International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(6): 930-934

Received: 13-09-2019
Accepted: 15-10-2019

Aditya Mishra
Assistant Professor, Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Pragati Patel
VAS, Government of Madhya Pradesh, India

Anand Jain
Assistant Professor, Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Jyotsana Shakkarpude
Assistant Professor, Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Amir Amin Sheikh
Ph D Scholar, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu and Kashmir, India

Corresponding Author:
Aditya Mishra
Assistant Professor, Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur, Madhya Pradesh, India

Effect of ascorbic acid supplementation on corticosterone levels and production parameters of white leghorn exposed to heat stress

Aditya Mishra, Pragati Patel, Anand Jain, Jyotsana Shakkarpude and Amir Amin Sheikh

Abstract
Meteorological factors such as high ambient temperature and high relative humidity exert adverse effects on poultry production. Stressful stimuli, such as environmental, pathological and nutritional disturbances, to mention a few, can cause stress like impairing poultry performance and evoking the behavioural and physiological chain reactions. Thermal stress not only reduces production but also causes higher mortality in poultry thus, inflict considerable economic losses. In the present research investigation a possibility was explored to investigate the effect of ascorbic acid on corticosterone level and production parameters during heat stress in White Leghorn birds.A total of 96 White Leghorn birds were randomly divided and maintained in comfort (26±1.0 °C) and heat stressed (40±5.0 °C) conditions. Layers were divided into 4 groups and each group consisting of 12 birds in 2 replicates. G1 group was kept as control. G2, G3 and G4 group was supplemented with 100, 200 and 300 mg ascorbic acid (AA) respectively. The overall mean concentration of plasma HSP70 concentration showed non-significant difference between comfort and heat stressed birds. The overall mean concentration of corticosterone between comfort and heat stressed condition in egg type grower showed significant difference ($p<0.01$) in G1, G2 and G3 groups. In egg type growers, feed efficiency ratio and performance index were significantly higher ($p<0.05$) in G3 group. In layers maximum hen housed egg production (%) was observed in G3 group. The overall mean egg weight (g) of layers in G2 group showed significant ($p<0.05$) difference in comfort condition as compared to heat stressed layers. In the present investigation, on supplementation of ascorbic acid the corticosterone level reduces with improved production performance of White Leghorn birds indicates beneficial effects of ascorbic acid during heat stress.

Keywords: Ascorbic acid, corticosterone, white leghorn birds

1. Introduction
Nearly one third of world-wide broiler and layer stock placement is in Asian countries such as India and China and these countries are emerging as important locations for the production and trade of poultry product. India has distinctly different seasons with variable temperature and humidity. Meteorological factors such as high ambient temperature and high relative humidity exert adverse effects on poultry production. They also cause heat stress in poultry during the hot dry season (Ayo *et al*., 2014) [1]. Heat stress results in poor performance in growth, feed efficiency and egg production as well as higher mortality. Thus, acute exposure of chickens to extreme conditions results in major economic losses.

During heat stress, the most vital hormonal response is an increase in blood level of corticosterone, the primary glucocorticoid hormone produced by the adrenal gland. Ascorbic acid supplementation improved performance of heat challenged broiler chickens and has been associated with a lower plasma level of corticosterone. The inhibitory action of ascorbic acid on adrenal steroidogenesis is mediated via modulation of steroid hydroxylating enzymes in the adrenal gland (Kitabchi, 1967) [6].

Substantial attention has been paid to the role of nutritional additives to minimize the effects of heat stress. The withholding of feed as well as the manipulation of dietary protein content, energy density, calcium, use of carbonated water and usage of vitamin C and E are believed to alleviate the effects of heat stress and upregulation of HSP gene (Mishra, 2015b) [10]. The most significant increase in ascorbic acid demand take place during acute environmental stress such as excessive hot or cold weather and stress conditions increases the metabolic need for this vitamin or that decrease the innate capacity of biosynthesis. Under such conditions, supplementing the poultry diet with vitamin C may have a beneficial effect on performance (Yigit *et al*., 2002) [16].

