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

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Abstract

The average surface temperature is predicted to rise 0.5 to 6˚C by the year 2100. When Japanese quail (*Coturnix coturnix japonica*), a source of protein for many, are subjected to heat stress, their blood acid-base equilibrium and ability to regulate electrolytes 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, the following treatments were applied (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, siblings of TNS with high feed conversion ratios (FCR), 31.1˚C). Body weights (BW), blood gases, and electrolytes of quail were measured during the first 4 hours (acute) and after 3 weeks (chronic) of heat exposure (31.1˚C) in generation 10. ANOVA was used to determine statistical significance at $P \leq 0.05$. Models included treatments, length of exposure, sex, and their interactions. Results showed that acute and chronic heat stress did not have a clear effect on blood electrolytes, acid-base regulation, and oxygen transport. However, 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 compared to sexually immature quail and sexually mature females. Future studies using higher temperatures (32 to 34˚C) could inform producers when to expect significant physiological changes in quail, lending to adaptions of feeding regiments according to environmental temperature and age.

Introduction

Data from the 1880 to 2018 suggest that the average surface temperature of earth will rise 0.5 to 6˚C by 2100 [1]. This rise in temperature will affect food systems, which are currently

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straining to sustain the human population of 8 billion people [2]. The disparity of food distribution is emphasized in tropical areas such as Ethiopia, South Sudan, and Yemen which reported that 1 in 300 people were in famine-level starvation in 2021 [2]. Additionally, there will be increased pressure on food systems to adapt to extreme weather conditions due to climate change and to the continuing rise in human population as was seen in 8 African countries that reported that 23.5 million people were in a food crisis due to extreme weather [2]. Protein is the most sought-after and expensive nutrient of all nutrient classes due to its importance as the building block of many biological functions. As areas of the world become more economically stable, the demand for protein from animal sources has been shown to increase which emphasizes the importance of meat products such as poultry [3]. In fact, poultry products such as meat and eggs have seen the most increase in demand in economically developing countries [3]. Others have begun researching the benefits of using local agriculture to sustain the food system in areas of the world that are vulnerable to varying weather conditions caused by climate change [4].

The quality and quantity of animal production are sensitive to weather conditions produced by climate change and can be negatively impacted during times of heat stress. In general, animals experience heat stress when internal heat production exceeds the ability of the animal to dissipate heat to the external environment [5]. Due to the significance of poultry as a source of animal protein, it is important to understand changes in concentration of blood gases and electrolyte in response to rising climatic temperatures. These parameters are important for understanding the physiological response to heat stress in quail which can decrease production yield and harm breeding efforts.

Japanese quail (*Coturnix coturnix japonica*) are of particular interest due to their importance as an option for meat and eggs [6,7]. Tropical areas such as Bangladesh and Egypt have several successful quail farms, indicating that quail can adapt to hot and humid weather [6,7]. The thermoneutral zone of Japanese quail is 18 to 30˚C with optimal temperatures between 21 to 27˚C, whereas upper critical temperatures of chicken, turkeys, and ducks are 23.86 to 25.46˚C, 27˚C, and 25.5 to 27.4˚C, respectively [8–12]. Japanese quail also have short generation intervals and can reach sexual maturity at 5 to 6 weeks of age allowing for many generations to be studied in a shorter period of time relative to other poultry species.

When quail and other domesticated avian species are subjected to heat stress, their blood acid-base equilibrium may change as well as their ability to regulate electrolytes such as sodium (Na^+), potassium (K^+), and calcium [13]. Typically, as laying hens from several species experience temperatures outside of their thermoneutral zone, they will perform rapid shallow breathing, also known as gular fluttering. This behavior increases air passage in the upper respiratory tract and can cause blood alkalosis and moderate to severe dehydration [14]. This disequilibrium may influence eggshell quality, enzyme functions, and synthesis of tissue proteins [13]. Eggshell quality is reliant on blood bicarbonate which may decrease due to decreased calcium intake or blood alkalosis [14].

Na^+ and K^+ are the main electrolytes fed to maintain acid/base balance for osmotic pressure [15]. Na^+ accounts for 93% of total cation content in blood plasma [16]. It is used in many physiological processes such as muscle cell contractions, adrenal gland function, and carbohydrate absorption and energy turnover [16]. 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^+ [16]. 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^+ into urine [16]. 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 [17]. 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 [17]. However, once they were supplemented with vitamins and minerals, their feed conversion ratios were significantly higher than control [17]. 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 [17].

Climate change produces a multitude of weather conditions and includes many environmental factors; however, rising temperatures is important for production of poultry in several parts of the world. Thus, the objective of the current study was to determine if there was an effect on concentrations of blood gases and electrolytes in whole blood using Japanese quail as the model animal after 10 generations of cyclic heat stress. It was hypothesized that there will be little 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. It was determined that selection for high performance at 31.1˚C did not incur an overall fitness advantage when considering parameters measured in this study.

Materials and methods

Ethics statement

Animal care and use was approved by the Institutional Animal Care and Use Committee at the University of California Davis (Protocol #22728; Davis, CA).

Experimental design

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 (Eq 1).

