



## Biochemical Parameter Derangement in Commercial Broilers during Heat Stress and Their Mitigation using Ascorbic Acid Supplementation

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Received: 05 Jan., 2021

Revised: 21 Jan., 2021

Accepted: 28 Jan., 2021

### ABSTRACT

Poultry production suffers huge losses due to heat stress, which is caused by high temperatures in many areas during the summer. Therefore, maximum production requires the elimination of the deleterious impacts of environmental stressors. The aim of this study was to check the amelioration of heat stress in commercial broilers by ascorbic acid supplementation on biochemical parameters viz., effect on plasma ascorbic acid, plasma albumin, plasma glucose, breast muscle pH, thio-barbituric acid value. A total number of 96 birds were randomly divided into 8 groups, 4 groups of birds were kept at (37±5.0°C) in heat stress conditions, while 4 other groups of birds were kept at 26±1.0°C (comfort temperature) using an air conditioner and each group consist of 12 birds. G1 was taken as control whereas G2, G3 and G4 were supplemented with 100 mg, 200 mg and 300 mg of ascorbic acid respectively. G4 group supplemented with 300 mg of ascorbic acid showed better results with overall plasma ascorbic acid values (6.84 ± 0.14) as compared to other supplemented and non-supplemented groups. However, G3 group supplemented with 200 mg of ascorbic acid showed better results with overall plasma glucose (310.39± 3.60) and plasma albumin (4.07±0.14) values. On 45<sup>th</sup> day, G3 group supplemented with 200 mg of ascorbic acid showed better results with respect to Breast muscle pH (6.2) and thiobarbituric acid (2.18) values. This shows Vitamin C/Ascorbic acid has potential antioxidant effect able to modulate physiological adjustments to mitigate the undesirable effects of exposure of broilers to high temperatures.

### HIGHLIGHTS

- Supplementation with 300 mg of ascorbic acid showed better results with overall plasma ascorbic acid values as compared to other supplemented and non-supplemented groups.
- Supplementation with 200 mg of ascorbic acid showed better results with overall plasma glucose and plasma albumin values.
- On 45<sup>th</sup> day, supplementation with 200 mg of ascorbic acid showed better results with respect to Breast muscle pH and thiobarbituric acid.

**Keywords:** Ascorbic acid, Biochemical parameters, Broilers, Heat Stress

During the current century, climate change, defined as the long-term imbalance of environmental factors such as temperature, radiation, wind and rainfall characteristics of a given region, is likely to be one of the main challenges facing mankind. The climate of the earth has warmed in the last century (0.74±0.18°C) with the 1990s and 2000s being

the warmest instrumental record. Changes in environmental

**How to cite this article:** Sheikh, A.A., Mishra, A., Nabi, B. and Sofi, O.M. (2021). Biochemical parameter derangement in commercial broilers during heat stress and their mitigation using ascorbic acid supplementation. *J. Anim. Res.*, 11(1): 41-49.

**Source of Support:** None; **Conflict of Interest:** None



variables, such as temperature, humidity and radiation, have been identified as possible threats to the growth and development of all domestic animal species, including poultry. The elevated ambient temperature accompanied by high humidity induced additional discomfort and increased the level of stress, resulting in physiological and metabolic activity depression. High ambient temperatures outside the range of the thermo-neutral zone in poultry have been recognised as one of the most fatal stressors among different environmental conditions, which adversely affect feed intake, growth rate, immunity and mortality (Niu *et al.*, 2009). Therefore, it is becoming increasingly important to avoid and alleviate heat stress (HS) toward summer high ambient temperatures. When chickens are exposed to high temperatures, to preserve body homeostasis, they try to reduce their body temperature within a limited range by self-thermal control. In particular, poultry exposure to summer HS is sufficiently stressful to induce their metabolic rate and physiological responses to deal with the thermal environment (Puthongsiriporn *et al.*, 2001). In fact, the processes of body homeostasis are involved in an endogenous cell defence mechanism that allows cells to deal with stressful HS that causes inflammation and oxidative stress (Molvarec *et al.*, 2011).

