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

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ABSTRACT In some areas of the world, climate-controlled poultry houses are not possible; thus, likely resulting in lower production measurements and poorer quality poultry products due to lipid oxidation during heat stress. 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. Endogenous antioxidant enzymes such as superoxide dismutase (**SOD**) and catalase (**CAT**) can prevent further oxidation. The goal of this study was to determine if 10 generations of selection for low feed conversion ratio (**FCR**) in Japanese quail at 31.1°C resulted in lower lipid oxidation and more antioxidant activity. The experimental design for adult tissues was 4 treatments × 2 sexes × 4 tissue types and for egg yolks was 4 treatments × 3 wk of lay with varying storage conditions. Lipid oxidation was determined in brain, liver, kidney, thigh, and yolk. SOD and CAT activities were determined in brain, liver, kidney, and thigh. ANOVA indicated significance at $P \leq 0.05$. Results suggested that heat stress at 31.1°C and 10 generations of selection for low FCR did not significantly affect lipid oxidation and antioxidant enzyme activities across all tissues. Tissue differences occurred in lipid oxidation and antioxidant enzyme activity. Brain had the most oxidation, followed by liver > kidney > thigh ($P < 0.0004$). Kidneys had significantly more CAT activity than brain, liver, and thigh. Brain and thigh had similar CAT activities. Thus, poultry products from quail raised at this temperature may have similar quality to those that are raised within their thermoneutral zone (18 to 30°C). Future directions could include comparisons within the thermoneutral zone and incrementally higher temperatures to 1) to pinpoint the temperature when biochemical measurements in tissues associated with lipid oxidation begin to occur, 2) determine when total antioxidant capacity and lipid oxidation are significantly higher, and 3) ascertain SOD and CAT activity in day-of-lay yolks of eggs for future production to properly administer heat stress mitigation strategies.

Key words: lipid oxidation, antioxidant, multigenerational, heat stress, quail

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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 of lipids in the plasma, liver, and heart (Hosseini-Vashan and Raei-Moghadam, 2019). Specifically, Sahin, et al. (2004) noted that heat stress increased energy demands and corticosterone levels leading to higher levels of triacylglyceride (**TAG**) and nonesterified fatty acids (**NEFA**) in the plasma. TAG and NEFA contribute to energy production and can change membrane structure and fluidity which affects membrane potential during heat stress (Slimen et al., 2016). Heat stressed (32−34°C) Japanese quail have lower levels of antioxidants and higher levels of malondialdehyde (**MDA**), an indicator of lipid oxidation, than those housed in thermoneutral temperatures (Sahin et al., 2004; Orhan et al., 2020).

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 free radicals when an animal is experiencing heat stress. This is especially true for breeding avian females that experience more oxidative stress than nonbreeding females (Costantini et al., 2014). The allocation of antioxidants towards the yolk could be at the expense of her own need for antioxidants; thus, increasing the negative effects of oxidative damage in breeding females (Giordano et al., 2015).

During embryogenesis, yolk antioxidants are differentially transferred and partitioned among the tissues.

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Some researchers found that the embryonic liver has the highest concentration of antioxidants, and the brain has the highest concentration of MDA, followed by the liver, then the heart (Tsunekage and Ricklefs, 2015).

Superoxide dismutase (**SOD**) and catalase (**CAT**) are important endogenous antioxidants that are highly susceptible to oxidation. CAT and SOD have been found in the liver, heart, lung, yolk sac membrane, thigh muscle, kidneys, and brain of embryos (Weydert and Cullen, 2010; Surai et al., 2016). SOD converts superoxide radicals to hydrogen peroxide and oxygen. However, hydrogen peroxide can also cause oxidative damage; thus, CAT converts it to water and oxygen. Chicks subjected to heat stress (37°C for 8 h/d) had decreased 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, and liver (Surai, et al., 1999, 2016). It seems essential to compare the effect of heat stress on tissues with different metabolic pathways to determine where heat stress causes the most damage, in the form of lipid oxidation; thus, possibly leading to lower production.