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

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

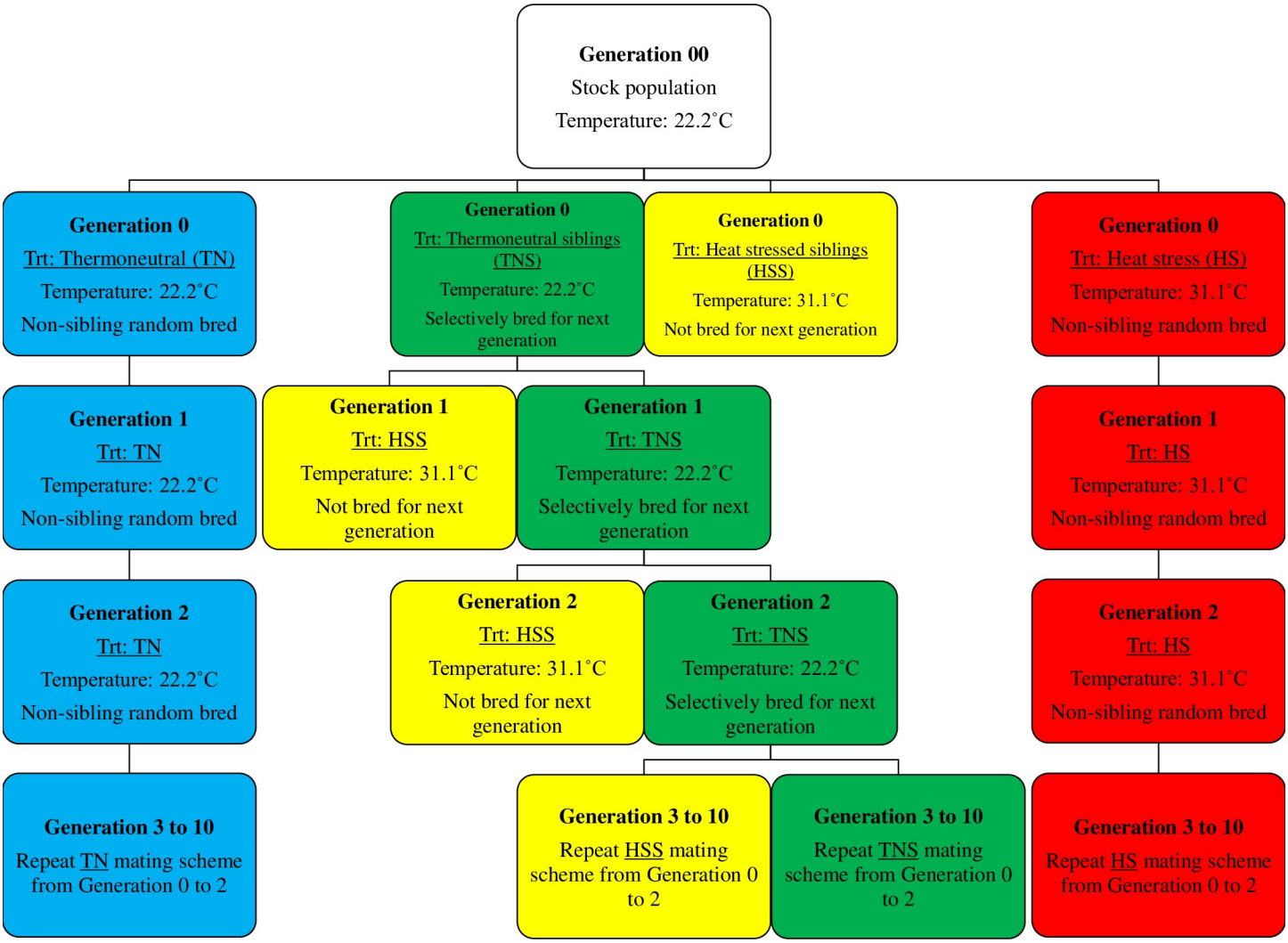

Fig 1. Schematic representation of treatments for 10 generations of Japanese quail.

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(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 (Fig 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 a total of 176 TN offspring, 194 HS offspring, and 254 TNS and HSS offspring.

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 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 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.

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 from 20 unique mating pairs for TN, HS, and HSS and 19 unique mating pairs for TNS. Quail were 69-day-old to 82-day-old at the time of egg collection, eggs were stored at 12.78˚C, incubated together for 15 days, then broken out. If an embryo failed to form, infertility was assumed. Death was defined as an embryo that expired prior to breakout and was counted over 15 days of incubation. Formation of multiple embryos or a physically defected quail was considered twins or deformed, respectively. Percentage of each abnormality for eggs in each treatment was determine.

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 Eq 2. With BW_{d0} as initial body weight and BW_{d7} as body weight after 7 days.

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

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 i-STAT-1 handheld blood analyzer (Abbott Laboratories, San Diego, CA) and CG8+ cartridges from treatments during the first 4 hours (acute) or after 3 weeks (chronic) of heat exposure (31.1˚C). Fresh, whole blood (100 μl) was applied to the i-STAT CG8+ cartridge immediately after drawing due to rapid coagulation. A sample size of 12 males and females per treatment and length of exposure were attempted; however, certain treatments had less than 12 samples due to cartridge malfunction or unsuccessful blood draws leading to prolonged stress and unreliable results (Table 1). The CG8+ cartridges detected electrolytes [Na^+ (mmol/L), K^+ (mmol/L), iCa (mg/dL)], glucose (mg/dL), hematology [(% PCV), hemoglobin (g/dL)], blood gases [pH, PCO_2 (mmHg), PO_2 mmHg), TCO_2 (mmol/L), HCO_3 (mmol/L)], and 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 (partial pressure of CO_2), PO_2 (partial pressure of oxygen), TCO_2 (total carbon dioxide) calculated from pH and PCO_2, HCO_3 (bicarbonate), and BE (calculated from HCO_3 and pH) were measured.

Statistical analysis

Analyses of data were performed in R 4.0.0 [18,19] to test significance ($P \leq 0.05$). Figures were created on Microsoft Excel 16.69 [20]. Birds were individually housed for body weight, FCR, and iSTAT data collection; therefore, birds were considered replicates and subjects. Birds were housed in male-female pairs for egg data collection; therefore, the females were considered replicates and subjects. FCR was analyzed using general linear model with sex, length of exposure, treatment, and their interactions as fixed effects. Egg collection data was analyzed using

Table 1. Sample size of individually housed birds for treatment x length of exposure x sex for body weight and all i-STAT[1] measurements.

Treatment[2]	Length of exposure[3]	Sex	N =
TN	Acute	Male	12
		Female	11
	Chronic	Male	12
		Female	12
TNS	Acute	Male	8
		Female	12
	Chronic	Male	10
		Female	11
HS	Acute	Male	9
		Female	10
	Chronic	Male	11
		Female	12
HSS	Acute	Male	11
		Female	11
	Chronic	Male	9
		Female	11

[1] VetScan i-STAT-1 handheld blood analyzer.

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

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general linear model with treatment as the fixed effect. Body weight and iSTAT data were analyzed using linear mixed models with sex, length of exposure, treatment, and their interactions as fixed effects and birds as the random subject effect. 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 $P \geq 0.05$. All data was determined have normal distribution and homogenic variances. For all data, analysis of variance (ANOVA) was used to assess significance. Tukey's method for comparison was used for analysis of significant pairwise differences of means, confidence level, and p-value adjustments. A confidence level of 0.95 was used. All data were reported as means with SD where appropriate.

