pregnenolone	Etiocholanolone[3]
	TN	Male	Acute	0.65±2.06	0.64±0.15^{ab}	0.47±0.34	12.29±13.38	0.14±0.13
			Chronic	3.47±2.63	1.24±0.19^{b}	0.54±0.44	41.10±17.28	0.42±0.16
		Female	Acute	1.11±1.86	0.28±0.14^{a}	0.48±0.31	44.74±12.22	0.33±0.12
			Chronic	5.80±1.73	0.26±0.14^{a}	1.01±0.29	20.13±11.31	0.58±0.11
	TNS	Male	Acute	1.95±1.74	0.33±0.15^{ab}	0.46±0.29	34.70±11.31	0.21±0.11
			Chronic	0.45±2.27	0.36±0.17^{ab}	0.57±0.38	23.98±14.96	0.44±0.14
		Female	Acute	0.34±1.87	0.35±0.14^{ab}	0.34±0.31	61.23±12.22	0.20±0.12
			Chronic	0.25±1.87	0.31±0.14^{ab}	0.36±0.31	30.32±12.22	0.36±0.12
	HS	Male	Acute	0.70±2.03	0.45±0.15^{ab}	0.55±0.34	14.88±13.38	0.33±0.13
			Chronic	6.28±1.74	0.77±0.13^{ab}	1.66±0.29	39.14±11.31	0.62±0.11
		Female	Acute	1.04±1.54	0.58±0.11^{ab}	0.44±0.26	15.35±09.97	0.29±0.10
			Chronic	7.01±1.85	0.42±0.15^{ab}	1.23±0.34	23.64±12.22	0.48±0.11
	HSS	Male	Acute	0.17±2.25	0.41±0.16^{ab}	0.55±0.38	57.61±14.96	0.36±0.14
			Chronic	2.37±1.64	0.46±0.12^{ab}	1.27±0.27	18.61±10.58	0.34±0.10
		Female	Acute	0.26±1.54	0.25±0.11^{a}	0.64±0.26	27.34±09.97	0.33±0.09
			Chronic	0.25±2.26	0.42±0.15^{ab}	1.05±0.38	30.65±13.38	0.33±0.12
P-value				0.14	0.18	0.89	0.07	0.86

[a-b] Superscripts indicate significant differences at P≤0.05.

[1] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[2] Acute, exposure to respective temperature for 4 hours; chronic, exposure to respective temperatures for 3 weeks.

[3] Transformation of log(x) was used in the analysis but presented mean ± SE are presented in their original scale.

Table 7.6. Concentrations of neurosteroids in sera compared by treatment × sex, treatment × exposure, sex × exposure, treatment, sex, and exposure (ng/mL).

Treatment × Sex							
	Trt	Sex	Pregnenolone[3]	Allo-pregnanolone	β-pregnanolone[3]	17-OH pregnenolone	Etiocholanolone[3]
	TN	Male	1.71±1.88	0.86±0.13^{b}	0.50±0.28^{ab}	23.10±10.94	0.24±0.11
		Female	3.85±1.59	0.27±0.12^{a}	0.77±0.22^{ab}	31.50±08.58	0.46±0.09
	TNS	Male	1.42±1.64	0.34±0.12^{ab}	0.50±0.24^{ab}	30.80±09.33	0.29±0.09
		Female	0.29±1.62	0.33±0.11^{ab}	0.35±0.23^{a}	45.80±08.93	0.28±0.09
	HS	Male	4.13±1.62	0.64±0.11^{ab}	1.20±0.23^{b}	29.00±08.93	0.50±0.09
		Female	3.46±1.50	0.53±0.11^{ab}	0.73±0.21^{ab}	18.70±07.99	0.36±0.08
	HSS	Male	1.46±1.68	0.46±0.12^{ab}	1.03±0.23^{ab}	31.60±08.93	0.35±0.09
		Female	0.26±1.50	0.31±0.10^{a}	0.76±0.22^{ab}	28.50±08.27	0.33±0.08
P-value			0.69	0.09	0.49	0.49	0.16
Treatment × Exposure							
	Trt	Exposure[2]	Pregnenolone[3]	Allo-pregnanolone	β-pregnanolone[3]	17-OH pregnenolone	Etiocholanolone[3]
	TN	Acute	0.85±1.33^{ab}	0.47±0.11	0.47±0.22^{ab}	30.00±9.18	0.24±0.08^{ab}
		Chronic	5.09±1.39^{b}	0.61±0.12	0.87±0.23^{ab}	26.40±9.63	0.52±0.09^{ab}
	TNS	Acute	1.20±1.23^{ab}	0.34±0.11	0.40±0.21^{a}	47.00±8.44	0.20±0.08^{a}
		Chronic	0.37±1.39^{ab}	0.33±0.11	0.44±0.23^{ab}	27.80±9.63	0.39±0.09^{ab}
	HS	Acute	0.87±1.18^{ab}	0.57±0.10	0.48±0.20^{ab}	15.20±8.13	0.31±0.07^{ab}
		Chronic	6.67±1.22^{b}	0.60±0.10	1.48±0.21^{b}	32.00±8.44	0.56±0.08^{b}
	HSS	Acute	0.04±1.22^{a}	0.31±0.10	0.61±0.21^{ab}	36.70±8.44	0.34±0.08^{ab}
		Chronic	1.70±1.27^{ab}	0.44±0.10	1.20±0.21^{b}	23.20±8.44	0.34±0.08^{ab}
P-value			0.09	0.81	0.69	0.19	0.37
Sex × Exposure							
	Sex	Exposure	Pregnenolone[3]	Allo-pregnanolone	β-pregnanolone[3]	17-OH pregnenolone	Etiocholanolone[3]
	Male	Acute	0.92±1.02^{ab}	0.47±0.08^{ab}	0.50±0.17^{ab}	29.00±6.78	0.25±0.06^{a}
		Chronic	3.47±1.00^{b}	0.64±0.08^{b}	1.17±0.16^{b}	29.20±6.62	0.45±0.06^{b}
	Female	Acute	0.71±0.86^{a}	0.37±0.06^{ab}	0.49±0.14^{a}	34.00±5.67	0.29±0.05^{ab}
		Chronic	3.66±0.97^{b}	0.34±0.07^{a}	0.89±0.16^{ab}	25.70±6.34	0.45±0.06^{ab}
P-value			0.72	0.13	0.22	0.51	0.27
Treatment, Sex, and Exposure							
			Pregnenolone[3]	Allo-pregnanolone	β-pregnanolone[3]	17-OH pregnenolone	Etiocholanolone[3]
Trt		TN	2.93±1.18	0.53±0.09	0.66±0.17^{ab}	28.30±6.70	0.38±0.06
		TNS	0.85±1.12	0.34±0.09	0.42±0.17^{a}	38.60±6.40	0.29±0.06
		HS	3.74±1.07	0.59±0.08	0.94±0.16^{b}	23.30±5.91	0.42±0.06
		HSS	0.80±1.08	0.38±0.08	0.89±0.16^{b}	29.90±6.02	0.34±0.06
		P-value	0.15	0.13	0.02	0.37	0.26
Sex		Male	2.23±0.83	0.57±0.06^{a}	0.84±0.12	29.10±4.71	0.36±0.05
		Female	1.95±0.76	0.36±0.06^{b}	0.66±0.11	30.30±4.20	0.36±0.04
		P-value	0.74	0.02	0.14	0.85	0.92
Exposure		Chronic	0.81±0.65^{a}	0.43±0.05	0.49±0.11^{a}	31.90±4.31	0.28±0.04^{a}
		Acute	3.56±0.69^{b}	0.49±0.05	1.03±0.12^{b}	27.40±4.54	0.45±0.04^{b}
		P-value	0.00	0.31	0.02	0.47	0.00