If these environmental changes may not be reversed or changed by the animal or the cell, homeostasis may be disrupted and even death can occur. The withholding of feed, as well as the manipulation of the content of dietary protein, energy density and calcium, the use of carbonated water and the use of vitamins C and E are the approaches that are believed to reduce the effects of heat stress (Pardue and Taxton, 1986). The magnitude of the deleterious effects of heat stress is determined not only by its magnitude, but also by the status of cellular systems, particularly cellular antioxidant enzymes such as superoxide dismutase and glutathione peroxidase, as well as water or lipid soluble antioxidants such as ascorbic acid (AA) and  $\alpha$ -tocopherol (vitamin E) (Pigeolet *et al.*, 1990). Thus, this experiment focused on the effect of ascorbic acid supplementation on certain plasma biochemical parameters in broiler chickens during cyclical heating episodes.

## MATERIALS AND METHODS

The proposed 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.), India. Jabalpur is located at latitude 23°10'N and longitude 79°56'E at an altitude of 411 meters in central India. It has a humid subtropical climate, typical of North-Central India.

## Birds and management

A total of ninety-six (96) day-old chicks of commercial broiler birds were collected from private hatcheries. With prior approval from the Institutional Animal Ethics Committee, the birds were housed in the battery cage system in a well-ventilated room in the poultry experimental unit at the college. During the early era (brooding period) of growth artificial heat was given to chicks using thermostatically controlled electric brooders. By blow lamping, the battery brooders were cleaned, washed and disinfected and the complete house was fumigated four days before the start of the experiment using formaldehyde and potassium permanganate. With detergent, feeders and waterers were carefully washed. In each enclosure, aluminium plates of a reasonable size and small tin boxes were used to provide water during the early weeks. Due care was taken so that the chicks reach the feeder and waterer in the first week of age. In the first week of maturity, due care was taken so that the chicks hit the feeder and waterer. Later, feeders and waterers of large sizes were added to each cage in the opposite direction. During the experiment, the all-mash feeding system was introduced. Birds were made available all the time with new and safe drinking water. Thus, uniform, brooding, feeding, watering and housing conditions were provided for all the experimental groups throughout the entire period of research. Broiler birds were randomly divided into eight groups. Four groups of birds were kept at heat stress ( $37\pm 5.0^{\circ}\text{C}$ ) in heat stress conditions (May to June), while four other groups of birds were kept at  $26\pm 1.0^{\circ}\text{C}$  (comfort temperature) using an air conditioner. During the experimental phase, broilers were held closed in a ventilated system for 45 days. The temperature and moisture of the experimental poultry unit was recorded using a digital thermo-hygrometer. Diets were developed in accordance with the requirements of the NRC (1994) provided in table 1.

**Table 1:** Formula and chemical composition of broiler ration

Ingredients	Starter %	Finisher %
Maize	58.805	59.50
Soybean	28	26
Sunflower meal	5	2.5
Fish meal	5	3
Limestone	1.0	0.8
Di-calcium phosphate	1.5	1.1
Salt	0.2	0.2
DL- Methionine	0.06	0.04
Trace mineral Premix	0.1	0.1
Vitamin premix*	0.15	0.15
Vitamin B complex**	0.015	0.015
Choline chloride	0.05	0.05
Toxin binder	0.05	0.05
Protexim	0.02	—
Coccidiostat	0.05	0.05
De-oiled rise bran	—	1.42
Rape seed meal	—	5
Lysine	—	0.02
Total	100	100
<b>Nutrient Composition</b>		
Crude protein (%)	21.66	18.98
Metabolizable energy (Kcal. ME/Kg)***	2843	2850
Calcium (%)	1.17	1.17
Available phosphorus (%)	0.496	0.5
Lysine (%)	1.24	1.22

\*Trace mineral Premix: Mg-300, mn-55, I-0.4, fe-56, Zn-30 and Cu-4kg-1; \*\* Vitamin premix: Vitamin A-8250 IU, Vitamin D<sub>3</sub>-1200 IU, Vitamin K-1mg, Vitamin B1-2mg, Vitamin B<sub>2</sub>-4mg; Vitamin B<sub>12</sub>-10mg, Percent of values specified by NRC, 1994, \*\*\* Calculated.