Results

Performance

There were no significant differences in percentages of infertile eggs, embryo deaths, and deformed/twin embryos across all treatments ($P > 0.05$; Table 2). There were no significant differences in body weight during acute heat stress when comparing males and females and across treatments (Fig 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 (Fig 2). The p-value for the interaction between length of exposure and sex was <0.001. However, overall, there was no treatment effect on body weight ($p = 0.12$).

Table 2. Percentage of infertile, embryo death, deformed/twins[1] at the 10th generation of Japanese quail of respective treatments[1].

Treatment[2aa2]	N =	Infertile[3]	Total Embryo Death	Deformed/Twins
TN	160	0.069 (0.037)	0.075 (0.054)	0.000 (0.000)
TNS	152	0.046 (0.033)	0.089 (0.070)	0.007 (0.020)
HS	160	0.069 (0.059)	0.038 (0.044)	0.000 (0.000)
HSS	160	0.044 (0.042)	0.081 (0.070)	0.006 (0.018)

[1] Eggs collected from the 3rd and 4th week of lay were incubated and embryos were sampled on days 9, 11, 13, and 15 of incubation.

[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] Means are presented with standard deviations (SD).

Superscripts indicate significant differences at $p \leq 0.05$.

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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 ($P<0.001$) and ADG ($P<0.001$) 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 ($P<0.001$) than HSS females; however, HSS males had a significantly higher FCR ($p = 0.0013$) than HSS females (Table 3).

pH and BE

As shown in Fig 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

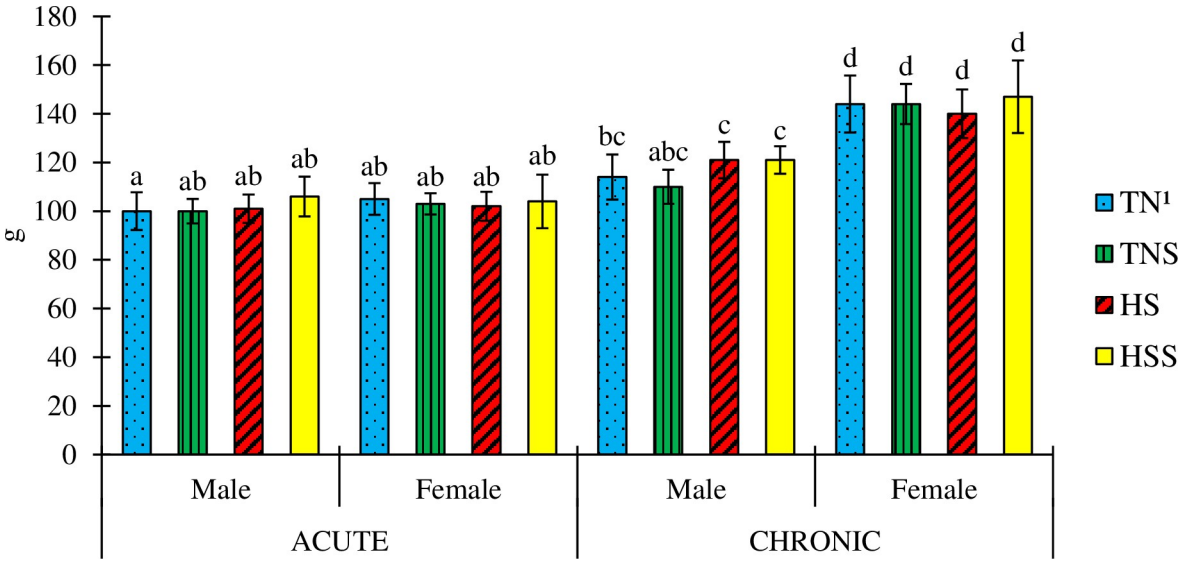

Fig 2. Body weight[2] of quail exposed to acute and chronic[3] temperatures. [1] Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational 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 $P \leq 0.05$. Means are presented with standard deviations (SD).

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Table 3. FCR[1] within treatments.

Treatment[2]	Sex	AFI (g)[3]	ADG (g)[4]	FCR
HS	Male	17.30 (2.11)[a]	0.19 (0.80)[a]	1.09 (42.86)[a]
HS	Female	22.60 (3.97)[b]	2.74 (1.09)[b]	7.71 (1.78)[b]
HSS	Male	20.30 (2.51)[a]	2.39 (1.02)[a]	9.59 (3.93)[a]
HSS	Female	21.50 (2.62)[b]	3.04 (0.70)[b]	7.38 (2.27)[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 $P \leq 0.05$.

Means are presented with standard deviations (SD).

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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 ($P<0.001$; Fig 4).

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 4). 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 4). 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 ($P<0.001$; Table 4). 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 4. 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 ($P<0.001$), and chronic TN females (p = 0.014; Table 4). 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

