Veterinary Integrative Sciences 2024; 22(3): 831 - 846 DOI; 10.12982/VIS.2024.056

Vet Integr Sci

Veterinary Integrative Sciences

Faculty of Veterinary Medicine
Chiang Mai University

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

Impact of heat stress on blood physiological parameters, body temperature, respiratory frequency, antioxidant status, carcass quality, production performance, and egg quality of Japanese quails

Nguyen Thi Kim Khang[1*], Ngo Thi Minh Suong[1], Masashi Takahashi[2]

[1]Department of Animal Science, College of Agriculture, Can Tho University, Can Tho 900000, Vietnam
[2]Department of Animal Science, Hokkaido University, Kita9 Nishi9, Kita-ku, Sapporo, Hokkaido, 060-8589, Japan

Abstract

The study was performed with three experiments. The first experiment studied respiratory rate (RR), body temperature (BT), blood physiological parameters (BPP), serum antioxidant enzyme activities (SAEA), and meat quality of 240 quails under three temperatures (38 °C, 25 °C, and environmental temperature 30 °C) and two bodyweights (heavy and lighter). Heat stress (HS) increased RR and BT of quails but had no effect on BPP; superoxide dismutase (SOD) activity was decreased in group 38 °C (P<0.05), and a trend of increasing glutathione peroxidase (GSH-Px) was also observed in group 38 °C (P=0.06); meat quality was not affected by HS except yellow index of meat color was increased (P<0.05). The second experiment was conducted with 168 quails (experiment 1) to record reproductive performance and egg quality. HS negatively affected feed intake, egg weight, egg height, albumen height, yolk weight, yolk color, and Haugh unit (P<0.05). The third experiment was performed to evaluate the effect of HS on growth and reproductive performance, and SAEA of the first generation (G1) from 84 quails (experiment 2). Feed intake, weight gain, and feed conversion ratio in the 38 °C H – G1 quail line were not significantly different from other groups. The higher egg weight and egg production were discovered in heat-stressed G1 groups. The lowest value of SOD and GSH-Px was reported in the 38 °C H – G1 quail line but no different in catalase (P>0.05). The 38 °C – G1 quail groups adapted to HS from G0 by expanding feed intake at the growing phase, improving egg weight and egg production, and maintaining the low SAEA.

Keywords: Antioxidant enzyme, Growth Performance, Heat stress, Quail, Reproductive Performance

***Corresponding author:** Khang Nguyen Thi Kim, Department of Animal Science, College of Agriculture, Can Tho University, Can Tho 900000, Vietnam. Email: ntkkhang@ctu.edu.vn

Funding; This study is funded in part by the Can Tho University Improvement Project VN14-P6 supported by a Japanese ODA loan.

Article history; *received manuscript: 20 May 2023*
revised manuscript: 10 December 2023
accepted manuscript: 20 December 2023
published online: 12 January 2024
Academic editor: *Korakot Nganvongpanit*

INTRODUCTION

Japanese quails (*Coturnix japonica*) were imported first in 1997 and became popular in husbandry in Vietnam (Lan, 2017) because of their growth and reproduction rapidly. In addition, they were considered cheap sources of poultry meat (Runjaić-Antić et al., 2010) with high-quality protein and low-caloric content (Genchev et al., 2008). They are also a preferable experimental animal model, which includes several advantages such as early sexual maturity time and short generation interval (NRC, 1991). In addition, Sousa et al. (2014) reported that meat-type quails have higher thermal comfort temperatures in comparison with chickens.

On the other hand, heat stress (HS) occurs when a quail's ability to remove excess heat from its surroundings exceeds its ability to produce heat (Alagawany et al., 2017) when the environment temperature is higher than 30 °C (Quinteiro-Filho et al., 2012). The quails' responses to HS can be illustrated as reduced feed intake, increased body temperature, poor nutrient digestibility, absorption, and metabolism, reduced reproductive function, disturbance in the structure and function of the intestinal epithelium, alteration in gut microbiota, and increased circulatory corticosterone and cortisol (Lara and Rostagno, 2013; Loyau et al., 2015) which initiates lipid peroxidation in cell membranes as a consequence of increased reactive oxygen species (ROS) and free radicals formation (Sahin et al., 2002). HS increases the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), three mainly antioxidant enzymes as biomarkers in serum response to increased ROS levels (Slimen et al., 2016). Nguyen et al. (2021) suggested that Japanese quails were highly adaptive to high temperatures and humid conditions in the Mekong Delta by changing their behaviors.

However, most investigations on quail exposed to HS have focused on one generation (G0) of birds. Information about the effects of heat stress on the next generation (G1) of quail remains poorly understood. Therefore, this study was conducted to evaluate the effects of temperature impact on blood physiological parameters, body temperature, respiratory frequency, antioxidant status, carcass quality, reproductive, and egg quality of six Japanese quail lines of G0 and growth performance, reproduction, and antioxidant status of G1.

MATERIALS AND METHODS

Experimental animals

The study was performed at the experimental farm of the Animal Science Department; Can Tho University. The research was ethically conducted, with approved protocols for animal care, housing, and blood collection adhering to the standards outlined in the Animal Welfare Assessment (DA2020-01/KNN).

Experiment 1

A total of 240 quails (120 males and 120 females) of G0 at 28 days old were randomly distributed in 6 treatments corresponding to three different temperature levels [38 °C, 25 °C, and environmental temperature (Et-30 °C)] and two different bodyweights (heavy and lighter), respectively. Each treatment

had 40 birds raised in individual cages, with 1 male and 1 female in each cage. The time of temperature impact was 4 h/day from 10 AM to 2 PM for 14 consecutive days. The data of bird body temperature, respiratory frequency, blood physiological parameters, and biomarkers of antioxidant status in serum were recorded. A total of 72 birds were taken randomly from six treatments for weighing, manual slaughtering, and carcass analysis.