[a-b] Superscripts indicate significant differences at P≤0.05.

[1] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively.

TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.
[2] Acute, exposure to respective temperature for 4 hours; chronic, exposure to respective temperatures for 3 weeks.
[3] Transformation of log(x) was used in the analysis but presented mean ± SE are presented in their original scale.

Table 7.7. Concentrations of mineralocorticoids in sera compared by treatment × sex × exposure (ng/mL).

	Trt[2]	Sex	Exposure[3]	Aldosterone	Cortexone[4]
	TN	Male	Acute	0.03±0.01	0.01±0.01
			Chronic	0.04±0.01	0.07±0.02
		Female	Acute	0.04±0.01	0.01±0.01
			Chronic	0.03±0.01	0.02±0.01
	TNS	Male	Acute	0.02±0.01	0.01±0.01
			Chronic	0.02±0.01	0.01±0.01
		Female	Acute	0.03±0.01	0.01±0.01
			Chronic	0.03±0.01	0.02±0.01
	HS	Male	Acute	0.03±0.01	0.01±0.01
			Chronic	0.03±0.01	0.04±0.01
		Female	Acute	0.02±0.01	0.01±0.01
			Chronic	0.04±0.01	0.01±0.01
	HSS	Male	Acute	0.02±0.01	0.01±0.01
			Chronic	0.02±0.01	0.03±0.01
		Female	Acute	0.03±0.01	0.01±0.01
			Chronic	0.01±0.01	0.05±0.01
P-value[4]				0.48	0.06

[1] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[2] Acute, exposure to respective temperature for 4 hours; chronic, exposure to respective temperatures for 3 weeks.

[3] Transformation of log(x) was used in the analysis but presented mean ± SE are presented in their original scale.

[4] Significant difference was determined at $P \leq 0.05$.

Table 7.8. Concentrations of mineralocorticoids in sera compared by treatment × sex, treatment × exposure, sex × exposure, treatment, sex, and exposure (ng/mL).

Treatment × Sex				
	Trt[1]	Sex	Aldosterone	Cortexone[2]
	TN	Male	0.03±0.01	0.03±0.01
		Female	0.03±0.01	0.01±0.01
	TNS	Male	0.02±0.01	0.01±0.01
		Female	0.03±0.01	0.01±0.01
	HS	Male	0.03±0.01	0.02±0.01
		Female	0.03±0.01	0.01±0.01
	HSS	Male	0.02±0.01	0.03±0.01
		Female	0.03±0.01	0.03±0.01
P-value			0.80	0.66
Treatment × Exposure				
	Trt	Exposure[3]	Aldosterone	Cortexone[2]
	TN	Acute	0.03±0.01	0.01±0.01
		Chronic	0.03±0.01	0.03±0.01
	TNS	Acute	0.02±0.01	0.01±0.01
		Chronic	0.02±0.01	0.01±0.01
	HS	Acute	0.02±0.01	0.01±0.01
		Chronic	0.03±0.01	0.02±0.01
	HSS	Acute	0.02±0.01	0.01±0.01
		Chronic	0.02±0.01	0.04±0.01
P-value			0.22	0.58
Sex × Exposure				
	Sex	Exposure	Aldosterone	Cortexone[2]
	Male	Acute	0.02±0.00	0.01±0.01[ab]
		Chronic	0.03±0.00	0.03±0.01[b]
	Female	Acute	0.03±0.00	0.01±0.01[a]
		Chronic	0.03±0.00	0.02±0.01[ab]
P-value			0.49	0.90
Treatment, Sex, and Exposure				
			Aldosterone	Cortexone[2]
Trt		TN	0.03±0.00	0.02±0.01
		TNS	0.02±0.00	0.01±0.01
		HS	0.03±0.00	0.02±0.01
		HSS	0.02±0.00	0.03±0.01
		P-value	0.43	0.13
Sex		Male	0.02±0.00	0.02±0.00
		Female	0.03±0.00	0.01±0.00
		P-value	0.34	0.24
Exposure		Chronic	0.03±0.00	0.01±0.00[a]
		Acute	0.03±0.00	0.03±0.00[b]
		P-value	0.85	0.01