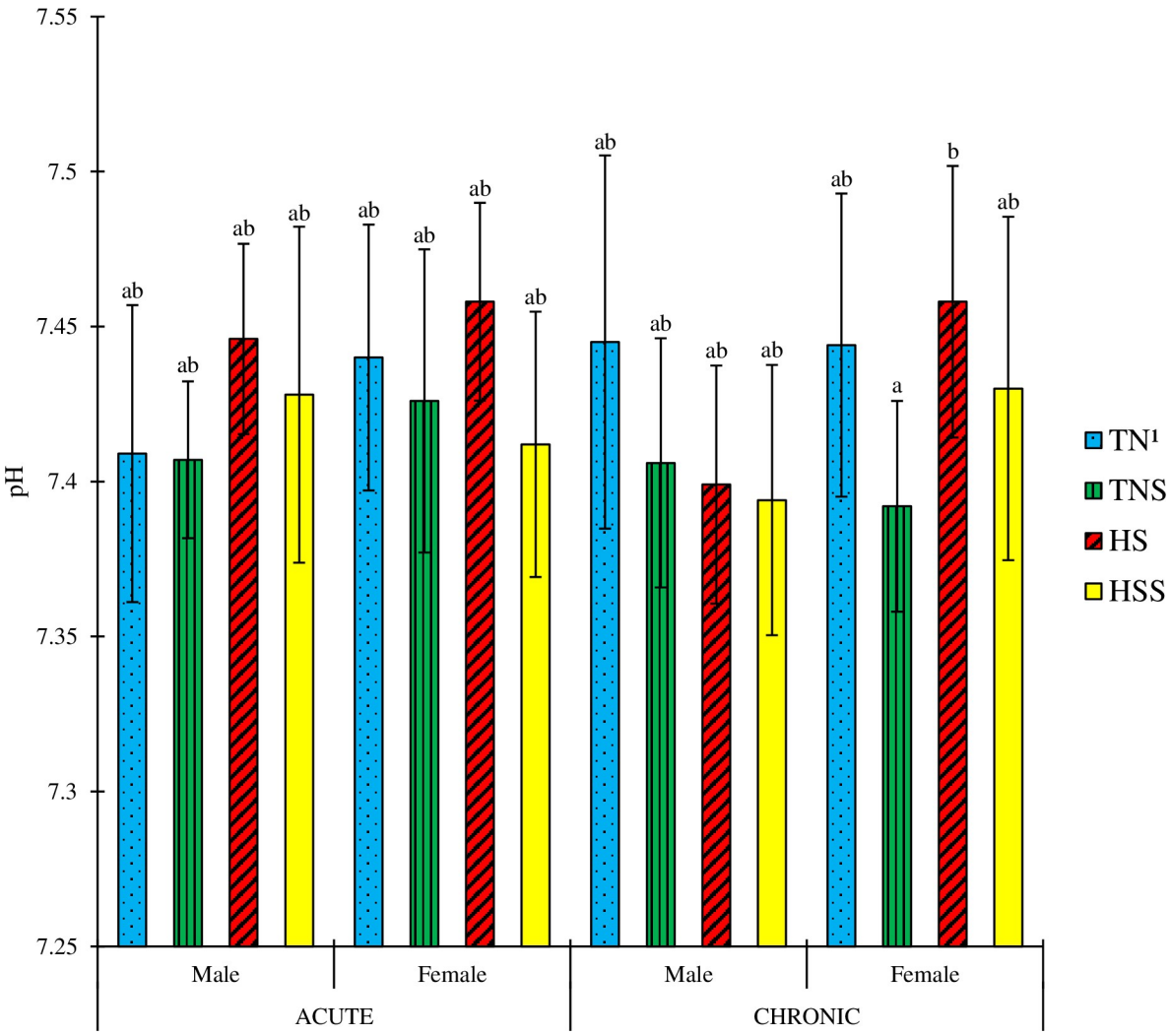

Fig 3. Blood pH[2] of quail exposed to acute and chronic[3] temperatures. [1] Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational 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) 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-b] Superscripts indicate significant differences at $P \leq 0.05$. Means are presented with standard deviations (SD).

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TN males (p = 0.043); however, it was not significantly different from that of all other treatments.

Blood electrolytes

Electrolyte levels are shown in Table 5. The results 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 ($P<0.001$). 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 ($P<0.001$, for all comparisons), chronic TNS

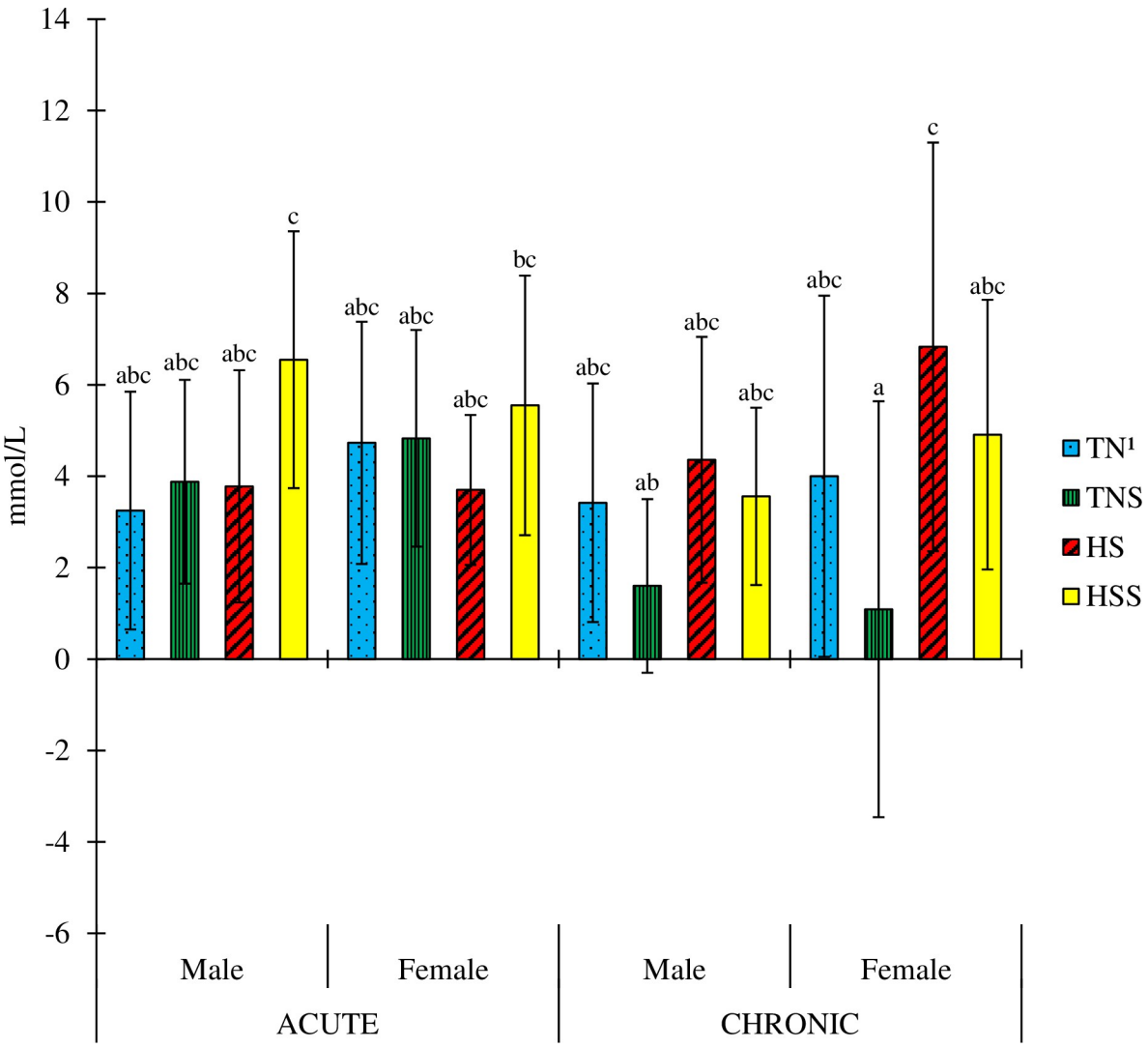

Fig 4. Blood base excess[2] of quail exposed to acute and chronic[3] temperatures. [1] Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational 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 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 $P \leq 0.05$. Means are presented with standard deviations (SD).

