

Original Research

The Effect of Heat Stress on Blood Picture of Japanese Quail

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Abstract

This study aimed to evaluate the effect of heat stress on quail hematological parameters. A total number of 300 Japanese quail chicks (*Coturnix Coturnix japonica*) of 28 days old were exposed to different levels of raising temperatures (22, 29, 34, 36 and 40 °C) for 15 days. At 42 days old, blood samples were collected from 14 birds (7 males + 7 females) that were randomly selected. The samples were used for determining red blood cells count, hemoglobin concentration, packed cell volume, total and differential white blood cells count, heterophils/ lymphocyte (H/L) ratio, mean corpuscular hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration. The obtained results indicated that, rearing quail birds under high environmental conditions during fattening period has a great adverse effect on these blood parameters.

Keywords: Heat stress; quail; RBCS; WBCS count; Hb; PCV; differential leukocytes count.

Introduction

Breeding of quail has rapidly increased in the last years. Quails enter a competition with the broilers as a source of meat as the demand for animal protein has increased (Jaap, 1964). The lower tolerance of birds to heat stress in the hot climate is a major limiting factor and a big problem for birds reared in tropic and subtropics regions. High ambient temperature in Egypt during the summer generates a status of stress and evokes a combination of behavioral, biochemical, immunological and physiological changes (Faisal et al., 2008). Exposure to high temperature significantly reduced live weight gain, feed intake, and feed conversion efficiency (Guo-YuMing et al., 1998). High temperature is enough to cause increased body temperature also change circulating leucocyte component in broilers and increased in H/L ratio (Altan et al., 2000a,b). Heat stress not only adversely affects

production performance but also inhibits immune function (Mashaly *et al.*, 2004) and cause a reduction in antibody production in young chicks (Zulk-ifli *et al.*, 2000).

Blood parameters are considered path-physiological indicators of the whole body. A number of hematological indices such as haematocrit value, hemoglobin concentration, red blood cells count and so on, are used to asses the functional status of the oxygen carrying capacity of the blood stream (Maheswaran, 2008). This study aimed to evaluate the effect of heat stress on quail hematological parameters

Materials and methods

Birds

A total number of 300 chicks at one day old were raised up to 4 weeks old. During raising period, the temperature was initially set at 37 °C and gradually reduced at a rate of 3°C/week until 28th days old was reached, then 300 birds were exposed to dif-

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ferent levels of heat stress.

Disinfection

Housing

Birds were reared in an allocated laboratory animal house at the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Assiut University, Egypt.

Chicks were equally divided into 5 groups, each group included 60 chicks that were divided into 4 replicates, and each replicate contained 15 birds. Experimented quails were exposed to heat stress for 15 days (5th-6th weeks) as they were housed under different temperatures as shown in Table 1:

Table 1. Different housing temperature

Groups	Minimum (°C)	Maximum (°C)	Average temperature		
A (control)	21.21±0.98	22.08±1.017	21.62±1.08		
В	29.20±0.87	29.75±0.79	29.47±0.87		
С	33.71±0.78	34.59±.0.76	34.17±0.89		
D	36.03±.0.87	36.77±0.94	36.4±.0.97		
E	39.1±1.37	41.00±1.10	40±1.55		

Temperature

Ambient temperature was determined by maximum and minimum thermometer, where the readings were taken every day and the average of weekly and monthly readings were calculated. Heat was provided by using electrical heater.

Humidity

Indoor relative humidity was measured by using wall mount thermohygrometer. Average relative humidity ranged between 60 to 70%.

Birds were raised at multidecked batteries of 4 decks (only the upper deck of each battery was used in order to maintain the same level of light intensity at the head of all birds to avoid effect of light intensity). Each deck measured 90 x 50 x 30 cm. Square area per bird was maintained as 150 cm²/ bird till 6 weeks old.

A 7 mm square mesh was connected to floor and sides of the cages to prevent chicks escaping through side walls prevent legs injury and help bird's movement.

In the present study the all in –all out management system was utilized, where all birds reared during the experiment at the same age and sold at the end of the experiment Disinfection was carried out by using Biocid-30 (produced by Pfizer) following the procedure outlined by manufacturer.

All manure materials were removed and scrubbed from battery decks, wall and floors. Dust materials were removed from windows, floors, ceiling, walls and ventilation shafts using water and high pressure sprayers, then disinfected by Biocid-30 that were previously diluted to 1:400. All equipments (feeders and drinkers) and removable fittings were demounted and taken out of the building and soaked in 1:600 Biocid-30 dilution baths. The battery units were washed with 1:400 Biocid-30 dilutions.