### Blood sampling

Blood was obtained from individual birds on the stated day of the experiment for analysis of biochemical parameters, i.e. on the 15<sup>th</sup>, 30<sup>th</sup> and 45<sup>th</sup> days. By cleaning the area by plucking the feather and wiping the area with an ethanol swab, the blood samples were collected. For blood collection, a 22 gauge needle was used. Both samples of blood were centrifuged for 30 min at 3000 rpm and plasma was isolated. In the labelled storage vials of 2 ml size, the plasma obtained was held and stored at -20 °C until further examination.

### Biochemical analysis

The plasma ascorbic acid was estimated using DCIP method described by (Omaye *et al.*, 1979). Plasma glucose concentration was calculated by Trinder's method (Pileggi and Szuskeiweiz, 1974), using diagnostic kits obtained from Erba Diagnostics, Mannheim GmbH, Germany. The blood glucose concentration was represented as mg/dl. The concentration of plasma albumin was calculated using diagnostic kits obtained from Erba Diagnostics, Mannheim GmbH, Germany, according to the method defined by Dumas *et al.* (1972). The plasma albumin concentration was expressed in g/dl of albumin.

The pH was calculated using the iodoacetate method modification stated by Petracci *et al.* (2004). Approximately 2.5 g of breast meat was removed from the cranial end of each fillet, minced with a mincer, homogenised for 30 seconds in 25 mL of 5 mM iodoacetate solution containing 150 mM potassium chloride and a pH metre was used to assess the pH of the homogenate. Lipid peroxidation for TBA-reactive substances was calculated by a micro-method (TBARS). Approximately 100 mg of liver was incubated in 0.5 mL of 50% trichloroacetic acid containing 1.3% (wt/ vol) thiobarbituric acid (dissolved at 60 °C) and heated for 1 h at 60 °C, accompanied by a supernatant absorption determination at 532 nm. Tetraethoxypropane, which spontaneously decomposes to form malondialdehyde (MDA) in an aqueous setting, was used as a norm and MDA equivalents were expressed as absorbance. MDA equivalents were determined after blank (water) subtraction, turbidity correction estimated at 650 nm and TBA reagent dilution from the water present in the meat.

### Statistical analysis

The reported data was analysed statistically using Completely Randomized Design (Snedecor and Cochran, 1994). Using the Duncan Multiple Range test, various conditions and treatment groups were compared (DMRT).

### RESULTS AND DISCUSSION

The data recorded and analysed for plasma ascorbic acid are shown in Table 2. The overall mean concentration of AA showed non-significant difference between comfort and heat stressed birds in ascorbic acid supplemented