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males ($P<0.001$, for acute TN females, acute HSS males, and acute HSS females), chronic HSS males ($p = 0.014$, $p = 0.038$, $p = 0.047$ as above), chronic TN females ($P<0.001$, for all comparisons), and chronic TNS females ($P<0.001$ as above). Chronic TN males had significantly higher levels of Na^+ in the blood than all acute treatments ($P \leq 0.006$), 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 ($P<0.001$, for acute HS females and acute TNS females), 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 ($P<0.001$), acute TN males

Table 4. 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 (4.09)^{abcd}	45.20 (2.13)^{abcd}	40.30 (3.01)^{abc}	46.90 (4.34)^{cd}
		Female	42.60 (3.01)^{abcd}	44.70 (5.17)^{abcd}	38.90 (3.23)^{a}	47.40 (4.82)^{d}
	Chronic	Male	40.00 (5.91)^{ab}	42.20 (5.18)^{abcd}	47.40 (4.61)^{d}	46.60 (4.65)^{bcd}
		Female	40.70 (1.89)^{abc}	42.60 (5.71)^{abcd}	43.00 (3.58)^{abcd}	44.00 (5.24)^{abcd}
PO_2[4] (mmHg)	Acute	Male	39.40 (5.33)^{abc}	37.90 (4.55)^{abc}	34.10 (4.20)^{ab}	31.70 (5.27)^{a}
		Female	39.50 (3.59)^{abc}	36.10 (9.95)^{abc}	33.70 (7.89)^{ab}	35.20 (5.71)^{abc}
	Chronic	Male	43.00 (4.63)^{c}	40.70 (3.32)^{bc}	35.90 (4.91)^{abc}	40.40 (4.13)^{abc}
		Female	40.80 (5.61)^{bc}	38.90 (5.94)^{abc}	36.10 (4.74)^{abc}	35.90 (5.28)^{abc}
HCO_3[4] (mmol/L)	Acute	Male	27.90 (1.89)^{abc}	28.50 (1.89)^{abc}	27.70 (2.27)^{abc}	30.90 (2.28)^{c}
		Female	29.00 (2.15)^{abc}	29.30 (2.12)^{abc}	27.50 (1.43)^{abc}	30.10 (2.45)^{bc}
	Chronic	Male	27.40 (2.37)^{abc}	26.40 (1.82)^{ab}	29.20 (2.29)^{abc}	28.40 (1.49)^{abc}
		Female	28.00 (3.25)^{abc}	26.00 (4.20)^{a}	30.60 (3.89)^{c}	29.10 (2.55)^{abc}
TCO_2[4] (mmol/L)	Acute	Male	29.20 (1.80)^{abc}	29.90 (2.03)^{abc}	28.90 (2.20)^{abc}	32.30 (2.41)^{c}
		Female	30.40 (2.20)^{abc}	30.80 (2.18)^{abc}	28.80 (1.40)^{abc}	31.60 (2.50)^{bc}
	Chronic	Male	28.80 (2.42)^{abc}	27.70 (1.95)^{ab}	30.60 (2.42)^{abc}	29.90 (1.54)^{abc}
		Female	29.20 (3.19)^{abc}	27.20 (4.38)^{a}	31.90 (4.03)^{c}	30.50 (2.54)^{abc}
sO_2[4] (%)	Acute	Male	73.00 (8.43)^{abc}	71.00 (7.29)^{abc}	67.60 (7.40)^{abc}	60.70 (9.19)^{a}
		Female	75.70 (4.73)^{abc}	65.60 (20.30)^{ab}	66.20 (15.90)^{abc}	66.10 (10.50)^{abc}
	Chronic	Male	80.10 (3.94)^{c}	75.80 (5.97)^{abc}	66.80 (9.77)^{abc}	73.90 (5.09)^{abc}
		Female	77.00 (6.54)^{abc}	71.60 (10.10)^{abc}	71.20 (7.95)^{abc}	68.90 (11.30)^{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 $P \leq 0.05$.

Means are presented with standard deviations (SD).

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(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 quail across treatments and sex, acute quail had significantly more K^+ than chronic quail (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 5).

Glucose, hematocrit, and hemoglobin

As seen in Fig 5, 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

Table 5. 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]
Na	Acute	Male	144.00 (4.05)^{abc}	143.00 (2.20)^{abc}	143.00 (1.39)^{ab}	142.00 (1.22)^{a}
		Female	142.00 (2.33)^{a}	143.00 (1.83)^{ab}	143.00 (1.83)^{ab}	142.00 (1.17)^{a}
	Chronic	Male	150.00 (3.34)^{f}	148.00 (2.83)^{def}	146.00 (2.77)^{abcde}	146.00 (2.65)^{bcdef}
		Female	149.00 (5.37)^{ef}	147.00 (3.56)^{cdef}	145.00 (2.16)^{abcde}	145.00 (2.07)^{abcd}
K	Acute	Male	5.23 (0.71)	5.35 (0.43)	5.26 (0.52)	5.29 (0.74)
		Female	5.32 (0.62)	5.09 (0.92)	5.17 (0.60)	5.32 (0.65)
	Chronic	Male	4.71 (0.61)	4.77 (0.48)	5.49 (0.57)	5.57 (0.75)
		Female	4.70 (0.63)	4.77 (0.60)	4.81 (0.70)	4.81 (0.35)
Ionized Ca	Acute	Male	1.52 (0.037)^{ab}	1.51 (0.038)^{ab}	1.49 (0.082)^{ab}	1.5 (0.053)^{ab}
		Female	1.53 (0.038)^{ab}	1.48 (0.061)^{ab}	1.48 (0.043)^{ab}	1.49 (0.037)^{ab}
	Chronic	Male	1.46 (0.067)^{ab}	1.45 (0.046)^{ab}	1.45 (0.052)^{ab}	1.49 (0.052)^{ab}
		Female	1.53 (0.14)^{b}	1.48 (0.17)^{ab}	1.42 (0.19)^{a}	1.49 (0.10)^{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 $P \leq 0.05$.