Experiment 2

One hundred sixty-eight quails (84 males and 84 females) of six treatments from experiment 1 continued to be kept to monitor reproductive performance parameters from 42 to 112 days of age. Feed intake and egg weight were recorded every day. When quails were 77 days old, 252 fresh eggs from six groups during 3 consecutive days were collected and labeled by pencil to assess egg quality.

Experiment 3

Seven hundred forty-seven G1 chicks of six treatments from experiment 2 were kept to collect data on feed intake, live weight, weight gain, and feed conversion from 1 to 35 days old. Egg production, feed consumption, and egg weight of hens were recorded from 42 to 112 days of age.

Housing and feeding

All experimental quails were kept in open – housing and consumed feed and fresh water ad libitum. From 1 to 14 days of age, birds were housed in a shed with a rice hull with infrared heating lamps for up to 14 days and 24 h of continuous light, and they were fed mixed feed with a nutritional value of 20 % CP and ME 2,750 kcal/kg. From 15 to 35 days of age, quails received feed with a nutritional value of 18 % CP and ME 2,900 kcal/kg. Breeding quails were fed mixed feed (ME is 2,900 kcal/kg and 18 % CP) with 14 h of light per day and were provided for quails to maintain maximum egg production and fertility (Randall et al., 2008).

Body temperature and respiratory frequency

Quail body temperature at 0, 2, and 4 h after thermal application using a thermocouple rectal thermometer with a 3-cm insertion probe. The respiratory rate was counted manually using a stopwatch every 30 seconds at 0, 2, and 4 h after treatment.

Blood physiological parameters

On the day 14th of thermal application, blood samples were collected on a random basis from 12 quails/ treatment. Whole blood samples were collected directly from the jugular vein in vacutainer tubes containing EDTA and kept at 5 °C for routine hematological examination, and into heparinized vacutainer tubes for the detection of antioxidant enzyme status.

Hematological analysis was carried out using an Auto Haematologyanalyzerr Mindray BC-2800 Vet® (Shenzen Mindray Bio-Medical Electronics Co., Ltd, Hamburg, Germany) with 15 physiological parameters.

Detection of antioxidant enzyme status in the serum

The serum biochemistry determinations were carried out using commercial test kits and followed the protocol of the production company, including Genlisa TM Chicken Catalase Lot CCAT0720 (Krishgen BioSystems, USA), Genlisa TM Chicken Super Oxidase Dismutase Lot CSOD0720 (Krishgen BioSystems, USA), and Chicken Glutathione Peroxidase (GSH-Px) Elisa Lot GSHPX0720 (Kinesisdx, USA).

Carcass quality

The carcasses were weighed, and the weights of the liver were recorded. Parts of the thigh and breast meat were weighed and calculated as the percentage of carcass weight. Breast meat color at 15 minutes, 24 h, and 48 h after slaughter was measured using a Konica Minolta Chroma Meter CR-400 (aperture 8 mm) (Konica Minolta, Tokyo, Japan). The CIELAB coordinates (L*, a*, and b*) were recorded. The parameter a* takes positive values for reddish colors and negative values for greenish ones, whereas b* takes positive values for yellowish colors and negative values for bluish ones. L* is an approximate measurement of luminosity, which is the property according to which each color can be considered equivalent to a member of the grey scale between black and white (Pathare et al., 2013). Thigh and breast meat pH at 15 minutes, 24 h, and 48 h after slaughter were recorded using the Hanna instrument (penetration pH electrode, H18314, membrane pH meter, 115V/60Hz. Cod. 1.1176). Drip loss of thigh and breast meat were counted by changing the weight before and after storing at 5 °C at 24 h and 48 h.

Egg quality

Egg weight was measured using the Sartorius 1202 MP balance with an accuracy of 0.01 g. Egg length and width (mm) were measured using an electronic digital caliper. The egg shape index is calculated as egg width/egg length × 100 (Das et al., 2010). Eggshell thickness (mm) was measured using an electronic digital caliper, taken as the mean of measures from the equator and both ends of the egg. Eggshell weight (g) was measured, as well as an eggshell percentage as shell weight/egg weight × 100 (Sezer et al., 2007).
The measurements of the internal qualities were obtained by gently broking the egg and using a scalpel, and the contents were taken on flat surface. The yolk was carefully separated from the albumen for weighing. The albumen weight was calculated by subtracting the weight of the yolk and shell from the weight of the whole egg. The albumen and yolk height and width (mm) were measured using an electronic caliper (Reddy et al., 1979). Yolk weight ratio (%) = yolk weight (g)/egg weight (g) × 100. Similarly, albumen weight ratio (%) = albumen weight (g)/egg weight (g) × 100. Yolk index (%) = yolk height (mm)/yolk diameter (mm) × 100 (Romanoff and Romanoff, 1949). Haugh unit = 100 logs (albumen height (mm) + 7.57 – 1.7 × egg weight (g) 0.37 (Haugh, 1937). Egg color was detected by using a Konica Minolta Chroma Meter CR-400 (aperture 8 mm) (Konica Minolta, Tokyo, Japan).

Data analysis

All data were analyzed by using the General Linear Model (GLM) procedure of Minitab (version 16.0) software, and the Tukey test was used to compare mean differences among treatments at $P \leq 0.05$.