**Table 2:** Mean plasma ascorbic acid concentration (mg/dl) of broilers at different intervals

Period	Condition	G1	G2	G3	G4
15 <sup>th</sup> day	Comfort	4.89 <sup>B</sup> ± 0.29 (12)	5.63 <sup>B</sup> ± 0.17 (12)	6.85 <sup>A</sup> ± 0.21 (12)	7.29 <sup>A</sup> ± 0.21 (12)
	Heat	5.35 <sup>B</sup> ± 0.21 (12)	6.08 <sup>AB</sup> ± 0.22 (12)	6.56 <sup>A</sup> ± 0.28 (12)	7.20 <sup>A</sup> ± 0.20 (12)
30 <sup>th</sup> day	Comfort	4.09 <sup>B</sup> ± 0.19 (12)	6.57 <sup>A</sup> ± 0.31 (12)	6.58 <sup>A</sup> ± 0.31 (12)	7.33 <sup>A</sup> ± 0.29 (12)
	Heat	5.87 ± 0.26 (12)	6.77 ± 0.21 (12)	6.47 ± 0.19 (12)	6.27 ± 0.16 (12)
45 <sup>st</sup> day	Comfort	4.09 <sup>B</sup> ± 0.20 (12)	6.77 <sup>A</sup> ± 0.30 (12)	6.71 <sup>A</sup> ± 0.16 (12)	6.95 <sup>A</sup> ± 0.23 (12)
	Heat	5.19 <sup>B</sup> ± 0.23 (12)	6.27 <sup>AB</sup> ± 0.26 (12)	7.43 <sup>A</sup> ± 0.25 (12)	7.06 <sup>A</sup> ± 0.27 (12)
Overall mean	Comfort	4.36 <sup>C</sup> ± 0.15 (36)	6.32 <sup>B</sup> ± 0.17 (36)	6.71 <sup>AB</sup> ± 0.14 (36)	7.19 <sup>A</sup> ± 0.14 (36)
	Heat	5.47 <sup>b</sup> ± 0.14 (36)	6.37 <sup>ab</sup> ± 0.14 (36)	6.82 <sup>a</sup> ± 0.16 (36)	6.84 <sup>a</sup> ± 0.14 (36)

Means bearing different superscripts within same row differ significantly (<sup>ABC</sup>;  $p < 0.01$ , <sup>ab</sup>;  $p < 0.05$ ); Means bearing different superscripts within same column differ significantly (<sup>PQ</sup>;  $p < 0.01$ , <sup>Pq</sup>;  $p < 0.05$ ); Comfort (26±1°C), Heat (37±5°C), G1 (Control), G2 (100 mg AA), G3 (200 mg AA), G4 (300 mg AA).

groups. Maximum (7.19±0.14 mg/dl) and minimum (4.36±0.15 mg/dl) AA concentrations were found in the G4 and control groups, respectively, which vary significantly ( $p < 0.01$ ). However, a non-significant difference was observed in G4 and G3 and a similar trend was observed between G3 and G2. Maximum (6.84±0.14 mg/dl) and minimum (5.47±0.14 mg/dl) AA concentrations were observed in the G4 and control groups, respectively, which differ significantly ( $p < 0.05$ ). In addition, a non-significant difference was observed in G2, G3 and G4, while a similar trend was observed in observed between G1 and G2.

In addition to the varying concentration of AA, the overall mean concentration of plasma AA showed a non-significant difference between comfort and heat-stressed broilers in all groups. Mahmoud *et al.* (2004) reported that dietary AA supplementation increased plasma AA and maintained it at high levels after heating but in non-ascorbic acid (N-AA) broiler birds, only heat elevated plasma AA, although the increase was non-significant, which corresponds to the present findings. AA supplementation resulted in a significant increase in plasma AA (18.8 to 26.6 µg/ml) compared to the N-AA group (8.7 to 16.4±0.93 µg/ml) which is not consistent with our findings. As per our reports, maximum (7.19 mg/dl) and minimum (4.36 mg/dl) AA concentrations were observed in the 300 mg AA complemented group and control group, respectively, which differ significantly. In the present research, plasma AA content has been shown to be significantly higher in birds fed AA supplemented diets compared to non-supplemented ones. Similar findings were also reported by Lohakare *et al.* (2005).

With the levels supplied and their plasma levels during the investigation, an important linear positive trend in AA was observed. AA is actively transported into tissues and its use increases during stress periods such as heat and the synthesising ability of the bird can become inefficient, thus reducing plasma AA concentrations, which may be the case in the current investigation in the non-supplemented groups. Under stressful conditions, the effectiveness of supplementing AA in birds depends on its ability to increase plasma AA concentrations, thus avoiding tissue depletion. By decreasing heat output, the AA either decreases heat load or increases heat loss by affecting thermal exchange avenues between the body and the environment.

