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Original article

Effects of heat stress on fattening performance, carcass traits, oxidant/antioxidant status, and hepatic heat shock protein 70 levels in different plumage colors of Japanese quail (*Coturnix coturnix japonica*)

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Abstract

The aim of this study was to investigate the impact of heat stress on production performance and oxidative stress in different plumage colors of Japanese quail. For this purpose, a total of 100 birds were used in this study. The 25 birds belonged to Wild-type (n=25, grey), Tuxedo (n=25, black), Golden (n=25, yellow) and Recessive white (n=25). The birds were reared for 42 days in an environmentally controlled room at 39°C and relative humidity of 60-65%. The body weight, body weight gain (g/bird/day), and feed conversion ratio were not different between the groups ($p>0.05$). However, the feed intake (g/bird/day) of the Wild-type had a higher value than the Tuxedo (black) group counterparts between 15 and 21 days different ($p<0.05$). There was no significant effect of heat stress on the carcass traits ($p>0.05$). Spleen weights were different between the groups ($p<0.05$). The yellow group had the highest spleen weight. The highest MDA level was found in the Recessive White variety, followed by Wild-type (grey), Golden (yellow) and Tuxedo (black), respectively. However, there were no statistical differences amongst the groups ($p>0.05$). There was also no statistical significance in glutathione (GSH) and superoxide dismutase (SOD) levels ($p>0.05$). The heat shock protein 70 kDa (HSP70) level was significantly different between the groups ($p<0.001$). The highest percentage was observed in the Golden (5.06%) and the lowest in the White (1.43%) variety.

There was no superior color variety of Japanese quail regarding fattening performance and carcass traits. It is conceivable that when considering the stress response of the different colors, the Golden group is more sensitive to stress due to the hepatic and cellular level of HSP70.

Key words: biochemistry, carcass yield, heat stress, quail

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Introduction

Quail is a bird species that belongs to the Phasianidae family. The most common domesticated type is the Coturnix quail also known as the Japanese quail, which has a crucial place in poultry farming of developing countries and rural areas with its remarkable features. Some essential features of the quails are fast-growing (maturity at 4-5 weeks), requiring small space for husbandry, short incubation period, and short generation interval (Ophir et al. 2005). 18 different plumage color mutants have been previously described for the Japanese quail. The Wild-type (Pharoah) is the most common one and has grey plumage. The other common varieties are Manchurian Golden, Recessive White (English White), and Tuxedo (Somes Jr 1979).

Raising quails for meat production is a genuine alternative to other animals raised as sources of animal protein (Faitarone et al. 2005). Quail meat is recommended for the low-fat diet because it contains a low amount of fat and cholesterol thanks to its thin skin and low fat accumulation between its tissues (Gecgel et al. 2015). It is an ideal food for all ages due to its high meat yield, low shrinkage during cooking, and being more effortless to cook and easier to serve (Khalifa et al. 2016).

High ambient temperature is one of the most serious problems for poultry production. Heat acclimatization has been experienced in many poultry species for economic losses due to heat stress by thermal conditioning. In addition to poultry production efficiency, high ambient temperature harms also behavioral, physiological, hormonal, and molecular changes that occur during heat stress (Ohtsu et al. 2017). The main factors affecting crossbred farm animal selection by companies or breeders are availability, feed consumption efficiency, and easy adaptation to the existing rearing system (cage or free-range). On the other hand, animals that have different color varieties of species may be a reason for admission regarding the choice of livestock. Under stress conditions, some physiological and cellular changes occur. When an organism faces an oxidant agent such as free oxygen and/or nitrogen radicals one of the products of lipid peroxidation, malondialdehyde (MDA), exists. If unpaired oxygen fails to bind to lipids, it can damage proteins and even DNA for pairing itself (Yu et al. 2016). On the other hand, Glutathione (GSH) and Superoxide dismutase (SOD), which are known as antioxidants, play a role in scavenging oxidants such as MDA. The imbalance of oxidant and antioxidant agents causes "stress" in organisms (Pizzino et al. 2017). At the cellular level, heat shock proteins (HSPs) are activated to overcome stress. HSPs are a large family of molecular chaperones that have very important roles in the biosynthesis of proteins and the maintenance of protein homeostasis (Fernández-Fernández and Valpuesta 2018). The HSP superfamily consists of a series of proteins named according to their molecular weight. Heat shock protein 70 kDa (HSP70) is one of the most activated subfamilies that prevents protein damage in organisms under particular heat stress (Rosenzweig et al. 2019).