[a-b] Superscripts indicate significant difference at P≤0.05.

[1] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[2] Transformation of log(x) was used in the analysis but presented mean ± SE are presented in their original scale.

[3] Acute, exposure to respective temperature for 4 hours; chronic, exposure to respective temperatures for 3 weeks.

Table 7.9. Concentrations of estrogens in compared by treatment × sex × exposure (ng/mL).

	Trt[1]	Sex	Exposure[2]	Estriol[3]	Estradiol[4]	Estrone	2-Methoxyestradiol
	TN	Male	Acute	0.04±0.07[a]	0.19±0.05	0.44±0.11	0.63±0.18
			Chronic	0.73±0.09[b]	0.15±0.08	0.46±0.14	1.31±0.23
		Female	Acute	0.10±0.07[a]	0.15±0.04	0.40±0.10	0.65±0.17
			Chronic	0.16±0.06[a]	0.15±0.04	0.39±0.09	0.55±0.15
	TNS	Male	Acute	0.06±0.06[a]	0.10±0.04	0.52±0.09	0.30±0.15
			Chronic	0.11±0.08[a]	0.13±0.05	0.32±0.12	0.50±0.20
		Female	Acute	0.09±0.07[a]	0.18±0.04	0.43±0.10	0.33±0.17
			Chronic	0.11±0.07[a]	0.28±0.04	0.62±0.10	0.55±0.17
	HS	Male	Acute	0.08±0.07[a]	0.11±0.05	0.70±0.11	0.53±0.18
			Chronic	0.14±0.06[ab]	0.21±0.04	0.51±0.09	0.63±0.15
		Female	Acute	0.11±0.05[a]	0.13±0.04	0.36±0.08	0.51±0.14
			Chronic	0.11±0.07[a]	0.15±0.04	0.54±0.10	0.36±0.17
	HSS	Male	Acute	0.08±0.08[a]	0.20±0.05	0.21±0.12	0.81±0.20
			Chronic	0.25±0.06[ab]	0.15±0.04	0.39±0.09	0.66±0.14
		Female	Acute	0.08±0.05[a]	0.13±0.04	0.68±0.08	0.52±0.14
			Chronic	0.26±0.07[ab]	0.21±0.05	0.37±0.11	0.61±0.18
P-value				0.03	0.52	0.02	0.25

[a-b] Superscripts indicate significant differences at P≤0.05.

[1] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[2] Acute, exposure to respective temperature for 4 hours; chronic, exposure to respective temperatures for 3 weeks.

[3] Transformation of log(x) was used in the analysis but presented mean ± SE are presented in their original scale.

[4] Transformation of sqrt(x) was used in the analysis but presented mean ± SE are presented in their original scale.

Table 7.10. Concentrations of estrogens in sera compared by treatment × sex, treatment × exposure, sex × exposure, treatment, sex, and exposure (ng/mL).

Treatment × Sex						
	Trt[1]	Sex	Estriol[2]	Estradiol[3]	Estrone	2-Methoxyestradiol
	TN	Male	0.31±0.07	0.18±0.04	0.45±0.09	0.89±0.14
		Female	0.13±0.05	0.15±0.03	0.39±0.07	0.60±0.11
	TNS	Male	0.08±0.06	0.11±0.03	0.45±0.08	0.37±0.12
		Female	0.10±0.05	0.24±0.03	0.53±0.07	0.44±0.12
	HS	Male	0.12±0.05	0.17±0.03	0.59±0.07	0.58±0.12
		Female	0.10±0.05	0.14±0.03	0.43±0.07	0.45±0.11
	HSS	Male	0.20±0.05	0.17±0.03	0.33±0.07	0.71±0.12
		Female	0.15±0.05	0.16±0.03	0.57±0.07	0.55±0.11
P-value			0.70	0.07	0.05	0.54
Treatment × Exposure						
	Trt	Exposure[4]	Estriol[2]	Estradiol[3]	Estrone	2-Methoxyestradiol
	TN	Acute	0.08±0.05	0.17±0.03	0.42±0.08	0.64±0.12
		Chronic	0.33±0.06	0.15±0.04	0.41±0.08	0.78±0.13
	TNS	Acute	0.08±0.05	0.13±0.03	0.48±0.07	0.31±0.11
		Chronic	0.11±0.06	0.22±0.03	0.50±0.08	0.53±0.13
	HS	Acute	0.10±0.05	0.13±0.03	0.48±0.07	0.52±0.11
		Chronic	0.12±0.05	0.18±0.03	0.53±0.07	0.50±0.11
	HSS	Acute	0.09±0.05	0.15±0.03	0.53±0.07	0.61±0.11
		Chronic	0.25±0.05	0.17±0.03	0.38±0.07	0.64±0.11
P-value			0.40	0.31	0.55	0.78
Sex × Exposure						
	Sex	Exposure	Estriol[2]	Estradiol[3]	Estrone	2-Methoxyestradiol
	Male	Acute	0.07±0.04[a]	0.14±0.02	0.49±0.06	0.53±0.09
		Chronic	0.25±0.04[b]	0.17±0.02	0.43±0.05	0.71±0.09
	Female	Acute	0.10±0.03[ab]	0.14±0.02	0.48±0.05	0.51±0.08
		Chronic	0.15±0.04[ab]	0.19±0.02	0.48±0.05	0.51±0.08
P-value			0.10	0.86	0.55	0.33
Treatment, Sex, and Exposure						
			Estriol[2]	Estradiol[3]	Estrone	2-Methoxyestradiol
Trt		TN	0.20±0.04	0.16±0.03	0.41±0.06	0.71±0.09
		TNS	0.09±0.04	0.17±0.02	0.49±0.05	0.41±0.09
		HS	0.11±0.04	0.15±0.02	0.50±0.05	0.51±0.08
		HSS	0.17±0.04	0.16±0.02	0.46±0.05	0.63±0.08
		P-value	0.83	0.96	0.66	0.08
Sex		Male	0.17±0.03	0.15±0.02	0.46±0.04	0.62±0.06
		Female	0.12±0.03	0.17±0.01	0.48±0.04	0.51±0.06
		P-value	0.75	0.75	0.66	0.18
Exposure		Chronic	0.09±0.03[a]	0.14±0.02	0.48±0.04	0.52±0.06
		Acute	0.20±0.03[b]	0.18±0.02	0.46±0.04	0.61±0.06
		P-value	0.00	0.09	0.64	0.28