Day to day management was carried out for keeping the facility cleaned, where the ancillary equipments within the room were washed with water containing potassium permanganate and the contaminated wastes and dead carcasses were hygienically disposed by incineration.

After daily cleaning, cleaned feeders and drinkers were dried and filled with food.

Lighting

Continuous lighting program (23 hours lightning: 1 hour darkness) was used. 60 watt bulb was suspended 2.20 m at head height of the birds. Light intensity at the level of the birds was approximately 2.66 lux/m²/second

Diet and feeding

Ration was formulated and prepared in the Department of Nutrition and clinical Nutrition, Faculty of Veterinary Medicine, Assiut University.

Diet was formulated to contain approximately 24 % Cp and 2.8 Mcal ME / Kg as recommended by NRC (1994).

The birds were fed adlibitum on the mesh diet and given free access to fresh and clean water throughout the experimental period.

The composition and metabolizable energy value of the diet were summarized in Tables 2 and 3.

Chicks were vaccinated with Lasota (Hipraviar-S manufactured by Laboratorios Hipra, S.A., 17170 Amer (Girona) Espana/spain) strain of Newcastle virus in drinking water at 6th days old.

Ingredients	(%)
Yellow corn (ground)	49.5
Soya bean meal	45.0
Dried fat	2.2
Ground lime stone	1.0
Dicalcium phosphate	1.6
Common salt	0.25
Mineral premix	0.10
Vitamin premix	0.12
Methionine	0.13
Lysine	0.10
	100%

	Chemical composition	%
1	Cp(%)	24.08
2	ME -1 (mcalkg ⁻¹)	2.83
3	Ca (%)	0.87
4	TP (%)	0.73
5	CF (%)	4.24

Blood parameters estimation

Blood parameters were estimated According to the recommendations of Magda (1999); Nadia (2003) and Sahin *et al.* (2005). At 42 days old, blood samples were collected from 14 birds (7 males + 7 females) that were randomly selected. About 2 cm³ of blood from each bird was collected in heparinized tube for hematological analysis (Hb, PCV, RBC count, WBCS count, and differential leucocytic count).

Haemoglobin concentration was assayed by a colorimetric method using a commercial kit (Spectrum hemoglobin diagnostic kits manufactured by Egyptian Company for biotechnology, Cairo, Egypt). Hematocrit value was determined by using microhematocrit method. Only 10 blood samples (5 males + 5 females) were used for RBCs and WBCs count according to Natt and Herrick (1952).

Fourteen blood smears (7 females + 7 males), from each treatment group, were prepared and stained using Giemsa stain "EDM" (Egyptian diagnostic media, made in Egypt). A total of 100 white cells were counted and heterophil/lymphocyte ratios were calculated according to Gross and Siegel (1983) and Parga *et al.* (2001). MCV, MCH and MCHC were calculated as reported by Mangrum (1975).

Statistical analysis

The results were expressed as the mean \pm SE. all data were analyzed using one way analysis of variances (ANOVA) followed by LSD TEST using SPSS 11.0 statistical software (Spss, Inc, Chicago, IL, 2001).

Results

The effect of heat stress on different hematological parameters were summarized in Tables 4, 5, 6 and 7.

Discussion

The results in Table (4) cleared that, there is an indirect relation between the heat stress and the decrease in number of RBCs in exposed quail. A significant (p<0.05) decrease in RBCs count was recorded in birds exposed to 40 °C followed by that exposed to 36°C then 34°C, in comparison with that exposed to 22°C. A non significant decrease in RBCs count was observed as temperature increased from 22 up to 29°C.

Similar results were obtained in Japanese quail exposed to 35°C or 42°C heat stress by Sturkie (1986), Osman (1996), Magda (1999) and Nadia (2003). This decrease in total number of RBCs may be due to the inhibition effect of heat stress on the life span of the present RBCs as well as the production of new RBCs from the bone marrow.

Table (4) revealed that, there is a significant relation between heat stress and decrease in number of WBCs. The same results were recorded previously in Japanese quail by McFarlane and Curtis (1989), Magda (1999) and Nadia (2003). These results may be related to atrophy of all lymphoid Organs (thymus, bursa, spleen, or liver) as their weights were significantly reduced by heat stress. This could have been a result of the reduction in feed intake, thereby providing less nutrients for the proper development of these organs (Bartlett and Smith, 2003). Moreover, Gross *et al.* (1980) reported that, exposure of birds to high environmental temperature causes an increase in the plasma corticosterone which subsequently depresses the activity of the lymphoid organs and total leucocytes count.