Means are presented with standard deviations (SD).

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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 (Figs 6 and 7), 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).

Discussion

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 by Omid, et al. [21]. Others have tested heat stress in quail at 33˚C or higher and have observed temperature effects on infertility and embryo mortality [21,22]. However, 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. These results demonstrated that multigenerational exposure to 31.1˚C did not significantly affect fertility or embryo development; thus, indicating that from 31.1 to 33˚C, there may be an inflection point in which reproduction is significantly affected by heat stress. As food systems become more vulnerable during climate change, it may be important to create early-intervention policies as temperatures approach 33˚C.

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 to 106 g and 102 to 105 g, respectively, for all treatments (Fig 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 [23].

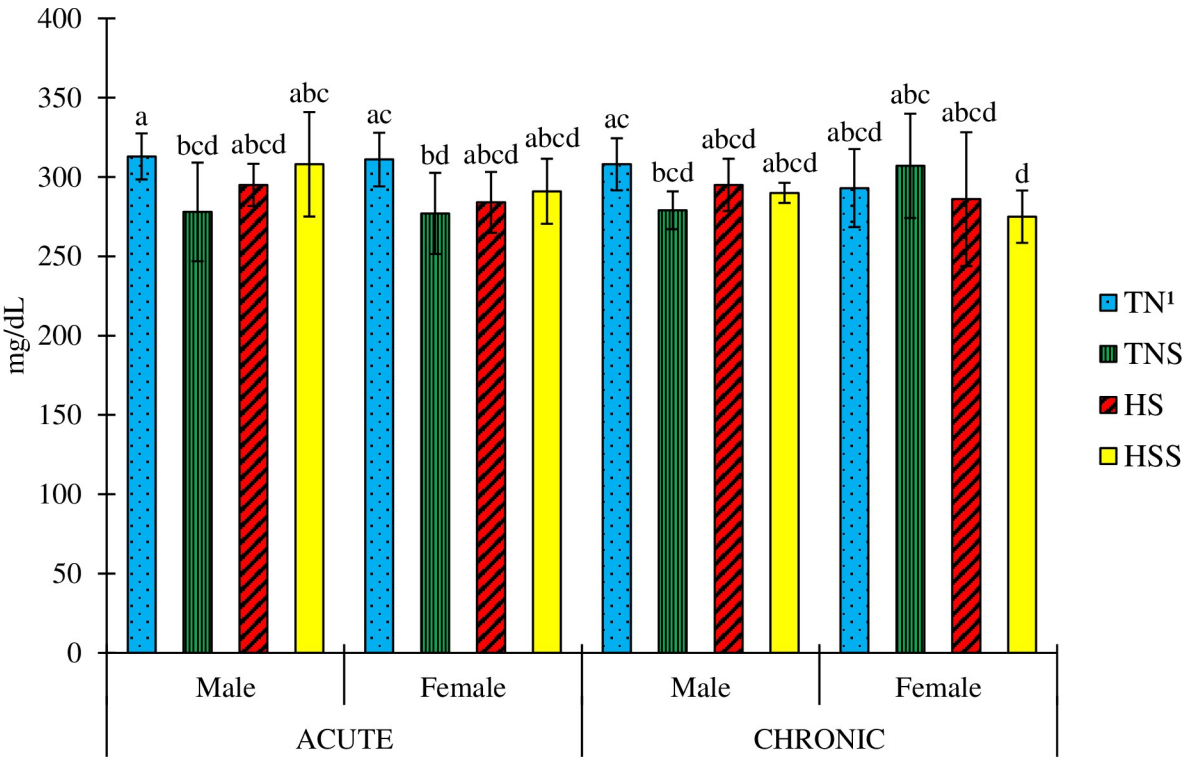

Fig 5. Blood glucose[2] of quail exposed to acute and chronic[3] temperatures. [1] Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational 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 $P \leq 0.05$. Means are presented with standard deviations (SD).

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Possibly, weight differences were due to genetic lines. Japanese quail used in the study by Sarica, et al. 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 [23,24]. In the current study, heat stress after 10 generations of 31.1˚C did not significantly affect body weight; however, other studies found that heat stress at 34˚C decreased body weight in quail [9,23,25].

The AFI for HS and HSS were similar to reports of others for 4- to 6-week-old quail [26]. However, the ADG for HS was lower than that reported for 5- to 6-week-old Japanese quail and ADG for HSS was similar to reports of 3.57 ADG of 4- to 5-week-old quail [26]. The FCR of all sexes within treatments, except HS males, was higher than that reported by others for quail at 5.60 (4- to 5-week-old) and 6.37 (5- to 6-week-old) [26]. 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 [26]. High FCR was also determined for 4-week-old broiler chicken that were housed at 32˚C [17,27,28]. Additionally, feed efficiency or FCR is recommended for selection during heat stress because when selecting for body weight alone, there is reduced heat tolerance [29]. As temperatures rise due to climate change, selecting for traits with increased heat tolerance will allow for continued use of animals as needed sources of relatively inexpensive protein.