Hence, in view of the above, the present research work was undertaken to investigate the effect of ascorbic acid on expression of heat shock protein (HSP70) gene, some hormonal and production parameters during heat stress in White Leghorn (WLH).

2. Material and Methods

The research was carried out in the Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, N.D.V.S.U., Jabalpur (M.P.). A total of 96 WLH egg type growers of 10 weeks age were randomly divided into eight groups in the experiment. Four group of birds was maintained in natural summer conditions (May to June) maintained in heat stress (40±5.0 °C) ambience, whereas other four group of birds was maintained in controlled conditions at 26±1.0 °C (comfort temperature) using an air conditioner. Temperature and humidity of the experimental poultry unit was recorded using a digital temperature and humidity recorder. G1 group was taken as control, whereas, G2, G3 and G4 groups were supplemented with 100 mg, 200 mg and 300 mg AA in feed respectively in both comfort and heat stressed condition. Diets were formulated as per NRC specifications.

2.1 Metabolic / hormonal parameters

Blood sampling was done from wing vein on 27th, 34th and 41st day of age. In the laboratory, all the blood samples were centrifuged at 3000 rpm for 30 min and plasma was separated. Plasma obtained was kept in the labeled storage vials of 2 ml capacity and stored at -20 °C till analysis for chicken HSP70, chicken corticosterone. Both parameters were analyzed by competitive inhibition enzyme immunoassay technique.

3. Production performance parameters

3.1 Body weight

The body weight was taken individually at the end of experiment i.e. 30 weeks to know their body weight gain. Weight gain in different groups of layers was calculated considering their body weights.

3.2 Egg production

The egg production was recorded from 18 weeks of age of the birds up to 30 weeks of age. Hen housed egg production (HHEP) was calculated using the following formula as per North (1984).

$$\text{HHEP (\%)} = \frac{\text{Total number of eggs laid during the period}}{\text{Total number of hens housed at the beginning of laying period}}$$

3.3 Egg weight

The egg weights were recorded on weekly basis starting from 18 weeks of age of the birds up to 30 weeks of age.

3.4 Mortality

Mortality was recorded on daily basis and the mortality rate was calculated for the period from 10 to 30 weeks.

3.5 Statistical Analysis of the Data

The recorded data was statistically analyzed using Completely Randomized Design. Various conditions and treatment groups were compared by using Duncan Multiple Range test (DMRT).

4. Results and Discussion

4.1.1 Metabolic / hormonal parameters

4.1.1.1 Plasma HSP70 of egg type of growers

The mean plasma HSP70 concentration of egg type growers has been presented in Table 01. The overall mean concentration of HSP70 showed non-significant difference between comfort and heat stressed birds in all the groups. However, in comfort condition, non-significant difference was observed between all the groups. A significantly ($p<0.01$) higher concentration of HSP70 (1.33±0.33 ng/ml) was observed in heat stressed control group as compared to other heat stressed treatment groups with varying concentrations of ascorbic acid supplementation.

In the present investigation exposure of control group of chickens to heat stress lead to significant increase in the concentration of HSP70, which was reduced subsequently after supplementation of varying concentration of AA. This might be the fact that HSP70 functions are energy dependent. The HSP70 functions protect newly synthesized proteins during and after heat stress. When HSP70 are induced as a result of cellular exposure to a stressor, the cell will decrease normal protein production and divert its energy resources toward mechanisms of survival (HSP70 induction). Present findings are in agreement with findings of Maak *et al*. (2003) [8]. They reported that moderate heat stress caused significantly increase in HSP70 levels as compared with the control groups in laying hens. Sahin *et al.* (2009) [13] reported that heat stress increased the level of HSP70 in brain and ovary of Japanese quails kept in control conditions, which was further reduced on supplementation of AA in different treatment groups. Present findings are in agreement to reports of Sahin *et al.* (2009) [13].