RESULTS

Experiment 1

Blood physiological parameters

The results in Table 1 indicated that mean platelet volume (MPV) of quails was statistically significant among lines (P=0.02), in which quails in 38 °C L had highest value (7.99 fl) than other lines (38 °C H – 7.9 fl, Et-(30 °C) L – 7.54 fl, 25 °C L – 7.49 fl, 25 °C H – 7.2 fl, Et-(30 °C) H – 7.14fl, respectively). However, different hematology parameters showed no significant on blood physiological parameters of experimental Japanese quails, with the minimum and maximum values in parenthesis were as follows: hemoglobin (HGB) [19.48-22.89 g/dl], mean corpuscular hemoglobin (MCH) [66.14-67.51 pg], mean corpuscular hemoglobin concentration (MCHC) [51.32-53.74 g/dl], red blood cell (RBC) [2.92-3.41×10^6/µl], mean corpuscular volume (MCV) [125.84-131.15 fl], red cell distribution with standard deviation (RDWs) [56.33-59.58 fl], red cell distribution with concentration (RDWc) [9.63-10.11 %], hematocrit (HCT) [36.76-41.98 %], platelet concentration (PLT) [11.83-15.23×10^3/µl], procalcitonin (PCT) [0.01-0.01 %], platelet disrabution width standard deviation (PDWs) [4.53-6.33 fl], platelet disrabution width concentration (PDWc) [23.48-29.49 %], platelet larger cell concentration (P-LCC) [1-2.5×10^3/µl] and platelet larger cell ratio (P-LCR) [7.73-14.18 %].

Table 1 Blood physiological parameters of six Japanese quail lines

Treatment	Et-(30 °C) H	Et-(30 °C) L	25 °CH	25 °C L	38 °C H	38 °C L	SEM	P
HGB (g/dl)	20.71	21.52	22.75	22.89	20.10	19.48	1.06	0.14
MCH (pg)	66.54	66.14	66.59	67.17	67.25	67.51	0.79	0.92
MCHC(g/dl)	51.82	51.95	52.43	51.32	52.38	53.74	0.82	0.41
RBC (10^6/µl)	3.24	3.29	3.41	3.41	2.99	2.92	0.17	0.18
MCV (fl)	128.72	127.51	127.70	131.15	128.43	125.84	2.15	0.65
RDWs (fl)	58.23	59.03	59.58	58.80	59.38	56.33	1.34	0.56
RDWc (%)	9.73	10.11	10.04	9.57	10.08	9.63	0.34	0.77
HCT (%)	41.98	41.98	43.93	44.74	38.36	36.76	2.45	0.16
PLT (10^3/µl)	13.04	12.14	13.67	14.56	15.25	11.83	1.29	0.38
PCT (%)	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.47
PDWs(fl)	5.56	4.53	5.23	5.12	6.33	4.74	0.52	0.20
PDWc (%)	27.51	23.48	25.88	26.35	29.49	23.99	2.00	0.30
MPV (fl)	7.14^{b}	7.54^{ab}	7.20^{ab}	7.49^{ab}	7.90^{ab}	7.99^{a}	0.20	0.02
P-LCC (10^3/µl)	1.25	1.00	1.25	1.53	2.50	1.42	0.42	0.19
P-LCR (%)	8.46	7.73	7.78	9.01	14.18	8.69	2.05	0.23

^{a,b} means on the same row with different superscripts are significantly differences at $P \leq 0.05$

Respiratory rate and Body temperature

The impact of thermal stress on Japanese quails' respiratory and body temperature is illustrated in Table 2. The obtained results revealed a significant (P<0.05) in respiratory rate and body temperature among six quail groups before the application of heat (at 0 h). The decrease in temperature of the bird body was found to be the highest value in group Et-(30 °C) L, next to 25 °C L, 25 °C H, Et-(30 °C) H, 38 °C H and the lowest value in group 38 °C L (41.84, 41.84, 41.83, 41.82, 41.79, and 41.69 °C, respectively) with P=0.001. Similarly, significant difference (P=0.001) in the rate of respiration, group 25 °C L showed the highest rate (33.91 times/ 30 seconds), then group Et-(30 °C) H (33.73 times/ 30 seconds), 25 °C H (33.04 times/ 30 seconds), Et-(30 °C) L (33.02 times/ 30 seconds), 38 °C H (31.07 times/ 30 seconds) and the lowest rate in 38 °C L (30.48 times/ 30 seconds).

Table 2 Respiratory rate and body temperature of six Japanese quail lines

Treatment	Time					
	0h		2h		4h	
	BT	RR	BT	RR	BT	RR
Et-(30 °C) H	41.82^{a}	33.73^{a}	41.78	30.86^{b}	41.82^{bc}	31.42^{b}
Et-(30 °C) L	41.84^{a}	33.02^{ab}	41.76	32.03^{ab}	41.84^{abc}	31.91^{b}
25 °C H	41.83^{a}	33.04^{ab}	41.81	32.36^{ab}	41.79^{bc}	33.02^{b}
25 °C L	41.84^{a}	33.91^{a}	41.79	30.67^{b}	41.74^{c}	32.00^{b}
38 °C H	41.78^{ab}	31.07^{bc}	41.18	33.34^{a}	41.88^{ab}	34.90^{a}
38 °C L	41.69^{b}	30.48^{c}	41.82	32.41^{ab}	41.93^{a}	34.94^{a}
SEM	0.03	0.59	0.18	0.51	0.02	0.55
P value	0.00	0.00	0.09	0.00	0.00	0.00

^{a,b,c} means on the same column with different superscripts are significantly different at P≤0.05

After 2 h of thermal stress application, the results showed significant effects (P<0.05) on respiratory rate, and the highest rate was observed in the quail group from 38 °C H (33-34 times/ 30 seconds). However, there are no significant differences in quail body temperature among the six groups. The results of post-4 h treatment revealed a strongly significant difference in the rate of respiration and body temperature (P=0.001 and P=0.001, respectively). The quails in group 38 °C increased their breath rate and body temperature higher in comparison to group 25 °C and Et-(30 °C), although the birds in group 25 °C and Et-(30 °C) were not different in statistics analysis. It is important to note that the birds in groups 38 °C H and 38 °C L had significantly higher body temperature and respiratory rates than other groups after 4 h of temperature affected.