### Plasma glucose

The data recorded and analysed for plasma glucose are shown in Table 3. In the groups supplemented with ascorbic acid, the average mean glucose concentration showed no significant difference between comfort and heat stressed birds. The G3 and G4 groups differ significantly ( $p < 0.01$ ) from G1 in comfort conditions, but no significant difference between the G2, G3 and G4 groups was observed. The G1 and G2 classes were also not significantly different. No significant difference between G3 and G4 was observed in heat-stressed conditions. Both in G2 and G4, a similar pattern was also observed. The control group G1 varies significantly ( $p < 0.01$ ) from all heat stressed groups treated with ascorbic acid, showing minimum glucose concentrations (216.19±6.79 mg/dl).

**Table 3:** Mean plasma glucose concentration (mg/dl) of broilers at different intervals

Period	Condition	G1	G2	G3	G4
15 <sup>th</sup> day	Comfort	266.5 <sup>B</sup> ± 11.6 (12)	283.9 <sup>AB</sup> ± 17.4 (12)	313.75 <sup>A</sup> ± 6.73 (12)	304.92 <sup>AB</sup> ± 7.71 (12)
	Heat	243.1 <sup>B</sup> ± 10.8 (12)	273.01 <sup>AB</sup> ± 8.84 (12)	313.00 <sup>A</sup> ± 7.84 (12)	291.00 <sup>A</sup> ± 18.5 (12)
30 <sup>th</sup> day	Comfort	252.51 <sup>B</sup> ± 7.78 (12)	292.2 <sup>AB</sup> ± 15.0 (12)	311.54 <sup>A</sup> ± 6.16 (12)	290.2 <sup>AB</sup> ± 16.1 (12)
	Heat	223.50 <sup>C</sup> ± 7.74 (12)	271.00 <sup>B</sup> ± 10.2 (12)	313.67 <sup>A</sup> ± 7.08 (12)	293.63 <sup>AB</sup> ± 9.75 (12)
45 <sup>st</sup> day	Comfort	238.83 <sup>B</sup> ± 7.55 (12)	269.8 <sup>B</sup> ± 13.8 (12)	310.00 <sup>A</sup> ± 9.85 (12)	307.50 <sup>A</sup> ± 3.13 (12)
	Heat	182.02 <sup>B</sup> ± 9.33 (12)	276.2 <sup>A</sup> ± 10.3 (12)	304.5 <sup>A</sup> ± 2.78 (12)	289.50 <sup>AB</sup> ± 6.77 (12)
Overall mean	Comfort	252.61 <sup>B</sup> ± 5.48 (36)	281.94 <sup>AB</sup> ± 8.80 (36)	311.94 <sup>A</sup> ± 4.35 (36)	300.86 <sup>A</sup> ± 6.01 (36)
	Heat	216.19 <sup>C</sup> ± 6.79 (36)	273.40 <sup>B</sup> ± 5.50 (36)	310.39 <sup>A</sup> ± 3.60 (36)	291.38 <sup>AB</sup> ± 7.11 (36)

Means bearing different superscripts within same row differ significantly (<sup>ABC</sup>;  $p < 0.01$ ); Comfort (26 ± 1 °C), Heat (37 ± 5 °C); G1 (Control), G2 (100 mg AA), G3 (200 mg AA), G4 (300 mg AA).

Supplementation of 100 mg, 200 mg and 300 mg AA in feed, it was found that the overall mean glucose concentration showed a non-significant difference between comfort and heat-stressed birds in all groups. Similar to the present investigation, several workers reported blood glucose levels in broilers under different thermal stress conditions (Aksit *et al.*, 2006; Olanrewaju *et al.*, 2010). In this study, significantly higher levels of plasma glucose were observed in 200 mg AA complemented birds compared to heat-stressed chickens. In comfort, the present plasma glucose levels on the 45th day with AA supplementation (310.00 ± 9.85 mg/dl) were comparable (Borges *et al.*, 2004) at 41 °C at 6 weeks of age Cobb broiler (283.4 mg/dl). Higher glucose levels have been reported by supplementation of AA during heat stress condition (Aksit *et al.*, 2006; Olanrewaju *et al.*, 2010). Sujatha *et al.* (2010) reported that the concentration of glucose in synthetic (100 g/tonne of feed) AA supplemented birds was significantly lower compared to the control group at 3rd and 5th week in broiler birds that is not similar to the findings. Khatibjoo *et al.*, (2014) reported that stress significantly changed glucose concentrations in broilers; however, it did not differ significantly, which is in disagreement with the findings.