Table 1: Mean plasma HSP70 concentration (ng/ml) of egg type growers at different intervals

Period	Condition	G1	G2	G3	G4
27th day	Comfort	$0.93^{a} \pm 0.29$ (12)	$0.56^{ab} \pm 0.14$ (12)	$0.42^{b} \pm 0.17$ (12)	$0.57^{ab} \pm 0.13$ (12)
	Heat	$1.10^{a} \pm 0.86$ (12)	$0.39^{b} \pm 0.13$ (12)	$0.26^{b} \pm 0.02$ (12)	$0.36^{b} \pm 0.072$ (12)
34th day	Comfort	$1.02^{a} \pm 0.45$ (12)	$0.85^{b} \pm 0.02$ (12)	$0.63^{b} \pm 0.26$ (12)	$0.71^{b} \pm 0.45$ (12)
	Heat	$1.43^{A} \pm 0.44$ (12)	$0.75^{B} \pm 0.01$ (12)	$0.56^{B} \pm 0.05$ (12)	$0.58^{B} \pm 0.33$ (12)
41st day	Comfort	$0.91^{a} \pm 0.70$ (12)	$0.63^{b} \pm 0.14$ (12)	$0.47^{b} \pm 0.22$ (12)	$0.66^{b} \pm 0.42$ (12)
	Heat	$1.36^{A} \pm 0.50$ (12)	$0.57^{B} \pm 0.20$ (12)	$0.41^{B} \pm 0.19$ (12)	$0.56^{B} \pm 0.03$ (12)
Overall mean	Comfort	0.97 ± 0.23 (36)	0.62 ± 0.09 (36)	0.55 ± 0.15 (36)	0.67 ± 0.17 (36)
	Heat	$1.33^{A} \pm 0.33$ (36)	$0.54^{B} \pm 0.10$ (36)	$0.41^{B} \pm 0.09$ (36)	$0.38^{B} \pm 0.05$ (36)

Means bearing different superscripts within same row differ significantly (AB; $p<0.01$, ab; $p<0.05$).
Comfort (26±1 ºC), Heat (40±5 ºC)
G1 (Control), G2 (100 mg AA), G3 (200 mg AA), G4 (300 mg AA)

4.1.2 Plasma corticosterone (CORT) of egg type growers
The mean plasma corticosterone concentration of egg type growers has been presented in Table 02. The overall mean concentration of CORT between comfort and heat stressed condition showed significant difference (p<0.01) in G1, G2 and G3 groups. In heat stressed birds, all the groups showed higher concentration of CORT as compared to those maintained in comfort condition. Significantly higher concentration of CORT was observed in birds maintained in heat stressed condition as compared to birds maintained in comfort condition. The present findings are in agreement with Sahin *et al.* (2003) [12] they, reported that at temperatures above or below thermoneutral zone, corticosteroid secretion increases as a response to stress. Supplementing the diet with AA before and during heat stress might elevate the adrenal stores and prevent or delay CORT depletion into the circulatory system (Wilbur and Walker, 1978) [15]. By decreasing synthesis and secretion of corticosteroids, vitamin C alleviates the negative effects of stress (Lara and Rostagno, 2013) [7]. Similar results were also found by Mishra *et al.* (2015a) [9]. They reported that Plasma CORT levels in control group of chickens were increased significantly in response to heat stress in comparison to levels seen in the AA-fed chickens.

Table 2: Mean plasma corticosterone concentration (ng/ml) of egg type growers at different intervals

Period	Condition	G1	G2	G3	G4
27th day	Comfort	5.07^{AQ} ± 0.18 (12)	4.21^{BQ} ± 0.16 (12)	2.26^{Dq} ± 0.28 (12)	3.10^{Cq} ± 0.16 (12)
	Heat	7.97^{AP} ± 0.11 (12)	5.90^{BP} ± 0.10 (12)	3.17^{Dp} ± 0.18 (12)	3.92^{Cp} ± 0.24 (12)
34th day	Comfort	6.61^{Aq} ± 0.08 (12)	5.40^{B} ± 0.22 (12)	3.38^{C} ± 0.39 (12)	4.04^{C} ± 0.11 (12)
	Heat	9.40^{Ap} ± 0.43 (12)	6.87^{B} ± 0.36 (12)	4.57^{C} ± 0.06 (12)	5.37^{BC} ± 0.37 (12)
41st day	Comfort	5.98^{Aq} ± 0.21 (12)	4.56^{Bq} ± 0.32 (12)	2.80^{C} ± 0.43 (12)	3.84^{BC} ± 0.15 (12)
	Heat	8.22^{Ap} ± 0.28 (12)	6.13^{Bp} ± 0.18 (12)	4.31^{C} ± 0.46 (12)	4.43^{C} ± 0.30 (12)
Overall mean	Comfort	5.56^{AQ} ± 0.24 (36)	4.52^{BQ} ± 0.17 (36)	2.59^{DQ} ± 0.24 (36)	3.44^{Cq} ± 0.16 (36)
	Heat	8.18^{AP} ± 0.15 (36)	6.08^{BP} ± 0.13 (36)	3.56^{DP} ± 0.22 (36)	4.21^{Cp} ± 0.23 (36)