Antioxidant enzyme activity

After 14 days of thermal treatment, three antioxidant enzymes in serum were detected and presented in Table 3. There were no significant differences in CAT among the six groups of quails (P=0.66). The minimum value was 27.9 ng/ml in group 25 °C L, and the maximum value was 53.68 mg/ml in group Et-(30 °C) H. In addition, the GSH-Px in serum were not significantly influenced by heat impact with a variety of value from 7.63 to 9.17 ng/ml (P=0.06). A trend of GSH-Px activity was found in four groups of birds at Et-(30 °C) H, Et-(30

°C) L, 38 °C H, and 38 °C L, which tended to be higher in GSH-Px than 25 °C H and 25 °C L (8.52, 9.7, 8.35, 9.17, 7.63, and 7.8 ng/ml, respectively). Inside the group Et-(30 °C) and 38 °C, these quail traits in low production indicated a higher value of GSH-Px in comparison with high production (9.7 versus 8.52 ng/ml, 9.17 versus 8.35 ng/ml, respectively). The thermal treatment affected the action of SOD in the serum of six Japanese quail lines (P=0.001). The highest SOD was shown in group Et-(30 °C) L (368.61 ng/ml), and the lowest value was found in group 38 °C H (203.74 ng/ml).

Table 3 Antioxidant enzyme activity in the serum of six Japanese quail lines

Treatment	Et-(30 °C) H	Et-(30 °C) L	25 °C H	25 °C L	38 °C H	38 °C L	SEM	P
CAT (ng/ml)	53.68	36.64	42.02	27.90	40.54	44.04	10.54	0.66
SOD (ng/ml)	235.77^{b}	368.61^{a}	224.16^{b}	220.66^{b}	203.74b	215.07^{b}	28.49	0.00
GSH-Px (ng/ml)	8.52	9.70	7.63	7.80	8.35	9.17	0.50	0.06

^{a,b} means on the same row with different superscripts are significantly different $P \leq 0.05$

Carcass yield and meat quality

Table 4 showed the carcass yield of six quail lines, live body weight, live body weight after 24 h stop feeding, and percentage of thigh meat were statistically significant (P<0.05), except for the percentage of breast meat and liver weight were not different among treatments (P>0.05). The thigh meat(%) in group 38 °C L is the highest (27.06 %), next to 38 °C H (26.23 %), 25 °C L (25.93 %), 25 °C H (24.81 %), Et-(30 °C) L (24.66 %), and Et-(30 °C) H(23.37 %). Especially the low production in the three group temperatures indicated a higher percentage of thigh meat than the high production lines.

Table 4 Carcass yield of six Japanese quail lines

Treatment	Et-(30 °C) H	Et-(30 °C) L	25 °C H	25 °C L	38 °C H	38 °C L	SEM	P
Live BW (g)	170.49^{a}	153.71^{ab}	169.16^{a}	156.81^{ab}	171.02^{a}	150.03^{b}	4.41	0.001
Live BW 24 h (g)	145.00^{ab}	133.50^{bc}	144.00^{ab}	130.08^{c}	150.58^{a}	136.50^{bc}	2.91	0
Dressing (%)	67.15	66.51	64.49	65.35	63.05	62.75	1.18	0.06
Breast meat (%)	61.55	59.97	64.66	61.19	59.95	60.25	2.11	0.61
Thigh meat (%)	23.37^{c}	24.66^{bc}	24.81^{abc}	25.93^{ab}	26.23^{ab}	27.06^{a}	0.55	0.00
Liver weight (g)	2.17	2.55	2.42	2.49	2.86	2.45	0.2	0.301

^{a,b,c} means on the same row with different superscripts are significantly different at $P \leq 0.05$

The meat quality parameters of six quail lines are noted in Table 5. Most of the parameters were not significantly different (P>0.05), while the breast meat b* value was found statistically significant (P=0.01) among six groups of birds.

Table 5 Meat quality of six Japanese quail lines

Treatment	Et-(30 °C) H	Et-(30 °C) L	25 °C H	25 °C L	38 °C H	38 °C L	SEM	P
pH 0 h	5.78	5.77	5.78	5.79	5.78	5.78	0.01	0.87
L* 0 h	41.14	40.11	40.90	39.07	39.44	41.20	0.87	0.37
a* 0 h	10.01	10.10	9.90	9.61	9.83	9.95	0.25	0.80
b* 0 h	25.83^{ab}	19.57^{b}	27.65^{ab}	29.97^{a}	28.65^{a}	27.02^{ab}	2.02	0.01
pH 24 h	6.00	6.00	6.01	6.01	6.00	6.01	0.01	1.00
L*24 h	41.47	40.86	41.54	41.96	41.67	41.05	0.68	0.88
a* 24 h	12.61	11.13	12.48	12.32	11.81	12.44	0.65	0.59
b* 24 h	33.31	31.25	33.03	33.82	32.93	32.00	1.85	0.94
pH 48 h	6.04	6.04	6.04	6.02	6.02	6.04	0.02	0.92
L* 48 h	42.38	41.93	42.58	43.25	42.73	42.19	0.81	0.90
a* 48 h	11.46	11.77	11.27	10.35	10.34	11.78	0.63	0.36
b* 48 h	33.48	33.07	32.35	33.51	32.94	32.29	2.87	1.00
Drip loss 24 h (%)	5.49	5.43	5.10	5.74	5.85	5.31	0.30	0.52
Drip loss 48 h (%)	6.37	6.42	6.24	6.62	6.74	6.07	0.29	0.60