In view of this, the current study aimed to evaluate the growth performance and adaptation features of four different Coturnix quail varieties under heat stress.

Materials and Methods

Animals and experimental design

The trial protocol was approved by the Institutional Animal Care and Use Committee of the Elazig Veterinary Control Institution of the Agriculture and Forestry Ministry (approval number 2020/01). A total of 100 *Coturnix coturnix japonica* quail, 25 males and 75 females, were used in this study. The 25 birds, belonging to each of the four plumage color groups were placed in 5-tiered plastic cages with 3 compartments in each tier, with subdivided 5 repetitions according to their initial body weights. The birds were kept in each cage compartment with a stocking density of 0.02 m^2 / 5 birds with one male and four females. The birds were reared for 42 days in an environmentally controlled room at 39°C and relative humidity of 60-65%. The ventilation was provided with a semi-automatic "air inlet window" (Jinmiran Machinery equipment, Shanghai, China). Soybean meal and maize-based standard mixture feed and freshwater were provided on an ad libitum basis (NRC 1994). Feed ingredients are presented in Table 1. The lighting program was implemented as 23L:1D for the first 7 days, 21L:3D for 7-14 days, 19L:5D for 14-21 days, 16L:8D for 21-42 days. Manure was removed weekly from the cages and other optimum rearing conditions were provided as appropriate. Bodyweight, feed intake, and feed conversion ratio were calculated weekly for 42 days for observing the growth performance of the all birds. At the end of 42 days, a total of 10 birds (5 males and 5 females) were slaughtered from each group based on similar body weights. Carcass weights were determined after plucking of feathers and evisceration of giblets. The carcass was cut into pieces as breast, whole leg, back-neck, and wing. The heart, liver, and spleen were weighed using a digital scale. Carcass yield (percentage) was calculated using the following formula:

Carcass yield, % = [dressed carcass weight/slaughter weight] × 100.

Table 1. Feed ingredients and nutritional composition of the feeds.

Ingredients	%	Nutritional composition	%
Maize	51.40	Dry matter	90.40
Wheat barn	9.00	Crude protein	18.00
Soy bean meal (44% CP)	22.00	Crude cellulose	4.40
Corn extract meal	2.00	Crude fat	5.35
Sunflower meal (45% HP)	4.30	Crude ash	10.19
Sunflower oil	3.50	Calcium[1]	2.50
Phosphate of lime	0.88	Phosphorus[1]	0.35
Calcium carbonate	4.50	Natrium[1]	0.18
Limestone	1.43	Lysine[1]	1.00
L-Lysine hydrochloride	0.16	Methionine+Cystine[1]	0.59
L-Threonine	0.12	Threonine[1]	0.76
Natrium bicarbonate	0.16	Triptophane[1]	0.25
Salt	0.20	ME, kcal/kg[1]	2800
Vitamin-mineral premix*	0.35		

* Vitamin-mineral premix (per 1 kg): Vitamin A 15.500 IU; Vitamin D3 3.500 IU; manganase 120 mg; ferrous 40 mg; zinc 100 mg; copper 16 mg; cobalt 200 mg; iodine1.25 mg; selenium 0.30 mg.
[1]: Calculated. Data are presented mean±standard error. [a,b]: mean values with different superscripts within a row differ significantly ($p<0.05$).