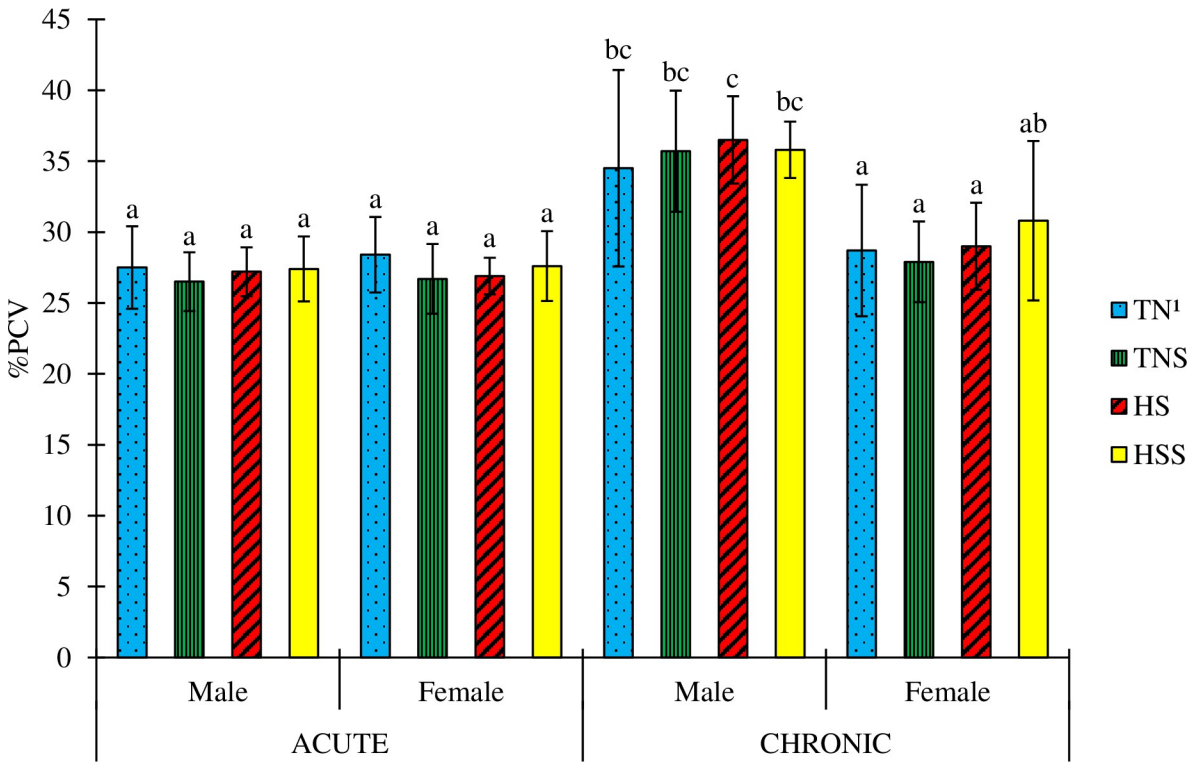

Fig 6. Hematocrit[2] of quail exposed to acute and chronic[3] temperatures. [1] Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational 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 $P \leq 0.05$. Means are presented with standard deviations (SD).

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pH, BE, and blood gases

Comparisons for the following parameters were made to studies that subjected random-bred quail to temperatures ≥32˚C; however, the current study subjected selectively bred quail to temperatures <32˚C. This could be the basis for inconsistencies seen between the current study and others. More work needs to be done on selectively bred and non-selectively bred lines during mild heat stress to determine (1) the effect on other parameters that can be the first indicator of heat stress and (2) the ideal avian phenotype that can adapt well to high temperatures.

The parameters of pH, PCO_2, TCO_2, HCO_3, and BE are closely related to each other and CO_2 [28]. CO_2 related parameters indicate the acid-base balance in blood [30]. BE is the amount of acid or base required to return the pH of the blood to 7.4 [31]. It can also be used as an indicator of non-respiratory metabolic acid-base imbalance [32]. Due to the mild heat stress applied in this study, it was hypothesized that metabolic dysregulation would not occur and there would be little changes in BE across treatments, which was proven to be true by the results. However, behavioral changes such as increased panting or gular fluttering was still expected; thus, it was hypothesized that HS and HSS would have more alkaline blood. This hypothesis was proven false by results that suggested that there was no significant difference among treatments, sex, and length of exposure. Thus, there was indication that behavioral changes at 31.1˚C did not incur changes in blood acid-base balance. When considering

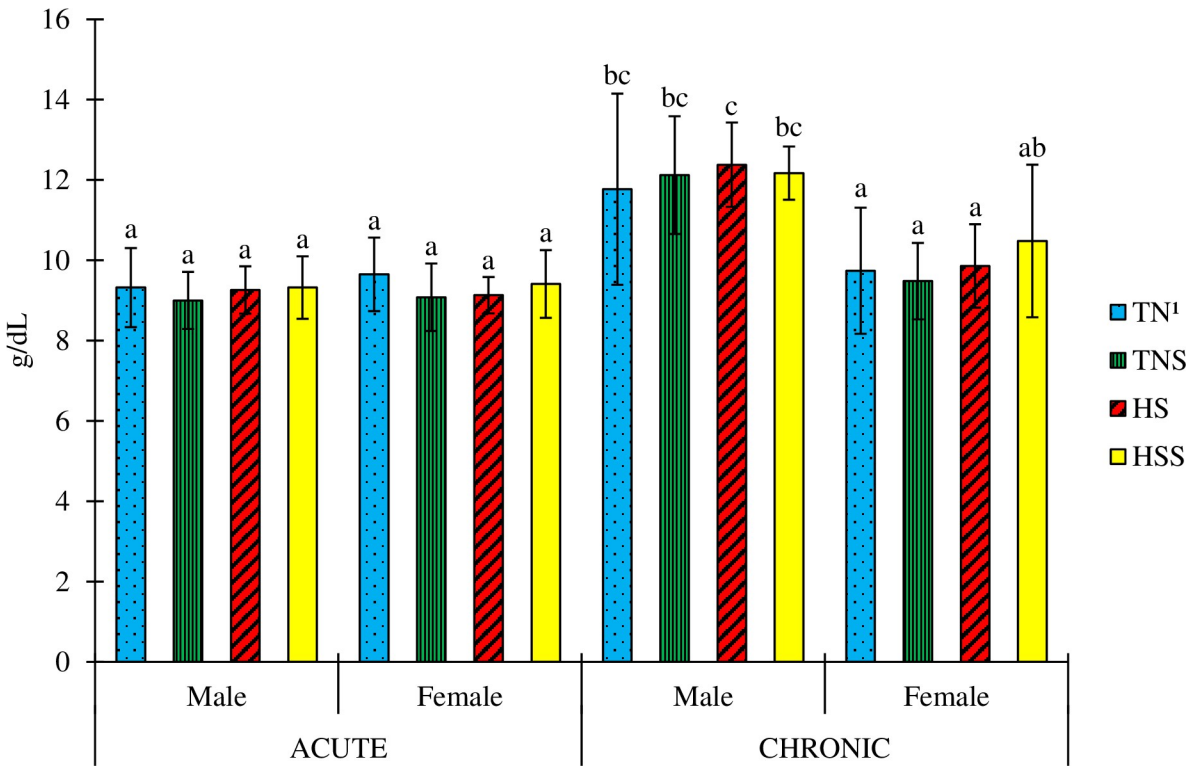

Fig 7. Hemoglobin[2] of quail exposed to acute and chronic[3] temperatures. [1] Four treatments were: (1) thermoneutral controls (22.2˚C, TN), (2) thermoneutral siblings (22.2˚C, TNS), (3) heat stress (31.1˚C, HS), and (4) heat stressed siblings (31.1˚C, HSS) TN and HS were obtained through generational 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 $P \leq 0.05$. Means are presented with standard deviations (SD).