^{a,b} means on the same row with different superscripts are significantly differen at $P \leq 0.05$

Experiment 2

Reproductive performance

The reproductive performance of experimental quails is presented in Table 6. The number of eggs, egg production, feed conservation ratio, and egg mass were not affected by the heat factor ($P>0.05$). However, a significantly decreased egg weight showed in group 38 °C L (10.23 g) in comparison to others. A significant difference was also found in feed intake ($P<0.05$), with the lowest amount of feed consumption by birds in group 38 °C L (23.69 g/bird/day).

Table 6 Reproductive performance of six Japanese quail lines from 35 to 105 days old

Criteria (per week)	Treatment						
	Et-(30 °C) H (n=13)	Et-(30 °C) L (n=14)	25 °C H (n=13)	25 °C L (n=13)	38 °C H (n=13)	38 °C L (n=13)	P
Number of eggs	5.26±0.18	5.32±0.18	5.44±0.19	5.09±0.18	5.5±0.18	5.1±0.18	0.52
Egg production (%)	78.02±2.77	77.52±2.71	78.8±2.81	72.77±2.79	78.57±2.75	73.99±2.73	0.51
Egg weight (g)	10.73^{a}±0.11	10.61^{ab}±0.12	10.83^{a}±0.11	10.62^{ab}±0.11	10.48^{ab}±0.13	10.23^{b}±0.14	0.00
Feed intake (g/b/d)	25.02^{ab}±0.3	25.82^{a}±0.29	24.09^{bc}±0.3	23.79+±0.3	23.93^{bc}±0.3	23.69^{c}±0.29	0.00
FCR (g feed/g egg)	2.45±0.11	2.5±0.11	2.58±0.12	2.38±0.12	2.79±0.11	2.78±0.11	0.05
Egg mass (g egg/b/d)	8.56±0.33	8.44±0.32	8.74±0.33	7.88±0.33	8.42±0.33	7.79±0.32	0.24

^{a,b,c} means on the same row with different superscripts are significantly different at $P \leq 0.05$

Egg quality

Parameters of egg quality are presented in Table 7. The obtained results revealed the lowest ($P<0.05$) egg weight and egg height in the 38 °C L group (10.57 g and 30.89 mm, respectively), but no statistical difference was found in egg width and egg shape index. Accordingly, increased temperature affected yolk weight ($P=0.02$), albumin weight ($P=0.01$), albumin height ($P=0.01$), yolk index ($P=0.02$), Haugh unit ($P=0.01$), L* value of yolk ($P=0.001$), and b* value of yolk ($P=0.001$). In contrast, yolk ratio, shell weight, shell ratio, albumin ratio, shell thickness, yolk diameter, yolk height, and a* value of yolk were not influenced by the thermal factor.

Table 7 Egg quality of six Japanese quail lines from 77 to 80 days old

Treatment	Et-(30 °C) H	Et-(30 °C) L	25 °C H	25 °C L	38 °C H	38 °C L	SEM	P
Egg weight (g)	10.43^{b}	10.63^{b}	10.75^{b}	11.58^{a}	10.57^{b}	10.59^{b}	0.19	0.00
Egg height (mm)	31.57^{b}	31.75^{ab}	31.52^{b}	32.66^{a}	31.44^{b}	30.89^{b}	0.25	0.00
Egg width(mm)	25.19	24.90	25.20	25.52	24.84	24.95	0.18	0.10
Egg shape index (%)	79.90	78.45	80.00	78.18	79.02	80.90	0.82	0.17
Yolk weight (g)	3.25^{b}	3.38^{ab}	3.44^{ab}	3.62^{a}	3.29^{b}	3.43^{ab}	0.08	0.02
Yolk ratio (%)	30.66	31.81	32.02	31.27	31.15	33.17	0.75	0.25
Shell weight (g)	1.23	1.23	1.22	1.30	1.27	1.20	0.03	0.18
Shell ratio (g)	11.68	11.54	11.39	11.23	12.04	11.63	0.27	0.39
Albumin weight (g)	6.12^{ab}	6.02^{ab}	6.09^{ab}	6.66^{a}	6.01^{ab}	5.80^{b}	0.16	0.01
Albumin ratio (%)	57.66	56.65	56.60	57.49	56.82	55.20	0.88	0.43
Albumin heght (mm)	4.45^{a}	3.92^{ab}	3.99^{ab}	4.26^{ab}	3.39^{b}	3.54^{b}	0.22	0.01
Shell thickness (mm)	19.98	19.36	19.48	19.94	19.62	19.10	0.37	0.52
Yolk diameter (mm)	24.91	24.84	25.40	25.73	24.81	25.78	0.42	0.35
Yolk height (mm)	8.28	9.03	8.44	8.58	8.58	8.20	0.21	0.10
Yolk index (%)	33.44^{ab}	36.59^{a}	33.21^{ab}	33.44^{ab}	34.67^{ab}	31.86^{b}	0.97	0.02
Haugh unit	90.04^{a}	86.85^{ab}	87.18^{ab}	88.06+	83.36^{b}	84.47^{b}	1.27	0.01
L*	52.94^{cd}	50.82^{d}	58.77^{ab}	54.07^{bcd}	57.52^{abc}	59.76^{a}	1.34	0.00
a*	-1.39	-1.43	-1.67	-1.10	-1.13	-1.27	0.22	0.36
b*	31.79^{abc}	27.82^{c}	35.77^{ab}	36.26^{a}	33.81^{ab}	31.07^{bc}	1.23	0.00