Analysis of variance of the results In Tabel (4) cleared an indirect relation between the degree of temperature used in heat stress and decrease in packed cell volume in heat stressed quail. A significant (p<0.05) decrease in packed cell volume was recorded in birds exposed to high temperature in comparison with birds exposed to 22 °C. These results are in accordance with those of Yahav and Hurwitz (1996), Nadia (2003) and Gharib *et al.* (2005).

Vo *et al.* (1978) and Deyhim and Teeter (1991) suggested that, the reduced blood hematocrit in heat-stressed birds can be attributed to hemodilution, while, Nadia (2003) mentioned that heat stress lead to significant decrease in mononuclear cells.

Table (5) revealed that, there is indirect relation between the degree of temperature used in heat stress and the decrease in hemoglobin concentration of heat stressed quail. The elevation of the environmental temperature caused a significant decrease in hemoglobin concentration in birds exposed to high temperature, in comparison with birds exposed to 22°C. This result was consistent with the general trend observed in heat stressed quail by Osman (1996), Magda (1999) and Nadia (2003).

Heat stress leads to a decrease in RBCs numbers, the mean corpuscular hemoglobin concentration and live span of red blood cells. This leads to decreased hemoglobin concentration in blood due to the positive relation between the RBCs number and hemoglobin concentration in blood (Vo *et al.*, 1978).

The MCH value estimated the average hemoglobin content of each red cell. The overall means of the MCH values were presented in Table (5), cleared a significant decrease in MCH for heat stressed birds in comparison with birds exposed to 22°C. These results are in agreement with the report of Osman (1996), Magda (1999) and Nadia (2003) who indicated that, heat stress decreases the mean corpuscle hemoglobin value.

The results in Table (5) indicated an indirect relation between the degrees of temperature used in heat stress and the increase in MCV value in heat stressed birds. Only a significant (p<0.05) difference was observed between birds reared at 40 and 22 °C. similar results was obtained in Japanese quail and broilers exposed to 35 and 42°C heat stress by Vo *et al.* (1978), Yahav *et al.* (1997) and Nadia (2003).

MCHC is an index of the proportion of hemoglobin per average red blood corpuscle. The results in Table (5) cleared a significant decrease in MCHC for 34, 36 and 40°C groups in comparison with birds exposed to 22°C. While, a non significant decrease was presented in MCHC for birds exposed to 29 °C in comparison with birds exposed to 22°C. Similar results were obtained in Japanese quail by Osman (1996), Magda (1999) and Nadia (2003).

According to Vo *et al.*, (1978), heat stress leads to a decrease in RBCs numbers, the mean corpuscular hemoglobin concentration and live span of red blood cells. This results in decreased hemoglobin concentration in blood due to the positive relation between the RBCs number and hemoglobin concentration in blood.

The data demonstrated in Table (6) showed a direct relation between the temperature of heat stress and the increase in heterophil cells in exposed quail. The highest significant (p<0.05) value of heterophil cells was recorded in birds exposed to 40 °C, while, the lowest value was observed in birds exposed to 22°C. These results are in accordance with those of Mcfarlane and Curtis (1989), Nadia (2003), Mashaly *et al.* (2004) and Faisal *et al.* (2008).

The results in table (6) cleared a significant decrease in lymphocyte with increasing environmental temperature. Grey *et al.* (1989), Mcfarlane and Curtis (1989), Altan *et al.* (2000 a, b) and Nadia (2003) reported that, exposure of broilers or quail to heat stress results in decreased lymphocyte.

The results in table (6) cleared a positive relation between the degree of temperature used in heat stress and the increase in H/L in quail. Analysis of variance of the results cleared a significant increase in H/L with increasing environmental temperature. These results are in agreement with Osman (1996); Magda (1999); Altan *et al.* (2000a,b); Nadia (2003); Gharib *et al.* (2005); Faisal *et al.* (2008) and Al–Ghamdi (2008). They reported that, Heterophil /Lymphocyte ratio was significantly increased during heat stress. Usama T. Mahmoud et al. / Journal of Advanced Veterinary Research 3 (2013) 69-76