Means bearing different superscripts (^{ABCD}) within same row differ significantly (p<0.01).
Means bearing different superscripts within same column differ significantly (^{PQ}; p<0.01, ^{pq}; p<0.05).
Comfort (26±1 °C), Heat (40±5 °C)
G1 (Control), G2 (100 mg AA), G3 (200 mg AA), G4 (300 mg AA)

4.2 Production performance parameters
4.2.1 Body weight of layers
The mean body weight (g) of layers at 20 and 30 weeks of age has been presented in Table 03. Non-significant difference in mean body weight (g) was observed between comfort and heat stressed condition in all the groups. Puthpongsiriporn *et al.* (2001) [11] reported that vitamin C had no significant effect on body weight during heat stress in WLH layers, which is similar to present findings. In comfort condition, at 30 weeks of age, the maximum body weight (1343.66±38.40g) was attained in G3, supplemented with 200 mg AA and minimum body weight (1212.91±23.88 g) was attained in control group. In heat stressed condition, the maximum body weight (1328.41±28.04 g) was attained in G3, supplemented with 200 mg AA and minimum body weight (1180.91±26.35 g) was found in control group.

Table 3: Mean body weight (g) of layers at 20 and 30 weeks of age

Week	Condition	G1	G2	G3	G4
20	Comfort	1146.75±21.07	1177.33±18.09	1270.75±34.72	1201.83±19.80
	Heat	1121.00±23.08	1184.66±21.34	1245.75±23.23	1201.58±29.23
30	Comfort	1212.91±23.88	1244.40±20.79	1343.66±38.40	1268.08±23.39
	Heat	1180.91±26.35	1255.17±23.99	1328.41±28.04	1272.16±33.02

Comfort (26±1 °C), Heat (40±5 °C)
G1 (Control), G2 (100 mg AA), G3 (200 mg AA), G4 (300 mg AA)

4.2.2 Egg production of layers
The Hen housed egg production (HHEP) of birds has been presented in Table 04. Hen housed egg production (%) showed non-significant difference between comfort and heat stressed condition in all the groups during entire experimental duration. Kassim and Norziha (1995) [4] reported that egg production was slightly increased, though not significantly, when the layers received AA supplementation in the feed. However, in comfort condition maximum HHEP (92.25) was found in G3 group, supplemented with 200 mg AA and minimum HHEP (82.61) was found in G1 group. In heat stressed condition, maximum HHEP (89.27) was found in G3 group, supplemented with 200 mg AA and minimum HHEP (79.90) was found in control group.

Table 4: Hen housed egg production and mortality (%) of layers up to 30 weeks age

Parameter	Condition	G1	G2	G3	G4
Hen housed egg production (%)	Comfort	82.61	85.60	92.25	91.38
	Heat	79.90	83.45	89.27	88.64

Comfort (26±1 °C), Heat (40±5 °C)
G1 (Control), G2 (100 mg AA), G3 (200 mg AA), G4 (300 mg AA)