Tissue preparation, MDA, GSH, SOD and HSP70 analyses

Breast tissues were homogenized at 20000 rpm (WiseTis HG-15D, Witeg, Germany) with 30-sec intervals in an ice bath. 25 mM Tris-HCl (pH=7.4) was used as homogenization buffer at 1:10 (w/v). The MDA levels were determined spectrophotometrically (UV1280, Schimadzu Corp., Japan) according to Ohkawa et al. (1979). Its principle is based on the spectrophotometric measurement at 532 nm of the pink-colored complex formed by MDA that is the secondary product of lipid peroxidation, and thiobarbituric acid. Tissue GSH levels were determined spectrophotometrically (UV1280, Schimadzu Corp., Japan) according to the method of Ellman (1959). The principle is based on the 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) being reduced by sulfhydryl compounds to form a yellow-colored complex, which is a disulfide compound, and the GSH levels were then determined at a wavelength of 412 nm. SOD enzyme activity of the tissues was determined according to Sun et al. (1988). In this method, SOD activity is based on the reduction of nitro blue tetrazolium (NBT) by superoxide produced by the xanthine/xanthine oxidase system. The colored product formed by the reduction of NBT by the superoxide radicals is measured spectrophotometrically (UV1280, Schimadzu Corp., Japan) and this complex causes maximum absorbance at 560 nm. Semi-dry western blot (Trans-Blot Turbo, Bio-Rad, USA) analyses were performed according to Baykalir and Simsek (2018) for determining HSP70 levels of the liver tissues. β-actin levels of the liver tissues were also determined for normalization. NIH ImageJ image process software (National Institutes of Health, California, USA) was used for the calculation of the bands for obtaining "relative densitometric" (RD, %) data semi-quantitatively.

Statistical analysis

All data (growth performance, carcass traits, antioxidant/oxidant status, and RD values of the HSP70 bands) were subjected to a single-factor analysis of variance test (One-Way ANOVA) using IBM SPSS 22 software after the normality test (Kolmogorov-Smirnov). The Tukey test was chosen as post-hoc for determining the differences between the groups. Statistically, the significance level was accepted when $p\leq0.05$ (Petrie and Watson 2013).

Results

The fattening performance of the birds is presented in Table 2. The body weight, body weight gain (g/bird/day), feed conversion ratio were not different between the groups ($p>0.05$) except for the feed intake

Table 2. Effect of heat stress on fattening performance in four different color varieties of Japanese quail.

Traits	Golden (Yellow)	Tuxedo (Black)	White	Wild-type (Grey)	P value
Body weight, g					
Days 15-21	99.20±1.84	96.64±0.67	96.96±1.60	95.36±4.33	0.752
Days 22-28	143.20±2.14	144.92±0.70	143.00±2.88	140.48±5.02	0.792
Days 29-35	172.72±0.64	170.44±4.65	174.48±4.24	172.04±3.89	0.893
Days 36-42	204.00±4.25	196.48±4.86	193.68±9.59	195.88±5.73	0.689
Body weight gain, g / bird / day					
Days 15-21	2.83±0.05	2.75±0.01	2.77±0.04	2.72±0.12	0.734
Days 22-28	4.09±0.06	4.14±0.02	4.08±0.08	4.01±0.14	0.817
Days 29-35	4.93±0.01	4.87±0.13	4.98±0.12	4.91±0.11	0.897
Days 36-42	5.82±0.12	5.61±0.14	5.53±0.27	5.59±0.16	0.696
Feed intake, g / bird / day					
Days 15-21	16.80±0.62 ^{ab}	17.68±0.53 ^{a}	17.40±0.74 ^{ab}	14.40±1.01 ^{b}	0.028
Days 22-28	26.92±2.64	25.72±2.13	23.20±3.28	23.28±3.49	0.758
Days 29-35	36.12±0.50	37.24±0.21	34.80±1.73	36.28±1.12	0.472
Days 36-42	23.12±2.55	23.40±1.50	19.80±2.12	18.08±3.87	0.440
Feed conversion ratio, g feed / g gain					
Days 15-21	4.24±0.15	3.91±0.14	4.00±0.14	4.89±0.58	0.171
Days 22-28	3.91±0.29	4.12±0.31	4.71±0.57	4.80±0.85	0.613
Days 29-35	3.41±0.05	3.27±0.10	3.60±0.12	3.40±0.11	0.193
Days 36-42	6.59±0.69	6.12±0.52	7.23±0.67	8.98±1.65	0.233

Table 3. Effect of heat stress on some carcass characteristics in four different color varieties of Japanese quail.