^{a,b,c} means on the same row with different superscripts are significantly different at $P \leq 0.05$

Experiment 3

Growth performance

In the second generation of experimental Japanese quail lines, the growth performance (1-35 days of age) was illustrated in Table 8. Weight gain 1-14 d, feed conversion ratio 1-14 d, feed intake 15-35 d, and feed intake 1-35 d showed a significantly different among six groups of quails ($P<0.05$). By contrast, feed intake 1-14 d, weight gain 15-35 d, feed conversion ratio 15-35 d, weight gain 1-35 d, and feed conversion ratio 1-35 d were not different ($P>0.05$).

Table 8 Growth performance of the G1 generation of six Japanese quail lines

Treatment	Et-(30 °C) H (n=125)	Et-(30 °C) L (n=142)	25 °C H (n=134)	25 °C L (n=116)	38 °C H (n=192)	38 °C L (n=38)	P
Feed intake 1-14 d	5.88±0.01	5.88±0.01	5.88±0.01	5.88±0.01	5.87±0.01	5.89±0.02	0.86
Weight gain 1-14 d	2.71^{b}±0.07	3.01^{a}±0.06	2.80^{ab}±0.07	2.94^{ab}±0.07	2.71^{b}±0.05	2.84a±0.13	0.00
FCR 1-14 d	2.48^{a}±0.09	2.02^{c}±0.08	2.25^{abc}±0.09	2.08^{bc}±0.09	2.37^{ab}±0.07	2.11^{abc}±0.17	0.00
Feed intake 15-35 d	15.08^{a}±0.03	15.06^{a}±0.02	14.95^{b}±0.03	15.11^{a}±0.02	15.09^{a}±0.02	15.10^{ab}±0.05	0.00
Weight gain 15-35 d	4.04±0.07	4.13±0.06	4.08±0.07	4.00±0.07	4.06±0.05	4.21±0.13	0.61
FCR 15-35 d	3.85±0.08	3.78±0.07	3.74±0.08	3.86±0.08	3.72±0.06	3.63±0.16	0.59
Feed intake 1-35 d	11.40^{a}±0.02	11.39^{ab}±0.02	11.32^{b}±0.02	11.42^{a}±0.03	11.40^{a}±0.01	11.41^{ab}±0.04	0.01
Weight gain 1-35 d	3.51±0.05	3.68±0.05	3.57±0.05	3.58±0.05	3.52±0.04	3.66±0.1	0.09
FCR 1-35 d	3.34±0.14	3.16±0.12	3.23±0.14	3.22±0.13	3.11±0.11	3.14±0.26	0.85

^{a,b,c} means on the same row with different superscripts are significantly different at $P \leq 0.05$

Reproductive performance

The reproductive performance of quails in the second generation is presented in Table 9. Most of the reproductive performance parameters, such as the number of eggs, egg weight, egg production, and feed intake, were statistically significant among six quail lines ($P<0.05$), except feed conversion ratio and egg mass were not different ($P>0.05$).

Antioxidant enzymes

Table 10 presented the results of antioxidant enzymes of six quail lines in the second generation. There was no significant difference in CAT among the six groups of birds ($P=0.79$). However, the results showed significant effects ($P<0.05$) of SOD and GSH-Px on the second generation of six quail groups. The highest value of SOD and GSH-Px were found in group Et-(30 °C) L (363.16 ng/ml and 9.89 ng/ml, respectively), and the lowest value was in group 38 °C H (207.1 ng/ml and 7.77 ng/ml, respectively).

Table 9 Reproductive performance of the G1 generation of six Japanese quail lines from 35 to 105 days old

Criteria (per week)	Treatment						
	Et-(30 °C) H (n=14)	Et-(30 °C) L (n=13)	25 °C H (n=13)	25 °C L (n=11)	38 °C H (n=17)	38 °C L (n=7)	P
Number of eggs	5.85^{ab}±0.12	5.79^{b}±0.13	6.34^{a}±0.13	5.77^{b}±0.14	6.02^{ab}±0.11	5.86ab±0.17	0.02
Egg production (%)	83.59^{ab}±1.75	82.73^{b}±1.79	90.55^{a}±1.84	82.51^{b}±2.01	86.05^{ab}±1.6	83.64^{ab}±2.5	0.02
Egg weight (g)	11.49^{ab}±0.09	11.68^{a}±0.09	11.49^{ab}±0.09	11.44^{ab}±0.1	11.17^{b}±0.08	11.43^{ab}±0.12	0.00
Feed intake (g/b/d)	23.14^{abc}±0.13	22.66^{c}±0.14	23.49^{a}±0.14	23.28^{ab}±0.15	23.44^{a}±0.12	22.59^{bc}±0.19	0.00
FCR (g feed/g egg)	2.08±0.06	2.09±0.06	2.21±0.07	2.18±0.07	2.16±0.06	2.02±0.09	0.43
Egg mass (g egg/b/d)	9.55±0.23	9.71±0.23	10.22±0.24	9.44±0.26	9.39±0.21	9.63±0.33	0.16