[a-b] Superscripts indicate significant differences at P≤0.05.

[1] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[2] Transformation of log(x) was used in the analysis but presented mean ± SE are presented in their original scale.

[3] Transformation of sqrt(x) was used in the analysis but presented mean ± SE are presented in their original scale.

[4] Acute, exposure to respective temperature for 4 hours; chronic, exposure to respective temperatures for 3 weeks.

Table 7.11. Concentrations of androgens in sera compared by treatment × sex × exposure (ng/mL).

	Trt[1]	Sex	Exposure[2]	Testosterone glucuronide	Testosterone[3]	DHT[3,4]	Trans-Androsterone	Cis-Androsterone[3]	Andro-stanediol	Andro-stenediol	Andro-stenedione[3]	DHEA[4,5]	DHEA-S[3,4]
	TN	M	Acute	0.01±0.00	0.09±0.06	0.06±0.02	0.04±0.07	0.14±0.13	0.35±0.27	0.17±0.14^{a}	0.01±0.01	1.03±0.32^{abc}	1.14±0.27^{abcde}
			Chronic	0.02±0.00	0.09±0.08	0.05±0.03	0.29±0.10	0.42±0.16	1.46±0.34	1.27±0.17^{b}	0.03±0.01	0.22±0.41^{a}	0.92±0.34^{abcde}
		F	Acute	0.01±0.00	0.09±0.06	0.06±0.02	0.14±0.07	0.33±0.12	0.49±0.24	0.39±0.12^{a}	0.01±0.01	1.28±0.29^{abc}	1.22±0.24^{abcde}
			Chronic	0.01±0.00	0.08±0.05	0.05±0.02	0.10±0.07	0.58±0.11	0.58±0.22	0.49±0.11^{ab}	0.03±0.01	1.07±0.27^{abc}	0.98±0.22^{ac}
	TNS	M	Acute	0.01±0.00	0.06±0.05	0.05±0.02	0.26±0.06	0.21±0.11	0.63±0.22	0.28±0.11^{a}	0.02±0.01	1.45±0.27^{abc}	1.57±0.22^{abcde}
			Chronic	0.01±0.00	0.05±0.07	0.06±0.03	0.11±0.08	0.44±0.14	0.78±0.30	0.44±0.15^{ab}	0.01±0.01	1.07±0.35^{abc}	1.09±0.29^{abcde}
		F	Acute	0.01±0.00	0.07±0.06	0.04±0.02	0.09±0.07	0.18±0.12	0.62±0.24	0.22±0.12^{a}	0.02±0.01	0.99±0.29^{ab}	1.11±0.24^{abcde}
			Chronic	0.01±0.00	0.13±0.06	0.06±0.02	0.24±0.07	0.36±0.12	0.35±0.24	0.28±0.12^{a}	0.02±0.01	1.42±0.29^{abc}	1.42±0.24^{abcde}
	HS	M	Acute	0.01±0.00	0.06±0.06	0.05±0.02	0.03±0.08	0.33±0.13	0.60±0.27	0.35±0.13^{a}	0.01±0.01	0.90±0.31^{ab}	0.98±0.26^{ab}
			Chronic	0.01±0.00	0.23±0.05	0.12±0.02	0.16±0.06	0.62±0.11	0.93±0.22	0.46±0.11^{ab}	0.03±0.01	1.56±0.27^{abc}	1.74±0.22^{abcde}
		F	Acute	0.01±0.00	0.05±0.05	0.04±0.02	0.23±0.06	0.29±0.10	0.98±0.20	0.56±0.10^{ab}	0.06±0.01	0.93±0.24^{ab}	1.00±0.20^{a}
			Chronic	0.01±0.00	0.26±0.06	0.10±0.02	0.17±0.07	0.48±0.11	0.56±0.24	0.57±0.12^{ab}	0.03±0.01	1.46±0.29^{abc}	2.02±0.24^{bde}
	HSS	M	Acute	0.01±0.00	0.10±0.07	0.06±0.03	0.03±0.08	0.36±0.14	0.39±0.30	0.33±0.14^{a}	0.01±0.01	2.67±0.35^{c}	2.46±0.29^{cde}
			Chronic	0.02±0.00	0.17±0.05	0.06±0.02	0.19±0.06	0.34±0.10	1.12±0.21	0.47±0.11^{ab}	0.02±0.01	1.29±0.26^{abc}	1.58±0.21^{abcde}
		F	Acute	0.01±0.00	0.06±0.05	0.05±0.02	0.16±0.06	0.33±0.09	0.63±0.20	0.33±0.10^{a}	0.02±0.01	1.43±0.24^{abc}	1.10±0.20^{abcd}
			Chronic	0.01±0.00	0.28±0.06	0.11±0.02	0.07±0.07	0.33±0.12	0.92±0.27	0.26±0.13^{a}	0.02±0.01	2.36±0.31^{bc}	2.16±0.26^{e}
P-value				0.13	0.49	0.76	0.03	0.86	0.82	0.04	0.30	0.02	0.02