Previous research revealed that multiple generations of stress incrementally altered response to stress over time (Ambeskovic et al., 2019). 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 in the future (Costantini et al., 2012). Similarly, when male fruit flies were exposed to short term anoxia before emergence and at sexual maturity, they had higher concentrations of the endogenous antioxidant, SOD and lower lipid and protein damage (López-Martínez and Hahn, 2012; Laviola and Macrì, 2013). Thus, heat stress priming could be a viable strategy for prevention of heat stress during high production periods of poultry.

It is important to study heat stress from low temperatures just outside of the thermoneutral zone to a wider range to understand effects on lipid oxidation and endogenous antioxidants. For instance, in Nigeria, temperatures can range from 30.2°C to 32.1°C and quail are exposed to environmental conditions with no artificial simulation (Egbuniwe et al., 2021). However, most research on heat stress in poultry has been conducted at 32°C to 40°C (Sahin et al., 2004; Ramnath et al., 2008; Hosseini-Vashan and Raei-Moghadam, 2019; Orhan et al., 2020). Previous research showed that multigenerational exposure to cyclic heat stress at 31.1°C for 8 h/d did not significantly affect performance; however, there were blood gas differences when sex and length of exposure were considered (Truong et al., 2023).

In addition to studies with high temperatures, little research has been performed on multigenerational heat stress and antioxidant capacity. Using Japanese quail (*Coturnix coturnix japonica*) as a model, the objective of the current study was to discern if selection for 1) high performance as low feed conversion ratio (**FCR**) and 2) heat stress at 31.1℃, extremely close to the thermal neutral zone, 18°C to 30°C, affected antioxidant capacity and extent of lipid oxidation. 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.

MATERIALS AND METHODS

Experimental Design

As noted in Truong et al., 2023, animal care and use were approved by the Institutional Animal Care and Use Committee at the University of California Davis (Protocol #22728; 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 wk of age, when. sexual dimorphisms were apparent and 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 FCR in HSS (Equation 1).

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

Although, FCR was similar within the group after 10 generations, the variation of FCR decreased over each generation of selection. To have a criterion for selection for high fitness, the upper one- half of the numeric range of similar FCR for each generation of HSS was classified as having low FCR. The ratios were only compared to other families in HSS and within their respective generation. After determining FCR for 1 wk, a range of FCR was measured within treatment and sex. The lower $^1/_2$ of males and females were designated as having low FCR and the higher $^1/_2$ was designated 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. Nonsibling 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). Overall, generation 10 had 23 unique mating pairs for TN, 24 unique mating pairs for HS, and 25 unique mating pairs for TNS and HSS. These mating pairs produced total offspring as 176 TN, 194 HS, and 254 TNS and HSS.