^{a,b,c} means on the same row with different superscripts are significantly different at $P \leq 0.05$

Table 10 Antioxidant enzymes in serum of the G1 generation of six Japanese quail lines

Treatment	Et-(30 °C) H	Et-(30 °C) L	25 °C H	25 °C L	38 °C H	38 °C L	SEM	P
CAT (ng/ml)	42.78	38.25	37.18	47.62	49.37	29.60	10.65	0.79
SOD (ng/ml)	211.02^{b}	363.16^{a}	232.83^{b}	226.54^{b}	207.10^{b}	227.38^{b}	29.17	0.00
GSH-Px (ng/ml)	8.69^{ab}	9.89^{a}	7.88^{ab}	9.01^{ab}	7.77^{b}	7.91^{ab}	0.50	0.03

^{a,b} means on the same row with different superscripts are significantly different at $P \leq 0.05$

DISCUSSION

Hematological parameters were not affected by thermal stress in this study. No difference was observed among G0 quail treatments of most blood physiological indices. There was a lighter increase in MPV of quails that stayed in group 38 °C. According to Etim et al. (2014), blood constituents change in relation to the physiological status of an animal by several genetic and non-genetic factors. He reported that blood-based parameters of farm animals were often influenced by age, sex, breed, and management system. In addition, the results of blood physiology parameters were compared with reference values of the previous studies (Ali et al., 2012; Agina et al., 2017), and an increase in the RBC, HBC, MCV, MCH, and MCHC values were recorded. These increases could be attributed to different farm environmental variations consisting of geography, climate, housing condition, and feed composition. Besides, the age of quails at blood sampling was young (42 days), and they were healthy and without the disease. The difference in MPV in group 38 °C quails observed in this study is not clearly understood but may be attributed to an error in manual blood sampling.

After 4 h of heat application, body temperature and respiratory rate of 38 °C bird treatment showed the highest values in comparison to others. When increasing the thermal impact, the bird responds by raising their respiratory speed to increase CO_2 loss and maintain the cool humidity in their lungs (Borges et al., 2004), which can lead to a partial reduction of CO_2 in the blood (Etches et al., 1995). Durgun and Keskin, (1998) suggested that heat stress can reduce quail blood CO_2. In addition, the quails increase their respiratory rate to prevent water loss in the skin and lungs since water evaporation becomes an important resource to dissipate heat (Toyomizu et al., 2005). In the previous study about some behavioral traits of Japanese quails rearing in different air temperatures, the 38 °C bird groups expanded drinking and decreased eating behavior. This is evidence of the effect of environmental temperature on the rate of quail respiration. Therefore, the body temperature of birds increases because high metabolism level in their blood under high temperatures. The data on body temperature from our research agree with that obtained by Bobek et al. (1980) and show that the quail's body temperature was significantly elevated in the heat stress group compared to the control group.

Under high temperatures, the activity of CAT, SOD, and GSH-Px is increased significantly to remove excessive free radicals (Hu et al., 2019). However, chronic heat stress can reduce the metabolic capacity of mitochondria, down-regulating antioxidant enzymes, and deplete the body's antioxidant reserves of birds (Akbarian et al., 2016). In this study, the low level of SOD was found in 38 °C bird groups and higher in Et-(30 °C) and 25 °C bird groups, while CAT was similar among treatments, and GSH-Px had a tendency

to be higher in group 38°C and Et-(30 °C) as compared to group 25 °C. The SOD decreased in treatment 38 °C H and 38 °C L was affected by long-term thermal stress during fourteen days. This result is in agreement with Lu et al. (2017) reported that chronic heat stress (32 °C for seven days) reduced SOD. Similarly, our results about the GSH-Px level fitted with the study of Pamok et al. (2009) and Del Vesco et al. (2017) reported that heat stress increased the expression of the GSH-Px gene, which made the high activity of GSH-Px in poultry. However, the CAT level of the six groups of quails was not affected by temperature because their activities are not important as compared to GSH-Px. Catalase (CAT) is an iron-containing enzyme mainly found in different tissues that play a role in decomposing H_2O_2 and eliminating the toxic effect of H_2O_2 (Kumerova et al., 1998) via the Fenton reaction in peroxisomes (Chance et al., 1979) but the GSH-Px distributed widely in the bird's body and also decompose H_2O_2 into H_2O (Yang et al., 2010) by using reduced glutathione in a powerful manner (Dorval and Hontela, 2003). In addition, GSH-Px can promote the conversion of harmful substances produced in the lipid peroxidation reaction into corresponding alcohols and block the chain cycle reaction (Yang et al., 2010). The study of Chartchai and Saowaluck, (2016) revealed the level of CAT and SOD were similar in the chickens of both stocking densities (10 and 15 birds/m^2) and suggested using antioxidant factors in feed was necessary to reduce the detrimental effect of stress on poultry. However, the level of stocking density was 16 birds/m^2, and Charinya et al. (2018) reported a decrease in the level of CAT and SOD activities of chickens compared to the bird in semi-intensive with 10 birds/m^2 stocking density. Supplement tomato pomace (Chartchai and Saowaluck, 2016) and curcumin (Charinya et al., 2018) as antioxidants reduce oxidative stress, activated antioxidant enzyme activities, and improve the growth performance of broilers raised under stress conditions. Alghirani et al. (2022) supplemented 100 mg/kg of *Yucca shidigera* saponins in a broiler diet, was acted as an anti-stress for chicken raised in tropical regions. The percentage of thigh meat increased significantly in group 38 °C and 25 °C (heat stress and cold stress experimental designed, respectively), while carcass dressing (%) tends to be lower in these treatments in comparison to group Et-(30 °C). The reason was discovered that the quail showed more actively under cold stress conditions (25 °C) by increased eating, feather pecking, and jumping but reduced drinking. Heat stress increases birds' drinking (Nguyen et al., 2021) and breathing at high speed with high body temperature. However, thermal stress had a light effect on reducing the dressing of Japanese quails. In agreement with these results, Habibian et al. (2016) and Zeferino et al. (2016) concluded that heat stress can induce negative changes in the carcasses, breast, and thigh meat of quails. In addition, there was no significant difference in liver weight of experimental quails attacked with Bonfim et al. (2016) reported that the carcass and organ yields (except heart and gizzard) of quail were not influenced by environmental temperatures. The current result especially presented that high production traits responded with thermal stress more weakness than low production traits.