$^{a\text{-}e}$ Superscripts indicate significant differences at P≤0.05.

[1] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[2] Acute, exposure to respective temperature for 4 hours; chronic, exposure to respective temperatures for 3 weeks.

[3] Transformation of log(x) was used in the analysis but presented mean ± SE are presented in their original scale.

[4] Dihydrotestosterone, DHT; Dehydroepiandrosterone, DHEA; Dehydroepiandrosterone Sulfate, DHEA-S.

[5] Although DHEA can be classified as an androgen, it is also a precursor to estrogens.

Table 7.12. Concentrations of androgens in sera compared by treatment × sex (ng/mL).

	Trt[1]	Sex	Testosterone glucuronide	Testosterone[2]	DHT[2,3]	Trans-androsterone[2]	Cis-androsterone[2]	Andro-stanediol	Andro stenediol[2]	Andro stenedione[2]	DHEA[3,4]	DHEA-S[2,3]
	TN	Male	0.02±0.00	0.09±0.05	0.06±0.02	0.13±0.06	0.24±0.11	0.76±0.22	0.59±0.12	0.02±0.01	0.73±0.29	1.05±0.25
		Female	0.01±0.00	0.09±0.04	0.05±0.02	0.12±0.05	0.46±0.09	0.54±0.17	0.43±0.10	0.02±0.01	1.15±0.23	1.10±0.20
	TNS	Male	0.01±0.00	0.06±0.05	0.06±0.02	0.21±0.05	0.29±0.09	0.69±0.19	0.34±0.10	0.02±0.01	1.31±0.25	1.39±0.21
		Female	0.01±0.00	0.10±0.04	0.05±0.02	0.16±0.05	0.28±0.09	0.49±0.18	0.25±0.10	0.02±0.01	1.22±0.24	1.27±0.21
	HS	Male	0.01±0.00	0.16±0.04	0.09±0.02	0.11±0.05	0.50±0.09	0.79±0.18	0.42±0.10	0.02±0.01	1.30±0.24	1.43±0.21
		Female	0.01±0.00	0.13±0.04	0.06±0.01	0.21±0.04	0.36±0.08	0.81±0.16	0.53±0.09	0.04±0.01	1.15±0.22	1.38±0.19
	HSS	Male	0.01±0.00	0.15±0.04	0.06±0.02	0.14±0.05	0.35±0.09	0.88±0.18	0.42±0.10	0.01±0.01	1.75±0.24	1.88±0.21
		Female	0.01±0.00	0.14±0.04	0.07±0.01	0.13±0.05	0.33±0.08	0.73±0.16	0.31±0.09	0.02±0.01	1.75±0.22	1.47±0.19
P-value[5]			0.57	0.56	0.76	0.67	0.16	0.90	0.76	0.38	0.66	0.82

[1] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[2] Transformation of log(x) was used in the analysis but presented mean ± SE are presented in their original scale.

[3] Dihydrotestosterone, DHT; Dehydroepiandrosterone, DHEA; Dehydroepiandrosterone Sulfate, DHEA-S.

[4] Although DHEA can be classified as an androgen, it is also a precursor to estrogens.

[5] Significant difference was determined at $P \leq 0.05$.

Table 7.13. Concentrations of androgens in sera compared by treatment × exposure (ng/mL).