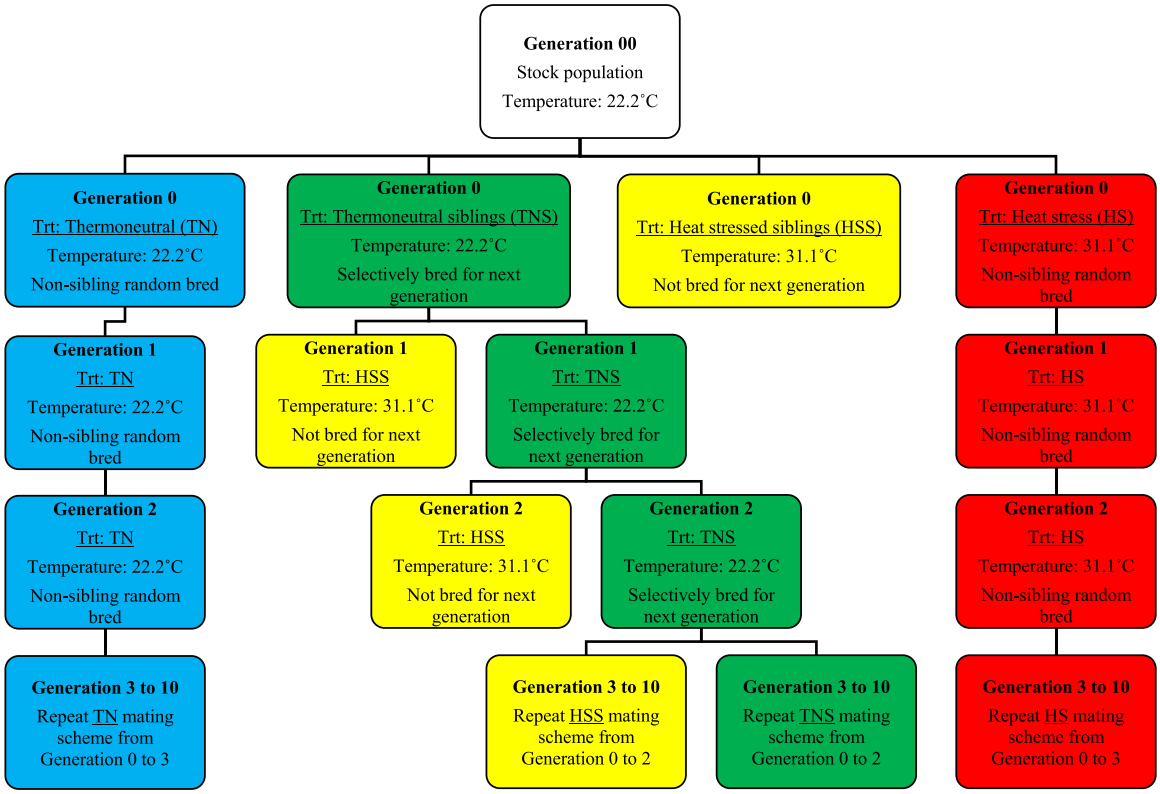

Figure 1. Schematic representation of treatments for 10 generations of Japanese quail.

The first day of heat exposure occurred 3 d 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 h), were maintained at 31.1°C between 1100h to 1630h (5.5 h), decreased from 31.1°C to 22.2°C between 1630h to 2200h (5.5 h), and were maintained at 22.2°C from 2200h to 0630h (8.5 h). The relative humidity remained constant at 50%. The chamber had at least 15 air exchanges/h with temperature 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 wk of age. A laying hen pellet (Purina Layena Pellets, Purina Animal Nutrition, Arden Hills, MN) was fed from 6 to 17 wk of age.

Sample Collection

All eggs were collected within 24 h after lay. Three groups of eggs with varying weeks of lay and storage conditions were analyzed. Group 1 eggs were from 7.86- to 8.86-wk-old females in their first week of lay and analyzed after storage for 13.86 wk at 4°C. Group 2 eggs were from 8.86- to 9.86-wk-old females in their second week of lay and analyzed after storage for 12.86 wk at 4°C. Group 3 eggs were from 16- to 17-wk-old females in their ninth week of lay and analyzed immediately after collection without storage at 4°C.

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.

Lipid Oxidation

A modified thiobarbituric acid reactive substances (**TBARS**) method was used to measure 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 min, followed by adding 1.0 mL of butanol, mixing, and centrifuging at 1,200 $\times$ g for 10 min. 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, VT).

A standard calibration curve was developed using 1 mM 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 are reported as absorbance (nm). Sample sizes for lipid oxidation analyses are presented in Table 1.

Table 1. Sample sizes of different tissues for lipid oxidation analysis.

Trt[1]	Egg yolk after 13.36 weeks in 4°C storage[2]	Egg yolk within 24 h after lay[3]	Thighs[4]	Brain	Kidneys	Livers
	N =	N =	N =	N =	N =	N =
TN[1]	100	30	8M, 4F[5]	15M, 15F	15M, 15F	15M, 15F
TNS[1]	97	30	6M, 5F	15M, 12F	15M, 12F	15M, 12F
HS[1]	99	30	8M, 6F	15M, 15F	15M, 15F	15M, 15F
HSS[1]	98	30	8M, 11F	15M, 15F	15M, 15F	15M, 15F

[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]Eggs were collected from 20 TN females, 14 TNS females, 20 HS females, and 17 HSS females.
[3]Eggs were collected from 18 TN females, 10 TNS females, 18 HS females, and 16 HSS females.
[4]Both thighs were homogenized for analysis.
[5]Males, M; females, F.

SOD and CAT Activity

Catalase activity (BioVision, Catalog #K773-100) and SOD activity (BioVision, Catalog #K335-100) were analyzed using commercially available kits and in accordance with the instructions of the manufacturer. For CAT, 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 SOD, activity, quantities of homogenate were 10 μL of 1:2 thigh, brain, and liver and 5 μL of 1:2 for kidney. Sample sizes for analyses of CAT and SOD activities are presented in Table 2.