Meat quality was not influenced by thermal stress except the yellow index (b*) at fifteen minutes after slaughter was observed to increase in cold stress and heat stress groups. It is related to the low concentration of SOD and GSH-Px in the serum of these birds. It means the free radical appeared highly in stressed birds, leading to the oxidation process of sarcoplasmic and

myofibrillar proteins (which reduces myoglobin protein and the redness value in the muscles) (Zhang et al., 2012). After that, the b* value was not different among treatments at recorded times because of the processing of normal physiological and biochemical changes of meat after slaughter and storage. Our results agreed with Gabriel et al. (2017), which reported an increase in the yellow index at the meat color of the chicken reared in pre-slaughter capture chase duration stress. Still, after 24 h of storage, the b* value was not different among treatments.

Heat stress is still influenced by egg weight and feed intake of laying hens in treatment and continues after finishing the time of thermal application in experiment 2. The data collected from females from 35 to 105 days of age showed the lowest egg weight and feed intake in group 38 °C L. These results are similar to Vercese et al. (2012) presented that high ambient temperature (36 °C) negatively affects bird performance with reduced feed intake; and consequent reductions in egg weight. Besides, Sahin et al. (2002) reported that the egg weight of laying Japanese quail reared under heat stress conditions (34 °C) was decreased by 23.3 % when compared with the control group (22 °C). Several factors, such as reducing appetite and feed intake mechanisms for birds to inhibit heat increment (Sohail et al., 2013). In addition, heat stress damaged intestinal morphology and the activity of digestive enzymes (Sohail et al., 2010) and thyroid hormones (Song et al., 2014) which caused low digestion and metabolism (Chen et al., 2014). Foud et al. (2016) suggested that heat stress negatively affected the reproductive performance of laying hens by decreasing ovary weight, oviduct weight, and oviduct length combined with low feed intake might reduce egg production and egg weight.

The continuous impact of heat stress was shown on the egg quality of experimental quails. Egg weight, yolk weight, albumen weight, egg height, albumen height, yolk index, and Haugh unit were the lowest values in birds in treatment at 38 °C L. It was noted that the low-production quail was influenced strongly by heat stress. Vercese et al. (2012) reported a reduction of egg weight of 5.1 %, 5.9 %, 6.9 %, and 11.9 % in the bird group under cyclic heat stress of 27, 30, 33, and 36 °C, respectively, in comparison with a comfortable temperature (21°C). Sahin et al. (2004) reported that Japanese quail reared under heat stress (34 °C) had significantly decreased Haugh units by 10.8 % and egg weight by 14.3 % in comparison with the control group (raised at 22 °C).

In the first generation (G1) of six quail lines from experiment 1 (G0), the experiment 3 birds revealed no effect of heat stress on growth and reproductive performance; and antioxidant status. In the growing period, the feed intake of G1 quail in group 38 °C L was higher than others, especially the feed consumption of the low-production trait seems higher than the high-production trait. It was explained that the quails G1 adapted to the heat stress from the heat impact of G0. However, In the laying period, the hen G1 in group 38 °C L also decreased feed intake, but their egg weight and egg production still increased compared to other traits. In addition, the bird G1 in group 38 °C expanded the level of SOD and GSH-Px lower than group 25 °C and Et-(30 °C). These results illustrated that heat stress influenced on antioxidant status of G0, which conveyed to G1 quails.

CONCLUSIONS

Under high temperatures (38 °C), G0 Japanese quail increased their body temperature, respiratory rate, and the yellow index in breast meat but reduced the concentration of SOD in serum. In the laying stage, the G0 quails in group 38 °C decreased feed intake, egg weight, and Haugh unit. In the first generation, the 38 °C quail group adapted to heat stress from the G0 generation by expanding feed intake during the growing phase and reducing the feed intake at the laying phase, but egg weight was improved. The antioxidant status of G1 is conveyed from G0.

ACKNOWLEDGEMENTS

This study is funded in part by the Can Tho University Improvement Project VN14-P6 supported by a Japanese ODA loan.

AUTHOR CONTRIBUTIONS

Ngo Thi Minh Suong; design the experexper, investigation, methodology, formal analysis, manuscript preparation
Nguyen Thi Kim Khang; Conceptualization and design the experiment, investigation, supervision, editing, and finalization
Masashi Takahashi; Supervision, editing, and finalization

CONFLICT OF INTEREST

We have no conflict of interest.

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How to cite this article;

Nguyen Thi Kim Khang, Ngo Thi Minh Suong, Masashi Takahashi. Impact of heat stress on blood physiological parameters, body temperature, respiratory frequency, antioxidant status, and carcass quality as well as production performance and egg quality of Japanese quails. Veterinary Integrative Sciences. 2024; 22(3): 831 - 846