	Trt[1]	Exposure[2]	Testosterone glucuronide	Testosterone[3]	DHT[3,4]	Trans-androsterone[3]	Cis-androsterone[3]	Andro-stanediol	Androstenediol[3]	Androstenedione[3]	DHEA[4,5]	DHEA-S[3,4]
	TN	Acute	0.01±0.00	0.09±0.04	0.06±0.02	0.09±0.05	0.24±0.08^{ab}	0.43±0.18	0.29±0.09	0.01±0.01	1.15±0.23	1.18±0.19^{ab}
		Chronic	0.02±0.00	0.08±0.04	0.05±0.02	0.17±0.06	0.52±0.09^{ab}	0.84±0.19	0.71±0.10	0.03±0.01	0.80±0.24	0.97±0.20^{a}
	TNS	Acute	0.01±0.00	0.06±0.04	0.05±0.01	0.18±0.05	0.20±0.08^{a}	0.63±0.17	0.27±0.09	0.02±0.01	1.22±0.21	1.35±0.18^{ab}
		Chronic	0.01±0.00	0.10±0.04	0.06±0.02	0.19±0.05	0.39±0.09^{ab}	0.52±0.19	0.33±0.10	0.02±0.01	1.32±0.24	1.30±0.20^{ab}
	HS	Acute	0.01±0.00	0.05±0.04	0.04±0.01	0.17±0.05	0.31±0.07^{ab}	0.84±0.16	0.48±0.08	0.04±0.01	0.96±0.21	0.97±0.17^{a}
		Chronic	0.01±0.00	0.24±0.04	0.11±0.01	0.16±0.05	0.56±0.08^{b}	0.76±0.17	0.48±0.09	0.03±0.01	1.51±0.21	1.87±0.18^{b}
	HSS	Acute	0.01±0.00	0.07±0.04	0.05±0.01	0.12±0.05	0.34±0.08^{ab}	0.55±0.17	0.32±0.09	0.02±0.01	1.86±0.21	1.55±0.18^{ab}
		Chronic	0.01±0.00	0.21±0.04	0.08±0.01	0.15±0.05	0.34±0.08^{ab}	1.04±0.17	0.40±0.09	0.02±0.01	1.64±0.21	1.74±0.18^{b}
P-value			0.20	0.09	0.19	0.67	0.37	0.19	0.28	0.28	0.16	0.00

[a-b] Superscripts indicate significant differences at P≤0.05.

[1] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[2] Acute, exposure to respective temperature for 4 hours; chronic, exposure to respective temperatures for 3 weeks.

[3] Transformation of log(x) was used in the analysis but presented mean ± SE are presented in their original scale.

[4] Dihydrotestosterone, DHT; Dehydroepiandrosterone, DHEA; Dehydroepiandrosterone Sulfate, DHEA-S.

[5] Although DHEA can be classified as an androgen, it is also a precursor to estrogens.

Table 7.14. Concentrations of androgens in sera compared by sex × exposure (ng/mL).

	Sex	Exposure[1]	Testosterone glucuronide	Testosterone[2]	DHT[2]	Trans-androsterone[2]	Cis-androsterone[2]	Andro-stanediol	Andro-stenediol[2]	Andro-stenedione[2]	DHEA[3,4]	DHEA-S[2,3]
	Male	Acute	0.01±0.00	0.07±0.03	0.05±0.01	0.12±0.04	0.25±0.06[a]	0.51±0.13[a]	0.30±0.07	0.01±0.01	1.46±0.17	1.51±0.15[ab]
		Chronic	0.01±0.00	0.16±0.03	0.08±0.01	0.18±0.04	0.45±0.06[b]	1.05±0.12[b]	0.56±0.07	0.02±0.00	1.15±0.17	1.43±0.14[ab]
	Female	Acute	0.01±0.00	0.07±0.03	0.05±0.01	0.16±0.03	0.29±0.06[ab]	0.71±0.11[ab]	0.38±0.06	0.03±0.00	1.16±0.15	1.09±0.12[a]
		Chronic	0.01±0.00	0.18±0.03	0.08±0.01	0.15±0.04	0.45±0.06[ab]	0.59±0.12[ab]	0.40±0.07	0.02±0.00	1.53±0.16	1.06±0.14[b]
P-value			0.56	0.30	0.66	0.14	0.28	0.01	0.11	0.21	0.03	0.06

[a-b] Superscripts indicate significant differences at P≤0.05.

[1] Acute, exposure to respective temperature for 4 hours; chronic, exposure to respective temperatures for 3 weeks.

[2] Transformation of log(x) was used in the analysis but presented mean ± SE are presented in their original scale.

[3] Dihydrotestosterone, DHT; Dehydroepiandrosterone, DHEA; Dehydroepiandrosterone Sulfate, DHEA-S.

[4] Although DHEA can be classified as an androgen, it is also a precursor to estrogens.

Table 7.15. Concentrations of androgens in sera compared by treatment, sex, and exposure (ng/mL).

		Testosterone glucuronide	Testosterone[1]	DHT[1,2]	Trans-androsterone[1]	Cis-androsterone[1]	Andro-stanediol	Androstenediol[1]	Androstenedione[1]	DHEA[2,3]	DHEA-S[1,2,3]
Trt[4]	TN	0.01±0.00	0.09±0.03	0.05±0.01	0.13±0.04	0.38±0.06	0.62±0.13	0.49±0.07	0.02±0.01	0.99±0.18[a]	1.08±0.15[a]
	TNS	0.01±0.00	0.08±0.03	0.05±0.01	0.19±0.04	0.29±0.06	0.58±0.13	0.29±0.07	0.02±0.00	1.26±0.17[ab]	1.33±0.15[ab]
	HS	0.01±0.00	0.14±0.03	0.08±0.01	0.17±0.03	0.42±0.06	0.80±0.12	0.48±0.07	0.03±0.00	1.22±0.16[ab]	1.40±0.14[ab]
	HSS	0.01±0.00	0.14±0.03	0.07±0.01	0.13±0.03	0.34±0.06	0.80±0.12	0.36±0.07	0.02±0.00	1.75±0.16[b]	1.66±0.14[b]
	P-value	0.23	0.74	0.41	0.33	0.26	0.46	0.14	0.25	0.01	0.03
Sex	Male	0.01±0.00	0.12±0.02	0.07±0.01	0.15±0.03	0.36±0.05	0.78±0.09	0.43±0.05	0.02±0.00	1.31±0.13	1.47±0.11
	Female	0.01±0.00	0.12±0.02	0.06±0.01	0.16±0.02	0.36±0.04	0.65±0.08	0.38±0.05	0.03±0.00	1.32±0.12	1.31±0.10
	P-value	0.42	0.81	0.42	0.18	0.92	0.30	0.42	0.10	0.94	0.24
Exposure[5]	Chronic	0.01±0.00	0.07±0.02[a]	0.05±0.01[a]	0.14±0.02	0.28±0.04[a]	0.62±0.08	0.34±0.05[a]	0.02±0.00	1.29±0.11	1.26±0.10[a]
	Acute	0.01±0.00	0.17±0.02[b]	0.08±0.01[b]	0.17±0.03	0.45±0.04[b]	0.81±0.09	0.47±0.05[b]	0.02±0.00	1.35±0.12	1.52±0.10[b]
	P-value	0.08	0.02	0.04	0.14	0.00	0.15	0.03	0.13	0.69	0.03

[a-b] Superscripts indicate significant differences at P≤0.05.