4.2.3 Egg weight
The mean egg weight (g) of layers has been presented in Table 05. The overall mean egg weight (g) of layers in G2 group showed significant (p<0.05) difference in comfort condition as compared to heat stressed layers. However, non-significant difference was observed between comfort and heat stressed layers in G1, G3 and G4 groups. In comfort condition, G1 showed significant (p<0.05) difference from G3 and G4, whereas, non-significant difference was noted between G1, G2; G2, G4 and G3, G4 groups. The maximum

egg weight (53.32±0.72 g) was found in G3 group which was supplemented with 200 mg AA and minimum (49.92±0.20 g) egg weight was found in G1 group which was kept as control group. In heat stressed condition, G1 and G2 showed significant ($p<0.01$) difference from G3 and G4, whereas, non-significant difference was found between G1, G2 and G3, G4 groups. The maximum egg weight (53.51±0.42 g) was found in G4 group which was supplemented with 300 mg AA and minimum egg weight (49.22±0.26 g) was found in G1 group which was kept as control group. Khan and Sardar (2005) [5] reported that the mean egg weight and egg number in layers supplemented with vitamin C was higher ($p<0.05$) than non-supplemented groups whereas, non-significant difference in egg production was also reported. This indicates that vitamin C was effective in improving egg weight consistently in layers during summer. These results are in accordance with the present findings

Table 5: Egg weight (g) of layers during experimental period

Week	Condition	G1	G2	G3	G4
18-20	Comfort	50.02 ± 0.80	50.76 ± 0.95	50.77 ± 2.27	49.48^{q} ± 0.52
	Heat	48.19 ± 0.37	50.57 ± 0.48	50.54 ± 1.39	52.40^{p} ± 0.08
21-23	Comfort	50.12^{b} ± 0.22	51.86^{ab} ± 1.17	53.69^{a} ± 0.31	54.07^{a} ± 0.65
	Heat	49.60^{B} ± 0.41	50.28^{B} ± 0.19	54.03^{A} ± 0.11	53.76^{A} ± 1.05
24-26	Comfort	49.83^{B} ± 0.58	51.38^{B} ± 0.78	54.45^{A} ± 0.20	54.08^{A} ± 0.18
	Heat	49.33^{B} ± 0.33	50.26^{B} ± 0.53	54.13^{A} ± 0.18	54.00^{A} ±1.05
27-30	Comfort	49.70^{B} ± 0.31	51.41^{B} ± 0.72	54.41^{A} ± 0.59	54.27^{A} ± 0.07
	Heat	49.76^{B} ± 0.20	50.02^{B} ± 0.02	54.37^{A} ± 0.16	53.89^{A} ± 1.07
Overall mean	Comfort	49.92^{c} ± 0.20	51.35^{bcp} ± 0.38	53.32^{a} ± 0.72	52.97^{ab} ± 0.77
	Heat	49.22^{B} ± 0.26	50.27^{Bq} ± 0.16	53.26^{A} ± 0.65	53.51^{A} ± 0.42

Means bearing different superscripts within same row differ significantly (^{AB}; $p<0.01$, ^{ab}; $p<0.05$).
Means bearing different superscripts within same column differ significantly (^{pq}; $p<0.05$).
Comfort (26±1°C), Heat (40±5 °C)
G1 (Control), G2 (100 mg AA), G3 (200 mg AA), G4 (300 mg AA)

4.3 Mortality
The mortality (%) of layers has been presented in Table 05. On perusal of data, it was observed that highest mortality in layers was found in heat stressed control group. In comfort condition, only G1 group (control group without any supplementation) showed mortality (09.09%), as compared to other treatment groups. However, in heat stressed condition, the highest mortality (18.18 %) of layers was found in G1 group (heat stressed control group without any supplementation) whereas; no mortality was observed in G2, G3 and G4 groups. Ciftci *et al.* (2005) [3] reported that mortality was significantly decreased in AA supplemented birds as compared to the controls ($p<0.05$), which is similar to present findings. It is speculated that levels of serum corticosteroids increase as the result of the increased activity of the adrenal gland due to stress, which then causes suppression of cell proliferation factor or interleukin-2 (Siegel, 1995) [14].