Statistical Analyses

Analyses of data were performed in R 4.0.0 (R Core Team, 2020, 2022) to test significance ($P \leq 0.05$). Lipid oxidation of egg yolks, adult tissues, and SOD and CAT activities were analyzed using one-way repeated measures ANOVA. The egg yolk model included treatment, week of lay, and their interaction as the fixed effects and females as the random effect. The adult tissues model included treatments, tissue types, sex, and their interactions as the fixed effects and individual bird as the random effect. The SOD and CAT activities models included treatments, tissue types, and their interactions as the fixed effects and the individual bird as the random effect. Sex was not determined as a significant effect and was not included in the SOD activity and CAT activity models. For all models, cage was the experimental unit. 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 $P \geq 0.05$. Tukey's method for comparing estimates was used if the interaction was significant. All values are presented as means ± standard error (**SE**).

Table 2. Sample sizes for superoxide dismutase and catalase analysis.

Trt[1]	Thighs[2]	Brain	Kidneys	Livers
	N =	N =	N =	N =
TN[1]	7M, 5F[3]	8M, 8F	8M, 8F	8M, 8F
TNS[1]	6M, 5F	8M, 8F	8M, 8F	8M, 8F
HS[1]	8M, 7F	8M, 8F	8M, 8F	8M, 8F
HSS[1]	9M, 10F	8M, 8F	8M, 8F	8M, 8F

[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]Both thighs were homogenized for analysis.
[3]Males, M; females, F.

RESULTS

Production Measurements

As delineated in Truong et al., 2023, there was no significant treatment effect on body weight; however, HS males had significantly lower FCR than females and HSS males had significantly higher FCR than females.

Lipid Oxidation of Egg Yolks

When egg yolks were compared by treatment × week of lay (Group, with varying storage conditions; Figure 2), Group 1 HS (first week of lay, storage for 13.86 wk at 4°C) had significantly more lipid oxidation than all weeks of TN and TNS and Groups 2 and 3 HS and HSS (second week of lay, storage for 12.86 wk at 4°C and ninth week of lay, no storage, respectively; $P < 0.008$). Group 2 HS had significantly less lipid oxidation than Group 1 and 2 TN and Group 1 HSS ($P < 0.01$). Group 1 HSS had significantly more lipid oxidation than Group 1 and 3 TNS ($P < 0.001$; Figure 2). Group 3 TNS also had significantly less lipid oxidation than Group 1 TN ($P = 0.30$). When Groups were compared without the treatment effect, yolks from Group 1 had significantly more lipid oxidation than Groups 2 and 3 yolks ($P < 0.001$; Table 3). When treatments were compared without Group effect, TNS had significantly less lipid oxidation than all other treatments ($P < 0.03$; Table 3).

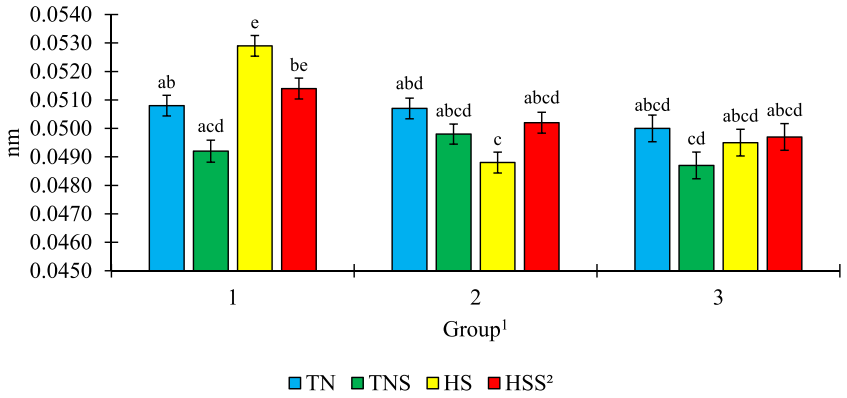

Figure 2. Lipid oxidation (absorbance at 532 nm) of egg yolks isolated after various weeks of lay and storage conditions. [a-e]Superscripts indicate significant differences at $P \leq 0.05$. Values were compared by treatment ×week. [1] All eggs were collected within 24 h after lay. Three groups of eggs with varying weeks of lay and storage conditions were analyzed. Group 1 eggs were from 7.86- to 8.86-wk-old females in their first week of lay and analyzed after storage for 13.86 wk at 4°C. Group 2 eggs were from 8.86- to 9.86-wk-old females in their second week of lay and analyzed after storage for 12.86 wk at 4°C. Group 3 eggs were from 16- to 17-wk-old females in their ninth week of lay and analyzed immediately after collection without storage at 4°C. [2] 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.