[1] Transformation of log(x) was used in the analysis but presented mean ± SE are presented in their original scale.

[2] Dihydrotestosterone, DHT; Dehydroepiandrosterone, DHEA; Dehydroepiandrosterone Sulfate, DHEA-S.

[3] Although DHEA can be classified as an androgen, it is also a precursor to estrogens.

[4] Detailed experimental design is delineated in **Chapter 2: Changes in Japanese Quail (*Coturnix coturnix japonica*) blood gases and electrolytes in response to multigenerational heat stress**. Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational mating at 22.2˚C and 31.1˚C, respectively. TNS and HSS were obtained by mating males and females from TNS and dividing their offspring evenly into chambers at 22.2˚C (TNS) and 31.1˚C (HSS). Only families from TNS that had high fitness in HSS were mated.

[5] Acute, exposure to respective temperature for 4 hours; chronic, exposure to respective temperatures for 3 weeks.

Conclusions and Future Directions

1. Overall conclusions

Many investigators have researched the effects of heat stress at 32˚C and above; however, little research on avian species has been conducted on heat stress at temperatures between 27 to 31.9˚C. The heat stress temperature (31.1˚C) used in this study were immediately outside the outer limit of the thermoneutral zone (TNZ) of Japanese quail (JQ; *Coturnix coturnix japonica*). For both domesticated and wild avian species, heat stress at this period may be changing metabolic pathways before it is detectable through larger scale measurements such as feed intake or body weight. Due to the importance of lipids for cell membrane integrity, cell signaling, and energy, it was important to determine early possible changes occurring for lipids as nutrients/biochemicals as well as changes in other metabolites of interest.

Through this current project, the measurements that may have detected early signs of heat stress were in PCO_2, PO_2, sO_2, Na, glucocorticoids, progestogens, and androgens in serum and long chain PUFA such as ARA, total SFA, total MUFA, and % TPA of TCA cycle proteins in tissue. These measurements had significant differences between HS and/or HSS and TN. However, other measurements such as: tissue lipid oxidation and antioxidant activity produced no significant differences between HS and/or HSS and TN which may indicate that they are not useful for early detection of heat stress. For others working in this field, it would be valuable to utilize these significant measurements to determine early signs of heat stress.

Another notable result from the current study was that quail selected for low FCR at 31.1˚C (HSS) showed highly significant protein expression differences in adult female livers when compared to those that were not selected for any trait at 22.2˚C (TN). This finding demonstrates the necessity of molecular analyses such as gene expression or protein expression for determination of differences among treatments.

More detailed conclusions for each chapter are presented below:

Chapter 2: Changes in JQ blood gases and electrolytes in response to multigenerational heat stress

1. Acute and chronic heat stress at 31.1˚C does not have a clear effect on blood electrolytes, acid-base regulation, and oxygen transport.
2. Across treatments, sexes, lengths of exposure, and their interactions, acute HSS males or females were significantly different than chronic TN males as follows: lower in body weight, PO_2, sO_2, and Na^+ and higher in PCO_2.
3. Chronic HS males and females did not have significantly different blood electrolytes, acid-base regulation, and oxygen transport than chronic HSS males and females. Thus, selection for low FCR in heat stress at 31.1˚C does not incur a fitness advantage when considering these parameters.
4. Sexually mature males had significantly higher levels of hematocrit and hemoglobin than sexually immature quail males and females and sexually mature females.

Chapter 3: Fatty acid composition of multigenerational heat stressed adult JQ

1. Overall, of all organs analyzed, livers experienced the most variations in concentrations of fatty acids when compared by treatment and sex as expected because it is the source of desaturation and elongation of fatty acids in avian species.
2. Most significant differences observed in the adult livers were also reflected in the adult thighs because the fatty acid composition of skeletal muscle is directly related to that in the diet and liver.
3. Males had significantly more stearic acid in brains, kidneys, and thighs and significantly more PA in all tissues than females.

4. TN brains had significantly less PUFA than that for both TNS and HSS, possibly indicating that selection for lower FCR in heat stressed environments reduced oxidation of PUFA or increased retention of PUFA in the brain.
5. TN or TNS had significantly more long chain PUFA such as DPA n-6, DPA n-3, DHA, and ARA and significantly less SFA such as PA or stearic acid than HS or HSS across all tissues.