Traits	Golden (Yellow)	Tuxedo (Black)	White	Wild-type (Grey)	P value
Slaughter weight, g	201.10±5.19	192.50±7.90	191.70±8.55	192.20±8.60	0.795
Carcass weight, g	130.19±2.78	130.01±3.01	131.69±4.91	132.10±5.09	0.978
Carcass yield, %	65.00	68.00	69.00	69.00	0.363
Breast weight, g	50.97±1.63	49.30±1.37	49.84±2.48	50.14±2.29	0.947
Whole leg weight, g	48.37±1.30	47.94±1.15	49.65±1.55	47.99±1.89	0.836
Wing weight, g	10.50±0.30	10.89±0.44	9.90±0.57	10.49±0.54	0.539
Back-Neck weight, g	20.34±1.61	21.86±0.68	22.28±1.16	23.48±1.67	0.439
Liver weight, g	5.82±0.30	4.64±0.53	5.01±0.70	4.25±0.49	0.207
Heart weight, g	1.50±0.06	1.51±0.06	1.55±0.07	1.63±0.06	0.556
Spleen weight, g	0.17±0.01^{a}	0.08±0.02^{b}	0.10±0.01^{ab}	0.09±0.01^{b}	0.007

Data are presented mean±standard error. ^{a,b}: mean values with different superscripts within a row different significantly ($p<0.05$).

(g/bird/day) between 15 and 21 days ($p<0.05$). The Wild-type (grey) (14.40 g) had a lower value than the Tuxedo (black) group counterparts (17.68 g).

The carcass traits of the birds are given in Table 3. The weights of slaughter, carcass, breast, whole leg, wing, back-neck, liver and heart were similar between

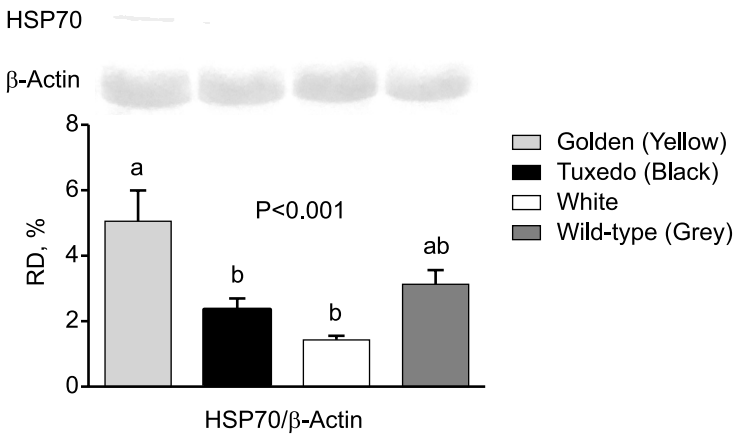

Fig. 1. Effect of heat stress on hepatic HSP70 level in four different color varieties of Japanese quail. Data are presented mean±standard error. [a,b]: mean values with different superscripts within a bar differ significantly ($p<0.05$).

Table 4. Effect of heat stress on oxidant/antioxidant status in four different color varieties of Japanese quail.

Traits	Golden (Yellow)	Tuxedo (Black)	White	Wild-type (Grey)	P value
MDA (nmol /mL homogenate)	3.77±0.38	3.47±0.49	4.18±0.30	3.97±0.26	0.582
GSH (nmol / mg protein)	22.72±1.66	19.06±1.54	19.97±1.00	19.40±1.59	0.300
SOD (% inhibition)	65.89±1.21	66.41±1.86	69.33±1.54	70.14±1.27	0.134

Data are presented mean±standard error.

the groups ($p>0.05$). Spleen weights were different between the groups ($p<0.05$). The yellow group had the highest spleen weight (0.17 g). The white (0.10 g), grey (0.09 g) and tuxedo (0.08 g) varieties had the lowest spleen weight.

The oxidant/antioxidant status of the groups is shown in Table. The highest MDA level was found in the Recessive White variety, followed by Wild-type (grey), Golden (yellow), and Tuxedo (black), respectively. However, there were no statistical differences amongst the groups ($p>0.05$). When GSH levels were examined, the highest value was found in the Golden (22.72 nmol/g protein) followed by White (19.97 nmol /g protein), Grey (19.40 nmol /g protein), and Black genotype (19.06 nmol /g protein), respectively. The SOD level resulted in 70.14% inhibition in the Grey group. It was determined in the White group as 69.33%, 66.41% in the Black, and 65.89% in the Yellow group. There was no statistical significance in GSH and SOD levels ($p>0.05$). The HSP70 level was determined to be significantly different between the groups ($p<0.001$; Fig. 1). The highest level was observed in the Golden (5.06%) and the lowest in the White (1.43%) variety. The Tuxedo (2.36%) and Wild-type (3.13%) varieties revealed similarity with the White group.