Lipid Oxidation of Adult Tissues

The interaction between treatment, tissue, and sex were significant at $P < 0.001$ (Table 4). 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.

Table 3. Lipid oxidation of egg yolks isolated after various weeks of lay and storage conditions compared by week and treatment separately.

Fixed effects	Egg storage groups and treatments	nm[1]
Groups[2]	1	0.051 ± 0.000^{b}
	2	0.050 ± 0.000^{a}
	3	0.050 ± 0.000^{a}
Treatment[3]	TN	0.051 ± 0.000^{a}
	TNS	0.050 ± 0.000^{b}
	HS	0.051 ± 0.000^{a}
	HSS	0.051 ± 0.000^{a}

[a-b]Superscripts indicate significant differences at $P \leq 0.05$. Values were compared by week and treatment separately.

[1]Absorbance was measured at 532 nm.

[2]All eggs were collected within 24 h after lay. Three groups of eggs with varying weeks of lay and storage conditions were analyzed. Group 1 eggs were from 7.86- to 8.86-wk-old females in their first week of lay and analyzed after storage for 13.86 wk at 4°C. Group 2 eggs were from 8.86- to 9.86-wk-old females in their second week of lay and analyzed after storage for 12.86 wk at 4°C. Group 3 eggs were from 16- to 17-wk-old females in their ninth week of lay and analyzed immediately after collection without storage at 4°C.

[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.

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 4). 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

Table 4. Lipid oxidation[1] measured in MDA[2] (nmol/mg wet weight) of adult brain, kidney, liver, and thighs compared on treatment × tissue × sex.

Tissues	Sex[3]	TN[4]	TNS[4]	HS[4]	HSS[4]
Brain	M	8.84 ± 0.26^{abcd}	9.31 ± 0.26^{cd}	10.01 ± 0.26^{d}	9.60 ± 0.26^{cd}
	F	9.87 ± 0.26^{d}	9.18 ± 0.29^{bcd}	9.83 ± 0.26^{d}	9.08 ± 0.26^{bcd}
Kidney	M	6.89 ± 0.26^{efg}	6.52 ± 0.26^{efgh}	6.43 ± 0.26^{efgh}	5.99 ± 0.26^{efh}
	F	5.91 ± 0.26^{eh}	6.58 ± 0.29^{efgh}	5.77 ± 0.26^{eh}	6.11 ± 0.26^{efh}
Liver	M	7.32 ± 0.26^{fgi}	8.32 ± 0.26^{abcj}	6.06 ± 0.26^{efh}	7.81 ± 0.26^{abgi}
	F	6.89 ± 0.26^{efg}	6.31 ± 0.29^{efh}	5.86 ± 0.26^{eh}	6.31 ± 0.26^{efh}
Thigh	M	5.02 ± 0.35^{hj}	7.18 ± 0.41^{aefgi}	5.03 ± 0.35^{hj}	6.86 ± 0.35^{efghi}
	F	5.21 ± 0.50^{efhj}	5.80 ± 0.45^{efhj}	5.30 ± 0.41^{ehj}	3.83 ± 0.30^{j}

[a-j]Superscripts indicate significant differences at $P \leq 0.05$. Values were compared on treatment × tissue × sex.
[1]Absorbance was measured at 532 nm.
[2]Malondialdehyde, MDA (nmol/mg wet weight). Results are presented as mean ± SE.
[3]Males, M; females, F.
[4]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.

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 ($P < 0.001$). TNS male thighs had significantly higher concentrations of MDA than TN and HS male thighs ($P < 0.04$).