The increase in glucose levels at higher ambient temperatures would be due to the endocrine stress regulation mechanism. Hypoglycemia caused by stress conditions led to the stimulation of the hypothalamus and the release of ACTH from the anterior pituitary gland, which resulted in increased secretions of the adrenal cortical steroid. Continuous stimulation of the adrenal

cortex led to chronically high levels of corticosteroids. These hormones are responsible for the formation of glucose from the body's carbohydrate, lipid and protein reserves. Among the 42 adaptive responses to ACTH, one of the responses reported was an increase in blood glucose levels (Borges *et al.*, 2004). Glucocorticoids also have primary effects on metabolism, stimulating gluconeogenesis leading to increased blood glucose levels (Khatibjoo *et al.*, 2014). These results could be explained by that, birds under environmental stress underwent an increase in plasma glucose which may be correlated with increase in corticosterone hormone secretion to supply the body with energy (Siegel, 1995).

#### Plasma albumin

The data recorded and analysed for plasma albumin concentrations are shown in Table 4. The overall mean concentration of albumin showed a non-significant difference between comfort and heat-stressed birds in groups supplemented by ascorbic acid. In comfort conditions, the G3 and G4 groups differ significantly ( $p < 0.01$ ) from the G1, but there was a significant difference between the G2, G3 and G4 groups. Also, G1 and G2 are not significantly different. A significant difference ( $p < 0.01$ ) between the groups G1, G3 and G4 was observed in the heat-stressed condition. A similar trend has also been observed between G2 and G3. However, a non-significant difference between G1 and G2 was observed. A similar trend has also been observed between the G2 and G4 groups.

**Table 4:** Mean plasma albumin concentration (g/dl) of broilers at different intervals

Period	Condition	G1	G2	G3	G4
15 <sup>th</sup> day	Comfort	1.65 <sup>B</sup> ± 0.08 (12)	2.42 <sup>AB</sup> ± 0.08 (12)	3.52 <sup>A</sup> ± 0.13 (12)	3.18 <sup>A</sup> ± 0.10 (12)
	Heat	1.33 <sup>b</sup> ± 0.87 (12)	1.91 <sup>ab</sup> ± 0.10 (12)	3.48 <sup>a</sup> ± 1.84 (12)	3.19 <sup>ab</sup> ± 0.15 (12)
30 <sup>th</sup> day	Comfort	1.76 <sup>b</sup> ± 0.14 (12)	2.91 <sup>ab</sup> ± 0.15 (12)	4.21 <sup>a</sup> ± 0.21 (12)	3.84 <sup>a</sup> ± 0.19 (12)
	Heat	1.60 <sup>B</sup> ± 0.06 (12)	2.58 <sup>AB</sup> ± 0.12 (12)	4.00 <sup>A</sup> ± 0.22 (12)	3.51 <sup>B</sup> ± 0.13 (12)
45 <sup>st</sup> day	Comfort	1.83 <sup>b</sup> ± 0.12 (12)	3.56 <sup>ab</sup> ± 0.16 (12)	4.94 <sup>a</sup> ± 0.19 (12)	4.47 <sup>ab</sup> ± 0.14 (12)
	Heat	1.60 <sup>C</sup> ± 0.09 (12)	2.67 <sup>BC</sup> ± 0.12 (12)	4.72 <sup>A</sup> ± 0.18 (12)	3.80 <sup>AB</sup> ± 0.18 (12)
Overall mean	Comfort	1.74 <sup>B</sup> ± 0.07 (36)	2.96 <sup>AB</sup> ± 0.11 (36)	4.22 <sup>A</sup> ± 0.14 (36)	3.83 <sup>A</sup> ± 0.12 (36)
	Heat	1.51 <sup>C</sup> ± 0.05 (36)	2.39 <sup>BC</sup> ± 0.09 (36)	4.07 <sup>A</sup> ± 0.14 (36)	3.50 <sup>B</sup> ± 0.10 (36)