Discussion

Coturnix quail are also known as the Japanese quail has an important place in poultry farming of the developing countries and rural areas regarding meeting the protein deficit with low cost (Baykalir and Aslan 2020). In poultry farming, heat stress is known to harm production performance as well as welfare and physiology (Erisir et al. 2020). Numerous studies have been conducted on how to eliminate the negative effects caused by heat stress. One of these strategies could be to select the animal material that is heat resistant in different varieties within the same breed. In this study, as a different approach from the other studies, it was investigated how resistant the four varieties of the Coturnix quail species were to heat stress. In this study, it was seen that no color group was superior to the others when all color groups were compared in relation to fattening performance. However, the feed intake between 15-21 days in the Tuxedo (black) group had a greater value than the other groups. It was followed by White, Yellow, and Wild-type, respectively. The same results were also reported by Inci et al. (2015), who found that Yellow (Golden), Wild-type (grey), Dark brown (Tuxedo), and White color groups had a similar fattening performance. In livestock, feed intake may vary during the growing period. Several factors have an impact on intake such as stress and environmental temperature. On the other hand, the physical characteristics of the diet and its nu-

trient and any anti-nutrient content change the feed intake of the animals. However, the feed intake may change at certain times in the growing period for an unknown reason. Therefore, it would be more appropriate to evaluate feed intake values cumulatively (Barwick et al. 2018). Similarly, the carcass traits had also been found fairly close between the groups in both the current study and in Inci et al. (2015). However, only spleen weight had a higher value in the Golden group. Spleen enlargement may have many causes. The spleen plays a significant role in hematopoiesis and immunosurveillance. On the other hand, spleen weight is highly correlated to body weight. In this study, the Golden group had the highest body weight. In this sense, high spleen weight in the Golden group can be explained by the positive correlation between body weight and spleen weight (Mathuramon et al. 2009).

Stress is described as the "nonspecific response of the body to any demand" (Selye 1950). Heat is one of the phenomena that cause a loss of performance, health, and welfare in livestock. Although there was no statistical difference between the groups in terms of MDA, GSH, and SOD, it was observed that the highest MDA was in the Golden, the highest GSH in the Tuxedo, and the highest SOD in the White group. These findings were intriguing due to different responses of the quail varieties to heat. For example, parallel to the high MDA level, GSH and SOD were not high enough in the Golden group when compared with other color groups. Therefore, these results were evaluated together with the HSP70. According to Gu et al. (2012), there is a negative correlation between HSP70 and MDA levels. Indeed, in the White group, the HSP70 level had the lowest correlation against the high MDA level. However, in this group, the oxygen that demanded to pair itself presented an initial affinity for lipids, and since it paired itself with lipids, HSP70 was not required to be activated. In addition, in the Golden group, overexpression of the HSP70 may support this situation: Unpaired oxygen caused depletion of lipids and after this stage, this fact led to overexpression of HSP70. It was noted that the Wild-type and Tuxedo groups resisted heat stress moderatly by comparison to the Golden and White groups in this study. Moreover, HSP70 may significantly reduce the damage of heat stress, effectively scavenge oxygen free radicals, and improve body oxidant/antioxidant system imbalance (Gu et al. 2012).

Conclusions

According to the findings of the current study, there was no superior color variety of Japanese quail regarding fattening performance and carcass traits. When considering the stress response of the different colors, the Golden group is more sensitive to stress due to the behavior of the unpaired oxygen at the first stage of the stress condition. This is cause unpaired oxygen exceeds the lipids and after reaching the cellular level it activates HSP70. On the other hand, plumage color may have pleiotropic effects on physiological characters that can be either positive or negative. More studies should be conducted to determine which variety of the Coturnix quail has the better performance. Moreover, HSP70 inspections should be used to observe adaption to heat stress in farm animals.

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