Treatment × Tissue When sex was removed from the model, the interaction between treatment and tissue was significant at $P < 0.001$ (Table 5). Brain from all treatments had significantly higher concentrations of MDA than all other tissues ($P < 0.001$). 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 >k idney > thigh at $P < 0.004$ (Table 6). When MDA concentration was compared by sex alone, females had significantly less than males ($P < 0.001$).

Table 6. Lipid oxidation[1] measured in MDA[2] (nmol/mg wet weight) of adult brain, kidney, liver, and thighs compared on tissue and sex, separately.

Tissue	
Brain	9.47 ± 0.11^{a}
Kidney	6.27 ± 0.11^{b}
Liver	6.88 ± 0.11^{c}
Thigh	5.40 ± 0.18^{d}
Sex	
M[3]	7.50 ± 0.13^{a}
F[3]	6.97 ± 0.13^{b}

[a-d]Superscripts indicate significant differences at $P \leq 0.05$. Values were compared on tissue and sex, separately.
[1]Absorbance was measured at 532 nm.
[2]Malondialdehyde, MDA (nmol/mg wet weight). Results are presented as mean ± SE.
[3]Males, M; females, F.

Table 5. Lipid oxidation[1] measured in MDA[2] (nmol/mg wet weight) of adult brain, kidney, liver, and thighs compared on treatment[3] × tissue.

Tissues	TN[3]	TNS[3]	HS[3]	HSS[3]
Brain	9.36 ± 0.20^{a}	9.25 ± 0.22^{a}	9.92 ± 0.20^{a}	9.34 ± 0.20^{a}
Kidney	6.40 ± 0.20^{bcde}	6.54 ± 0.22^{cde}	6.10 ± 0.20^{bcdf}	6.06 ± 0.20^{bcf}
Liver	7.11 ± 0.20^{e}	7.43 ± 0.22^{e}	5.96 ± 0.20^{bcf}	7.06 ± 0.20^{de}
Thigh	5.08 ± 0.32^{bf}	6.56 ± 0.34^{bcdef}	5.15 ± 0.30^{bf}	5.11 ± 0.26^{f}

[a-f]Superscripts indicate significant differences at $P \leq 0.05$. Values were compared on treatment ×tissue.
[1]Absorbance was measured at 532 nm.
[2]Malondialdehyde, MDA (nmol/mg wet weight). Results are presented as mean ± SE.
[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.

Table 7. 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-b]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]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.

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 7). However, when SOD activity was compared by treatment, tissue and their interaction, tissue had a significant effect at $P < 0.001$. 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 ($P < 0.001$; Figure 3). Across all treatments, brains and thighs had significantly less CAT activity than kidneys and livers ($P < 0.001$). HS kidneys had significantly more CAT activity than livers from all treatments and HSS kidneys ($P < 0.001$). 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 ($P < 0.001$). 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 ($P < 0.001$).

DISCUSSION

Lipid Oxidation of Egg Yolks

The significant effects observed in egg yolks stored at 4°C were likely due to the extended storage of the eggs. Jones and Musgrove (2005) noted that extending egg storage 30 d past their shelf-life or sell-by date would