Blood parameter Temperature	RBCS (X 1	0 ⁶ /mm ³)		WBCS (X 1	0 ³ /mm ³)		PCV (%)			
	Males	Females	Overall means	Males	Females	Overall means	Males	Females	Overall means	
22°C	3.77±0.21	3.43±0.09	3.60±0.12ª	22.91±0.26	22.84±0.26	22.88±0.17ª	48.20±1.32	49.60±1.29	48.90±0.90ª	
29 °C	3.60±0.28	2.97±0.06	3.29±0.17ª	20.75±0.48	20.55±0.59	20.65±0.36b	46.00±0.71	45.40±0.51	45.70±0.42b	
34 °C	3.35±0.17	3.07±0.19	3.21±0.13 b	20.45±0.42	20.60±0.42	20.53±0.28b	45.20±1.02	45.20±1.02	45.20±0.68b	
36 °C	3.35±0.13	3.02±0.15	3.19±0.11°	18.93±0.81	19.82±0.38	19.38±0.44b	46.60±0.51	44.00±0.71	45.30±0.59b	
40 °C	3.21±0.15	2.88±0.17	3.04±0.12b	17.20±1.50	17.72±0.98	17.46±0.85°	46.20±0.58	47.20±1.59	46.70±0.82 b	

Table 4 Effect of back status as DDCs as	ount, WBCs count and PCV % of quail chicks at 42 days old
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a-c Overall means with different superscripts in the same column significantly differ (p<0.05)

Table 5. Effect of heat stress on hemoglobin concentration, MCH, MCV and MCHC % of quail chicks at 42 days old

Blood parameters	Hemoglobin concentration (g/dl)			MCH (pg)			MCV (fl)			MCHC (%)		
Temperature	Males	Females	Overall means	Males	Females	Overall means	Males	Females	Overall means	Males	Females	Overall means
22°C	16.66±0.89	15.38±0.69	16.02±0.58ª	44.23±1.08	44.73±0.96	44.48±0.69ª	128.57±4.12	144.85±4.83	136.71±4.04b	34.49±0.98	31.13±1.85	32.81=1.13ª
29 °C	14.50±1.06	12.94±0.59	13.72±0.63b	40.39±1.20	43.46±1.23	41.92±0.96*	132.24±9.53	148.08±0.99	140.16±5.23*	31.04±1.94	29.36±0.95	30.20±1.061*
34 °C	13.91±0.72	12.37±0.25	13.14±0.44 ^{bc}	41.61±1.17	40.75±1.84	41.18=1.04bc	135.93±4.48	149.40±9.08	142.66±5.23*	30.70±1.00	27.40±0.63	29.05±0.783b
36 °C	12.97±0.54	11.96±0.88	12.47±0.52bc	38.78±1.33	39.39±1.09	39.08±0.82¢	138.39±3.68	156.55±2.79	147.47±3.73*	28.05±0.89b	25.24±1.15	26.65±0.830bc
40 °C	12.66±0.54	11.36±0.76	12.01±0.495	39.49±0.99	39.11±2.29	39.30±1.18bc	144.11±4.92	159.58±7.26	151.85±4.87ª	27.49±0.87	25.01±1.54	26.25±0.931 [±]

a-c Overall means with different superscripts in the same column significantly differ (p<0.05)

Differential Leucocytic count Temperature	Heterophils	%		Lymphocyte	es %		H /L ratio			
	Males	Females	Overall means	Males	Females	Overall means	Males	Females	Overall means	
22°C	19.63±0.56	19.75±0.98	19.69±0.55°	73.88±1.69	73.75±1.75	73.81±1.49ª	0.27±0.01	0.27±0.02	0.27±0.0 ^d	
29 °C	27.63±1.16	27.75±0.53	27.69±0.62b	59.75±2.13	59.63±1.98	59.69±1.72b	0.47±0.03	0.47±0.02	0.47±0.01¢	
34 °C	28.75±0.90	28.88±0.58	28.81±0.52 ^b	57.75±1.80	58.00±1.71	57.88±1.52°	0.49±0.02	0.50±0.01	0.50±0.01°	
36 °C	33.88±1.57	33.50±0.89	33.69±0.87ª	53.25±2.18	53.50±1.68	53.38±1.66 ^d	0.64±0.04	0.63±0.02	0.63±0.02b	
40 °C	34.00±1.02	33.88±0.97	33.94±0.68ª	46.00±2.38	46.25±1.65	46.13±1.74*	0.75±0.04	0.73±0.03	0.74±0.02 ³	

Table 6. Effect of heat stress on heterophil %, lymphocyte % and H /L ratio of quail chicks at 42 days old

a-c Overall means with different superscripts in the same column significantly differ (p<0.05)

Table 7. Effect of heat stress on esinophil %, monocyte % and basiophil % of quail chicks at 42 days old