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interactions effects, the pH and BE were significantly lower in chronic TNS females than chronic HS females indicating 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.

Another study on heat stressed turkeys at 32˚C found no significant differences in acid-base balance according to measurements of pH and PCO_2 [33]. 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 [30]. The results of the current study also indicated that overall CO_2 related parameters 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. This indicated that although quail were selectively bred in the current study, there is an overall resilience to heat stress in quail which allows for little changes in acid-base balance. Along with other studies performed on turkeys and chicken, it appears that temperatures >35˚C are required to elicit a significant change in acid-base balance [30,33].

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 [15]. Researchers found a decrease in blood PCO_2 in heat

stressed chicken broilers 43˚C 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 5) [34]. The significantly lower blood PCO_2 in acute HS male and females and significantly higher blood pH in chronic HS females suggests that HS experienced more CO_2 loss via the lungs and was less adapted to 31.1˚C than HSS [30].

In chicken, when there was high pH and low PCO_2, there was a decrease in Ca in the blood [35]. Heat stress at 32˚C also decreased the water-holding capacity of proteins due to oxidative damage and decreased meat quality [35]. However, heat stress caused increased decomposition of glycogen and increased rate of muscle glycolysis which will change meat quality due to a decrease in pH [35]. 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 [36]. Sources also found that addition of sodium bicarbonate increases bicarbonate levels in the blood [36].

The measurement of sO_2 determines the amount of O_2 bound to Hb. Therefore, with acute HSS males having significantly lower levels of sO_2 than acute TN females, chronic TN males, and chronic TN females suggested that HSS males were least prepared to handle the initial exposure to heat stress at 31.1˚C. As expected, those in the TN treatment had consistently higher levels of oxygen saturation in their blood because they came from a line of quail that was unaffected by heat stress. Thus, when considering parameters to measure for early detection of heat stress, producers should consider sO_2 instead of pH, PO_2, PCO_2, and HCO_3.

Blood electrolytes

When other livestock species and poultry experience heat stress, there is usually a deficiency in both Na^+ and K^+ which lead to metabolic alkalosis and acid-base imbalances [16,37]. In the current study, when considering all parameters measured, 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 acute 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. Thus, results suggested that quail subjected to chronic heat stress at 31.1˚C (slightly beyond the upper level of their thermoneutral zone) experienced 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 [16]. However, when the animal has adapted to the stress, glycogen is reestablished and K^+ levels are restored [16]. Others have found that male broiler chicks subjected to heat stress at 35˚C for 8 hours per day increased blood K^+ levels [38]. However, researchers have also found decreases in K^+ levels, uric acid, total protein, and globulin levels in heat stressed broilers at 35˚C for 6 hours per day [39]. 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. These results indicated that protein levels may not be affected when quails are heat stressed at 31.1˚C; thus, providing similar qualities of proteins to consumers. Further research into protein quantity and quality in connection to K^+ levels should be conducted in quail at 31.1˚C.

When birds experience heat stress, there is a decrease in activity of carbonic anhydrase and feed intake. Carbonic anhydrase is essential to make the carbonate ion ($CaCO_3$) 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 blood levels [15]. 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. Another study using 35˚C determined that iCa increased as broilers chicks aged, and this increase was higher in heat stressed birds than those housed at thermoneutral temperatures (21 to 22˚C) [39]. The results of the current study disagree with the results of others because iCa was significantly lower in chronic random-bred heat stressed females [39]. Typically, an increase in iCa in the blood could be due to bone growth demands at the time of sampling or mobilization of iCa to activate calcium-dependent ionic channels involved in maintaining homeostasis during periods of increased temperature [39,40]. However, decreased iCa at 31.1˚C may mean that vascular dilation develops later and at a higher temperature which allows for conservation of iCa until a threshold for activation of certain homeostatic mechanisms is reached.

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 with meat-type ducks that were heat stressed at 34 to 43˚C reported a decrease in Na^+, K^+, and Cl^- in their blood [34]. This decrease may have caused disruptions to Na^+/K^+ ATP pumps which are responsible for 30 to 60% of the body's energy and is important for maintenance of moisture balance in cells [34]. To adapt poultry to increasing temperatures, it will be important to consider the timing of electrolyte administration to prevent deficiencies. Therefore, as demonstrated in the current study, 31.1˚C may be too early for supplemental electrolyte administration and may incur unnecessary cost to producers that want to prevent heat-stress-induced decreases in production. However, perhaps more research should be conducted between 31 to 33˚C to accurately pinpoint when electrolytes are needed for quail production.

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 increases 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 [41]. Other researchers have found that exogenous testosterone increases red blood cell counts, hemoglobin concentration, and hematocrit levels in Japanese quail [41].

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 not challenged with heat stress (temperature unreported) [42]. This is contrary to others who reported that Japanese quail were sensitive to high environmental temperatures and the metabolic stress response was triggered at temperatures higher than 25˚C [15].

Contrary to the findings of the current study, hematocrit of heat stressed broilers at 43˚C were observed to decrease due to the damage of the red blood cells [34]. In meat-type ducks that were heat stressed at 34 to 43˚C, researchers found that there was a decrease in red blood cells and hemoglobin which led to iron deficiency [34]. 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. As noted by others, the development of monitoring and early warning systems for agriculture and health is important for climate adaptation policies [43]. Results of the present study can inform producers of parameters that provide early warning signals on heat stress in quails; thus, allowing for food security during climate change.

Conclusions

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 [7]. Producers in more developed countries will also benefit from this information due to current consumer demand for pastured poultry farming [44]. For farmers to make informed decisions on how to continue production of these nutritious animals, when to anticipate changes production, and how to mitigate the changes in the face of climate change, there must be an understanding of quail response to mild heat stress. It is also valuable to understand if selective breeding for high performance in mild heat stress will result in quails that are better adapted to heat stress. Others have recognized the importance of research into alternative livestock species and breeds to alleviate the pressures of climate change and the importance of the nexus of climate, agriculture, nutrition, and health [43].

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 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 quail 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 ($\geq$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 $\geq$33˚C is often difficult to obtain from institutional animal care and use committees, studies using the higher temperature 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.

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