Table 5: Hen housed egg production and mortality (%) of layers up to 30 weeks age

Parameter	Condition	G1	G2	G3	G4
Mortality rate (%)	Comfort	09.09	00.00	00.00	00.00
	Heat	18.18	00.00	00.00	00.00

Comfort (26±1°C), Heat (40±5 °C)
G1 (Control), G2 (100 mg AA), G3 (200 mg AA), G4 (300 mg AA)

5. Conclusion
In the present study, Plasma HSP70 and corticosterone status of egg type growers reveals that 200 mg ascorbic acid supplementation in feed is most effective in combating the rigors of heat stress, followed by 300 mg ascorbic acid supplementation. Improved production performance of chickens indicates beneficial effects of ascorbic acid during heat stress. No mortality was reported in groups supplemented with 200 mg and 300 mg ascorbic acid in both comfort and heat stressed chickens. However, control group recorded 18.18 % mortality in heat stressed layers.

6. Acknowledgements
The authors would like to acknowledge, M.P. Biotechnology Council, Bhopal (M.P) for providing financial assistance for the research project.

7. References
1. Ayo JO, Egbuniwe I, Kawu MU, Sinkalu VO. Ameliorative effects of betaine and ascorbic acid administration to broiler chickens during the hot dry season in Zaria: A Review. Afr. J Biotechnol. 2014; 13:2295-2306.
2. Chen XY, Wei PP, Xu SY, Gen ZYT, Jiang RS. Rectal temperature as an indicator of heat tolerance in chickens. Anim. Sci. J. 2013; 84:737-739.
3. Ciftci M, Nihat EO, Guler T. Effects of vitamin E and vitamin C dietary supplementation on egg production and egg quality of laying hens exposed to a chronic heat stress. Rev. Méd. Vét. 2005; 156:107-11.
4. Kassim H, Norziha I. Effects of ascorbic acid (vitamin C) supplementation in layer and broiler diets in the tropics. Asian Austral. J Anim. Sci. 1995; 8:607-610.
5. Khan SH, Sardar R. Effect of vitamin supplementation on the performance of Desi, Fayoumi and commercial white leghorn chicken exposed to heat stress. Pak. Vet. J. 2005; 25:163-166.
6. Kitabchi AE. Ascorbic acid in steroidogenesis. *Nature*, 1967; 215:1385-86.
7. Lara LJ, Rostagno MH. Impact of heat stress on poultry production. Animals, 2013; 3:356-369.
8. Maak S, Melesse A, Schmidt R, Schneider F. Effect of long term heat exposure on peripheral concentrations of HSP-70 and hormones in laying hens with different genotypes. Br. Poult. Sci., 2003; 44:133-38.
9. Mishra A, Patel P, Singh HS, Baghel RPS. Can ascorbic acid supplementation alter plasma HSP70 and corticosterone levels during heat stress in commercial broilers? Vet. Pract., 2015a; 16(2):250-253
10. Mishra A, Patel P, Singh HS, Baghel RPS, Goyal G, Pankaj PK. Effect of ascorbic acid supplementation on

mRNA expression of HSP70 gene in broilers exposed to heat stress. Ind. J Poult. Sci., 2015b; 50(3):282-287
11. Puthpongsiriporn U, Scheideler SE, Sell JL, Beck MM. Effects of vitamin E and C supplementation on performance, *in vitro* lymphocyte proliferation, and antioxidant status of laying hens during heat stress. Poult. Sci. 2001; 80:1190-1200.
12. Sahin K, Sahin N, Onderci M, Gursu MF, Issi M. Vitamin C and E can alleviate negative effects of heat stress in Japanese quails. J Food Agric. Environ. 2003; 2:244-249.
13. Sahin N, Tuzcu M, Orhan C, Onderchi M, Eroksuz Y, Sahin K. The effect of Vitamin- c and E supplementation on heat shock protein 70 response of ovary and brain in heat stressed quail. Br. Poult. Sci. 2009; 50:259-265.
14. Siegel HS. Stress, strains and resistance. Br. Poult. Sci., 1995; 36:03-22.
15. Wilbur VA, Walker BL. Dietary ascorbic acid and resistance of guinea pigs to cold stress. Nutr. Rep. Int., 1978; 17:315-320.
16. Yigit AA, Dikicioglu T, Yarim G. Effects of increases in vitamin C supplementation in the laying hen rations on serum concentrations of vitamin C and vitamin. Rev. Méd. Vét., 2002; 153:563-566.