Means bearing different superscripts within same row differ significantly (<sup>ABC</sup>;  $p < 0.01$ , <sup>abc</sup>;  $p < 0.05$ ); Comfort (26±1°C), Heat (37±5°C); G1 (Control), G2 (100 mg AA), G3 (200 mg AA), G4 (300 mg AA).

The overall mean concentration of albumin showed non-significant difference between comfort and heat stressed birds in all the groups supplemented with AA. In both heat-stressed and comfort conditions, a significant difference was observed in the 200 and 300 mg AA supplemented broilers compared to the control group. In the present findings, the AA supplemented birds had higher serum albumin levels compared to the control group of broilers. Sabah *et al.* (2008) reported that the 750 mg/Kg AA dose provided the highest serum albumin value followed by 500, 250 mg/Kg AA supplementation, which is at odds with our findings that 200 mg AA resulted in the highest albumin concentration followed by 300 mg and 100 mg complemented.

Results from the present investigation also indicate that dietary AA has beneficial effects on commercial broilers. Kutlu and Forbes (1993) reported that vitamin C supplementation increases the concentrations of serum albumin. The present study also recorded elevated concentrations of serum albumin in birds that received vitamin C. The probable reason for such an increase in albumin concentrations may be due to the fact that corticosteroid secretion increases as a response to stress at temperatures above the thermoneutral zone. Mckee *et al.* (1997) reported that ascorbic acid supplementation reduces the synthesis of corticoid hormones in birds under heat stress. As corticosteroids induce gluconeogenesis from non-carbohydrate precursors such as lactate, amino acids and glycerol (Linne and Ringsrud, 1999), a reduction in glucocorticoid secretion could reduce lipid and protein catabolism (Kucuk *et al.*, 2003). The increase in serum albumin concentrations observed in the

experimental groups of the present investigation could be partly explained by the fact that this increase could be due to the reduction of the synthesis (lipids and proteins through non-carbohydrate sources) and the secretion of corticosteroids in birds supplemented by vitamin C.

#### Breast muscle pH and thiobarbituric acid value

The data recorded for breast muscle pH and thiobarbituric acid value is shown in Table 5. On day 45, in comfort conditions, a higher breast muscle pH of 6.8 and 6.7 was observed in the control and G1 sacrificed broilers, while a lower pH of 6.4 was observed in G3, supplemented by 200 mg AA. On day 45, in a heat-stressed condition, a higher breast muscle pH of 6.6 was observed in the control sacrificed broiler group, while a lower pH of 6.2 was recorded in G3, supplemented by 200 mg AA.

On day 45, in both comfort and heat-stressed condition, supplementation with 200 mg AA recorded a numerically lower pH of the breast muscle compared to control broilers. The pH of the sacrificed birds is comparatively higher than the final pH obtained after a few hours of sacrifice, which indicates the beneficial effect of AA supplementation in broilers. Present findings are in disagreement with the findings of Kadim *et al.* (2009), who reported that ascorbic acid supplementation in drinking water affects the quality of the meat and that the *Pectoralis* muscles collected during the hot season had significantly higher pH levels than those collected during the cool season. They also reported that chickens reared in an open house had a significantly higher pH ( $p < 0.05$ ) than those reared in a closed house. The likely reason for such reports may