Chapter 4: Fatty acid composition of multigenerational heat stressed embryo JQ

1. In the yolk:
    a. HSS had significantly higher levels of LA and ARA than TN, indicating that HSS may have experienced more inflammation from eicosanoids derived from ARA.
    b. TNS had significantly more LA, ALA, DPA n-3, and total n-3 than TN and significantly more stearic acid and SFA than HSS indicating there may have been less susceptibility to oxidation of beneficial n-3 fatty acids in TNS.
2. In the embryo brain:
    a. DHA, total PUFA, and stearic acid increased over the duration of incubation as expected for proper brain development.
    b. PA decreased as incubation progressed, most likely due to its conversion to stearic acid.
    c. TN had significantly lower levels of MUFA than all other treatments.
3. In the embryo kidney:
    a. As was seen in the brain, PA decreased as incubation progressed.
    b. N-6:n-3 increased as incubation progressed.
    c. HS kidneys had more total SFA than TNS and HSS.
4. In the embryo liver:

a. PA, DPA n-6, DHA, and total SFA decreased, whereas LA increased as incubation progressed possibly due to the allocation of these fatty acids to other organs such as the brain.
b. HS had significantly more stearic acid, SFA:PUFA, and total SFA than TN and TNS, possibly as a result of less PUFA availability from the female layer.
c. TN and TNS had significantly more PUFA than HSS.

Chapter 5: **Lipid oxidation and antioxidant capacity in multigenerational heat stressed JQ**

1. Quail eggs stored at 4˚C for 13.35 weeks did not have significantly different MDA levels than those that were analyzed within 24 hours after lay, indicating that long-term storage of eggs did not significantly increase lipid oxidation.
2. CAT activity was highest in kidneys, followed by liver, then thigh and brain.
3. MDA concentration was as follows: brain>liver>kidney>thigh.

Chapter 6: Proteomics of adult female JQ livers subjected to multigenerational heat stress

1. Quail in a thermoneutral temperature had significantly less % TPA of antioxidants (SOD Cu/Zn and CAT), lipoprotein transport from liver to egg, and HSP4 than those in a heat stress temperature. However, TN had significantly more % TPA involved in adipogenesis and lipogenesis than HSS.
2. Chronic heat stress in both HS and HSS decreased % TPA involved in TCA cycle activity. TNS and/or TN had significantly more β-oxidation enzymes than HS and/or HSS. This downregulation of proteins involved in β-oxidation and the TCA cycle in HS and HSS could have been an effort to decrease energy production and prevent further oxidative damage.
3. Differences in protein abundance between HSS and HS, TNS and HS, and TNS and HSS were minimal or insignificant.

Chapter 7: Steroid hormones of multigenerational heat stressed JQ

1. Chronic males, particularly from TN, had significantly more glucocorticoids, progestogens, androgens than many of the other treatments, especially HSS. Thus, selective breeding over 10 generations for higher performance at 31.1˚C does not seem to change the quail's susceptibility to stress.
2. Of the 29 steroid hormones analyzed, 13 were significantly higher in chronically stressed than acutely stressed quail. As expected, this was also associated with hormonal shift in concentration when quail undergo sexual maturity.

2. Pitfalls

Prior inbreeding was a pitfall of this study (as it would be for any commercial population) because the original quail population were from a stock population of 80 male and 80 females. Therefore, statistically significant or insignificant treatment effects may be confounded with the relatedness of the quail.

Another pitfall was that there may have been uneven sample sizes due to infertile eggs for the embryo sampling. The embryonic kidney samples were very small; therefore, pooling samples was necessary and decreased the number of replicates.

3. Future directions

To further the current research, analyses should be conducted to detect genetic modifications to heat stress as it pertains to energy and lipid metabolism. As well, knowledge may be gained from determining the fatty acid composition of triacylglyceride, phospholipid, and cholesteryl ester separately. Once changes, if any, are understood, dietary lipid recommendations or feed additives can be modified to ensure optimal success of poultry species transitioning to a heat stress environment. Further explorations of the relationship between the liver and differences

in fatty acid partitioning within various avian tissues under heat stress should be conducted. In line with fatty acid partitioning in adult tissues is the analysis of the fatty acid composition enzymes involved in lipid metabolism in the yolk sac and yolk sac membrane in embryos from heat stressed dams.

An important question that is yet to be answered is: At which temperature does PUFA experience enough degradation before reaching the embryo that deficiencies in embryonic fatty acid composition are detrimental to fetal development? More experimentation on this topic would also address the question of whether lipid deposition into eggs is so tightly regulated that embryos of heat stressed quail do not have differing fatty acids than those that are not heat stressed. To examine this question, different treatments with incremental increases in temperature for JQ should be administered, followed by egg collection, and fatty acid analysis of non-incubated egg yolks.

Future research should also focus on blood analysis in Japanese quail selected for low FCR in a thermoneutral temperature (22˚C), mild heat stress temperature (30-31˚C), and high heat stress temperature (≥33˚C). Even though permission to conduct research at temperatures ≥33˚C is often difficult to obtain from institutional animal care and use committees, permitted research could inform producers when to expect physiological changes in their quail and how to adapt their feed to meet the animals' needs at different temperatures. As well outcomes of such research is important for human health where commercial quail, other commercial avian species, and backyard avian species are raised in increasingly higher temperatures.

For antioxidant and lipid oxidation, future directions could include heat stress at a higher temperature to determine if total antioxidant capacity is lower and lipid oxidation is higher in eggs laid in the first week than all subsequent weeks of lay. Findings could help producers adjust their feeding programs to reflect the demands of the females during critical times such as onset of lay.

Future studies should further investigate steroid hormones at temperatures immediately outside the thermoneutral zone of quail and other poultry species. Although it is important to understand hormonal response to more extreme temperature differences, it is also of value to understand what occurs as the animal is transitioning to more extreme temperatures.

Research in the present study was focused on domesticated birds. However, it is obvious that results are meaningful for wild birds as well, especially because mitigation of heat effects will become more challenging as global temperatures continue to rise.