Differential Leucocytic count Temperature	Eosinophils (%)				Monocyte (%)		Basophils (%)		
	Overall means	Females	Males	Overall means	Females	Males	Overall means	Females	Males
22°C	4.38±0.53	4.50±0.38	4.44±0.32 d	1.80±0.20	1.14±0.143	1.42±0.15¢	0.63±0.18	0.75±0.16	0.69±0.12¢
29 °C	7.63±0.65	7.63±0.42	7.63±0.38°	3.67±0.33	2.20±0.20	2.75±0.31 b	1.63±0.42	1.88±0.23	1.75±0.23 b
34 °C	9.25±0.92	9.13±0.44	9.19±0.49 b	2.17±0.17	2.40±0.25	2.27±0.14 b	1.88±0.29	1.75±0.31	1.81±0.21 ^b
36 °C	8.25±0.81	8.75±0.70	8.50±0.54 bc	2.33±0.21	3.17±0.31	2.75±0.22°	1.75±0.37	1.38±0.18	1.56±0.20b
40 °C	10.63±0.73	10.50±0.93	10.56±0.57ª	5.17±0.48	6.75±0.45	6.07±0.38ª	2.75±0.37	2.63±0.38	2.69±0.253

a-c Overall means with different superscripts in the same column significantly differ (p<0.05)

According to Aengwanich and Chinrasri (2003) and Abou El-Soud *et al.* (2006), the H/L ratio measures the physiological change in organs such as an atrophy of the bursa of fabricius and thymus that is influenced by the effect of corticosteroids, as corticosteroids cause the release of heterophils.

Heterophils (H) are granulated leucocytes formed from myelocytes in the bone marrow. They are phagocytic cells designed to define the organism against infection or foreign bodies, such as viruses, bacteria and other particles. They are present in abundance in infection sites to where they attached by chemotactic compounds from injured cells. Lymphocytes (L) are non-granulated leucocytes formed in lymphoid tissues. They lay an important physiological impact in immunity, particularly for the production of antibodies. One of the physiological responses to exposure to stress is the release of glucocorticoids, causing dissolution of lymphocytes in lymphoid tissues leading to lymphopenia. However, there is an increase in heterophil released by bone marrow, thus increasing their numbering circulation, but their phagocytic and bactericidal activity decreased. A result of increase in heterophil and reduction in lymphocyte, the heterophil to lymphocyte ratio altered and has been proposed as sensitive and reliable measures of stress in broiler (Gross and Sigel, 1983; Maxwel and Robertson, 1998).

Table (7) showed a significant increase in esinophil percentage with increasing environmental temperature. Only non significant differences were recorded between 34°C and 36°C groups. Similar results were obtained by Grey et al. (1989) who reported that, exposure of 8 weeks old white leghorn chickens to chronic heat stress (32°C for 4 days) resulted in an increase of about 2.5% in esinophils. On the contrary, these results were disagreed with the finding of Mcfarlane and Curtis (1989) and Altan et al. (2000 a,b) who observed an insignificant decrease in esinophils % in young chicks (10-17 days of age) that exposed to 30.4-34.8 °C hot environment. However, Nadia (2003) stated that, exposing Japanese quail to heat stress resulted in decreased esinophils %.

Concerning the effect of heat stress on Monocyte percentage, Table (7) cleared a significant increase in Monocyte percentage for birds exposed to 29, 34, 36 and 40°C in comparison with birds exposed to 22 °C. These results are in accordance with those of Mcfarlane and Curtis (1989) and Nadia (2003) who stated that heat stress increased monocyte %. The reverse opinion was for Altan *et al.* (2000a,b) who reported that, exposure of the broilers to acute heat stress resulted in decreased monocyte%.

Table (7) indicated a significant increase in basophile percentage for birds exposed to 29, 34, 36 and 40°C in comparison with birds exposed to 22 °C. These results are in agreement with the report of Grey *et al.* (1989) who found that, exposure of 8 weeks old white leghorn chickens to chronic heat stress (32 °C for 4 days) resulted in an increase of about 20 % in basophiles. On the contrary, these results disagreed with finding of Mcfarlane and Curtis (1989) and Nadia (2003) who stated that, heat stress induce significant decrease in basophiles %.

Conclusion

From the obtained results of this study, it could be concluded that, rearing quail birds under high environmental conditions (29, 34, 36 and 40°C) during fattening period lead to adverse effect on quail hematological parameters. Meanwhile, 22°C could be considered as the optimum degree for raising quail chicks. Exposure of Japanese quail to chronic heat stress decreased the number of WBCs, RBCs, PCV %, Hemoglobin concentration (g/dl), and lymphocyte% and increased the heterophils cells%, H/L ratio , eosinophil %, Monocyte %, basophile %.

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