**Table 5:** Breast muscle pH and thiobarbituric acid value in liver homogenates of broilers

Parameter	Condition	G1	G2	G3	G4
Breast Muscle pH	Comfort	6.8	6.7	6.4	6.6
	Heat	6.6	6.5	6.2	6.5
TBA (nmol MDA equivalent/mg wet tissue)	Comfort	5.01	2.91	2.82	3.51
	Heat	5.74	3.58	2.18	2.64

Comfort (26±1°C), Heat (37±5°C); G1 (Control), G2 (100 mg AA), G3 (200 mg AA), G4 (300 mg AA).

be that rearing broiler chickens for 5-6 weeks may not be long enough for ascorbic acid to have an effect on muscle metabolism, which may also be the reason for the findings.

The possible justification for the present findings may be that high ambient temperatures reduce bird feed intake and impose physiological stresses that activate glycogenolysis in the skeletal muscle. Physiologically stressed birds use glucose and gluconeogenic precursors as their main oxidative fuel. Low muscle glycogen content due to fatigue or chronic stress before death results in high pH values and minimal rigour reduction, which could be one of the major factors leading to deterioration in the quality of the meat. Ogunwale *et al.* (2013) reported that the pH of broiler meat was not statistically different ( $p>0.05$ ). The carcass and meat pH of birds in all dietary AA treatments had a higher final pH, which decreased gradually over time, which is consistent with the findings. This could be the result of declining metabolic glycolysis in the meat, as oxidative respiration with post-mortem passing time ceases. Swatland (2008) reported implicit interactions between temperature and pH because glycolysis is exothermic and pH effects are very severe when the muscle is still near body temperature.

In comfort, the higher TBA value of sacrificed broilers on day 45 was 5.01 nmol MDA equivalent/mg of wet tissue in the control group, while the lower TBA value was 2.82 nmol MDA equivalent/mg of wet tissue in G3, supplemented by 200 mg AA. In heat-stressed condition, the higher TBA value of sacrificed broilers on day 45 was 5.74 nmol MDA equivalent/mg of wet tissue in the control group, while the lower TBA value was 2.18 nmol MDA equivalent/mg of wet tissue in G3, supplemented by 200 mg AA. The thiobarbituric acid value (nmol MDA equivalent/mg wet tissue) of sacrificed broilers was numerically lower in 200 mg AA supplemented birds compared to control birds during comfort and heat

stress conditions. Results from the present investigation show that 200 mg AA and 300 mg AA supplementation in broilers have been shown to be beneficial in reducing lipid peroxidation in chickens. Young *et al.* (2003) reported that chickens fed a diet complemented by ascorbic acid (1,000 ppm) decreased TBA values in non-stressed birds in the major muscles of *Pectoralis*, which is agreed to be reported. Increased antioxidative status thus seemed to protect against stress-induced increase in lipid oxidation in ascorbic acid-supplemented chickens.

Jang *et al.* (2014) reported that dietary supplementation with vitamin C undergoing summer HS in broilers significantly decreased (TBA value) lipid peroxidation in the liver compared to the control group is consistent with our findings. Dietary vitamin C significantly decreased hepatic lipid peroxidation in this study. In particular, several studies have shown that dietary vitamin C has shown a significant decrease in the MDA value as an indicator of lipid peroxidation (Cherian *et al.*, 1996; Sahin *et al.*, 2002). Dietary vitamins may therefore have a positive effect in preventing lipid peroxidation in birds exposed to summer HS, indicating that there is a strong functional association between pro-inflammatory cytokines and the antioxidant system in birds.

## CONCLUSION

With dietary supplementation of ascorbic acid, a linear positive trend in plasma ascorbic acid, plasmagluucose and plasma albumin was observed this could be due to the reduction of the synthesis from non-carbohydrate sources and the secretion of corticosteroids in birds supplemented by vitamin C (ascorbic acid). Also reduction in breast muscle pH and thiobarbituric acid value indicates amelioration in oxidative stress via reduced lipid peroxidation in chickens by supplementation of ascorbic acid.



## ACKNOWLEDGEMENTS

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

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