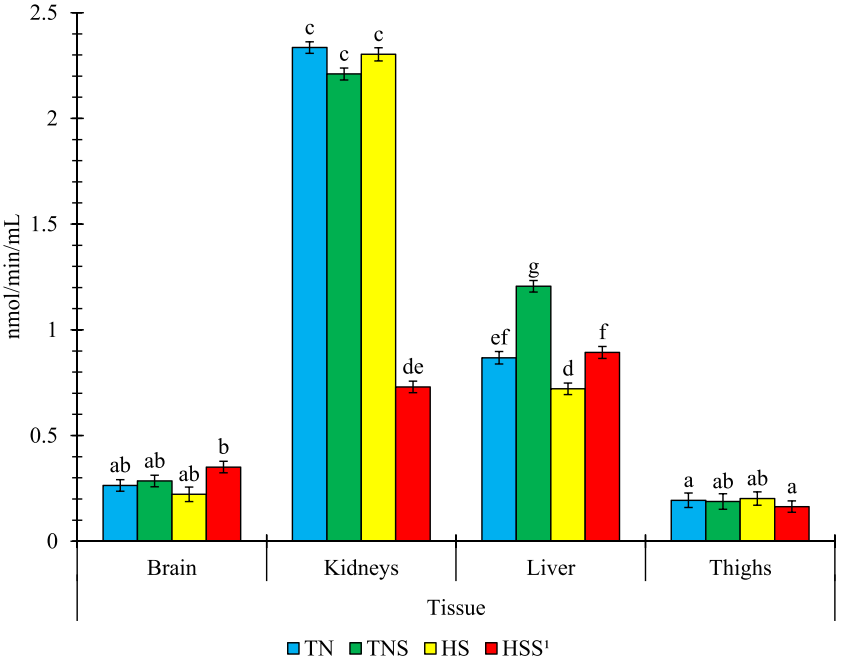

Figure 3. Catalase activity (nmol/min/mL) is presented in adult brain, kidney, liver, and thighs. [a-f]Superscripts indicate significant differences at $P \leq 0.05$. Values were compared on treatment ×tissue. [1] Four treatments were: 1) thermoneutral controls (22.2°C, TN), 2) thermoneutral siblings (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.

not compromise their physical-chemical quality, they noted that Haugh units and albumen height decreased after 10 wk of cold storage. Additionally, in another study, Jones et al. (2018), demonstrated that the quality of eggs stored at 4°C for 15 wk 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 wk 15 of storage as there were no significant indications of lipid oxidation between egg yolks analyzed at 24 h after lay and analyzed after 13.86 wk of storage.

Contrarily, Group 1 yolks had significantly more lipid oxidation than those in Groups 2 and 3, 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 d 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 the first week of lay.

Compared to HSS, TNS had significantly higher saturated fatty acids in the yolk than HSS, likely contributing to significantly less lipid oxidation for TNS yolk in the current study (L. Truong, unpublished data). 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 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.

Lipid Oxidation of Adult Tissues

Precursors of MDA and TBARS are derivatives of peroxyl radicals from polyunsaturated fatty acids (**PUFA**) with 3 or more double bonds (Frankel, 2005). Particularly, under acidic conditions as in the TBARS analysis, peroxyl radicals of α-linolenic acid are the most significant precursors 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). The brain contains a high concentration of PUFA, which could explain the high concentration of MDA found in the brain (Tsunekage and Ricklefs, 2015). The quail adult brain is comprised of approximately 11 to 14% docosahexaenoic acid, a long chain PUFA, whereas the quail kidney contains ~2%, the thigh contains ~1 to 2%, and the liver contains ~1 to 4% (L. Truong, unpublished data). Thus, 14% docosahexaenoic acid 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 of quail 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 adenosine triphosphate 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).

Although not significantly different, 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). More studies are needed to establish how 10 generations of selection for high FCR at 31.1°C affects male hormone production compared to females.

Others have reported that acutely heat stressed broilers (40°C for 5 h) 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.

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, CAT 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 h/d there was a significant increase in MDA in the serum, muscle, and liver; however, there was a significant decrease in SOD, CAT, and glutathione peroxidase (**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 h/d 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 (Azad et al., 2010), SOD activity in the current study also did not significantly change with heat stress at 31.1°C. Investigators reported that acute heat stress at 40°C caused an increase in SOD but did not affect other antioxidant enzymes like glutathione peroxidase (Ramnath et al., 2008). Broilers that were exposed to heat stress at 38°C for 3 h 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.

Future directions could include incremental heat stress from 30°C 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. The current study focused on treatment effect for tissues and sex; however, future studies should also determine antioxidant activities including CAT and SOD in day-old yolks of egg intended for future quail production at 31.1°C. 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. That heat stress at 31.1°C and 10 generations of selection for low FCR at 31.1 °C did not significantly affect lipid oxidation and antioxidant enzyme activities.
2. Quail eggs laid during the first week of lay (stored at 4°C for 13.86 wk) had significantly higher indications of lipid oxidation than those that were laid during the second week of lay (stored at 4°C for 12.86 wk) and during the ninth week of lay (no storage time; Table 3).
3. CAT activity was highest in kidneys, followed by liver, then thigh and brain.
4. MDA concentration was as follows: brain > liver > kidney > thigh.
5. Overall, it is extremely important to conduct studies on incremental heat stress from 30 to 35°C to accurately determine the temperature at which production and biochemical changes such as lipid oxidation begin to occur in quail and other domestic avian species.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the present study.

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