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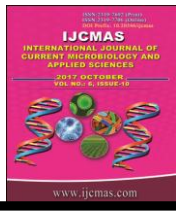

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Effect of Alpha-Tocopherol on Biochemical Parameters in Commercial Broilers during Heat Stress

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A B S T R A C T

Poultry production suffers huge losses due to heat stress, which is caused by high temperatures in many areas during the summer. Thus, the environment in which the broilers are reared is considered a key factor for success from the economic point of view. Therefore, maximum production requires the elimination of the deleterious impacts of environmental stressors. The objective of this study was to quantify plasma ascorbic acid, plasma albumin, plasma glucose, breast muscle pH, thio-barbituric acid value during heat stress in commercial broilers. A total number of 96 birds were randomly divided into 4 groups and each group consist of 12 birds in two replicates. Work was done in two conditions, heat and comfort. Heat stressed groups were maintained at 37±5.0°C whereas Comfort groups were maintained at 26±1.0°C. G1 was taken as control whereas G2, G3 and G4 was supplemented with 100 mg, 200 mg and 300 mg of vitamin E respectively. G4 group supplemented with 300 mg of alpha-tocopherol showed better results as compared to other supplemented and non-supplemented groups. This shows Vitamin E has potential antioxidant effect able to modulate physiological adjustments to mitigate the undesirable effects of exposure of broilers to high temperatures.

Introduction

Among various environmental conditions, high ambient temperature beyond the range of the thermoneutral zone in poultry has been known as one of the most fatal stressors, which adversely affects feed intake, growth rate, immunity and mortality (Niu *et al.*, 2009). Therefore, preventing and alleviating the heat stress (HS) against summer high ambient temperature is becoming increasingly important in the poultry industry.

When chickens are exposed to high temperature, they try to reduce their body temperature within a narrow range through self-thermal regulation to maintain body homeostasis. In particular, the exposure of poultry to summer HS is stressful enough to induce their metabolic rate and physiological responses to cope with the thermal environment (Puthpongsiriporn *et al.*, 2001). Therefore, vitamin E supplementation appears

to play a major role in the improvement of poultry production performance during heat stress.

Sahin *et al.,* (2001) reported supplementation of vitamin E alone has been reported to reduce the negative effects of heat stress in broilers. One of the most important properties of vitamin E is its antioxidant function. When animals fed diets rich in unsaturated fatty acids which are susceptible to peroxidation, the vitamin E deficiency is augmented (McDowell, 1989).

Materials and Methods

The experiment was conducted at college of veterinary science and animal husbandry, N.D.V.S.U., Jabalpur, Madhya Pradesh.

Birds and management

Total ninety six (96) day old chicks of commercial broiler birds were procured from private hatcheries of Jabalpur. The birds were maintained in the battery cage system in a well-ventilated room in the poultry experimental unit at college with prior permission from Institutional Animal Ethics Committee. Artificial heat was provided to chicks during early period (brooding period) of growth using thermostatically controlled electric brooders. The battery brooders were cleaned, washed and disinfected by blow lamping and complete house was fumigated using formaldehyde and potassium permanganate four days prior to start of the experiment. Feeders and waterers were carefully cleaned with detergent. Duration of experiment was six months. The feed was offered *ad-libitum* in linear chicks. Aluminum plates of appropriate size and small tin boxes were used in each cage to offer water during early weeks. Due care was taken so that the chicks reach the feeder and waterer in the first week of age. Later on, large size feeders and waterers were attached to each cage in opposite direction. All-mash system of feeding was practiced during the experiment. Fresh and clean drinking water was made available to birds all the time. Thus, during entire period of study uniform conditions of housing, brooding, feeding and watering was maintained for all the groups of the experiment. Broiler birds randomly divided into eight groups. Four groups of birds were maintained in summer conditions (May to June) maintained in heat stress (37±5.0°C) ambience, whereas other four groups of birds was maintained at 26±1.0°C (comfort temperature) using an air conditioner. Broilers were kept in closed ventilated system for 45 days during the experimental period. Temperature and humidity of the experimental poultry unit was recorded using a digital thermo-hygrometer.

Methods

Diets were formulated as per NRC (1994) specifications presented in table 1. For analysis of biochemical parameters, blood was collected from individual birds on specified day of experiment i.e. on 15th, 30th and 45st day. The blood samples were collected by cleaning the area by plucking the feather and wiping the area by ethanol swab. A 22 gauge needle was used for collection of blood. The blood samples were collected in heparinized polypropylene tubes (20 IU heparin/ml of blood) were kept in the ice bucket and carried back to the laboratory immediately. 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 biochemical parameters. The plasma ascorbic acid was estimated using DCIP method described by (Omaye *et al.*, 1979). Plasma glucose concentration was estimated by Trinder's method (Pileggi and Szuskeiweiz, 1974) using diagnostic kits

procured from Erba Diagnostics, Mannheim Gmbh, Germany. The concentration was expressed in mg/dl of blood glucose. Plasma albumin concentration was estimated as per method described by Doumas *et al.,* (1972) using diagnostic kits procured from Erba Diagnostics, Mannheim Gmbh, Germany. The concentration was expressed in g/dl of albumin. Automatic biochemistry analyzer was used for the determination of plasma ascorbic acid and albumin concentration.

The pH was determined using a modification of the iodoacetate method described by Petracci *et al.,* (2004). Lipid peroxidation was determined by a micro method for TBA-reactive substances (TBARS). Approximately 100 mg of liver was incubated in 0.5 mL 50% trichloroacetic acid containing 1.3% (wt/ vol) thiobarbituric acid (dissolved at 60°C) and heated at 60°C for 1 h, followed by determination of absorbance of supernatant at 532 nm. Tetraethoxypropane, which spontaneously decomposes in an aqueous environment to form malondialdehyde (MDA), was used as a standard and absorbance was expressed as MDA equivalents. MDA equivalents were calculated after subtraction of blank (water), correction for turbidity measured at 650 nm and dilution of the TBA reagent from water contained in the meat. The recorded data was statistically analyzed using Completely Randomized Design (Snedecor and Cochran, 1994). Various conditions and treatment groups were compared by using Duncan Multiple Range test (DMRT).

Results and Discussion

The data recorded and analyzed for plasma ascorbic acid is represented in (Table 2). The overall mean concentration of plasma ascorbic acid (AA) showed non-significant difference between comfort and heat stressed birds in alpha-tocopherol supplemented groups. In comfort condition, maximum (5.27±0.18 mg/dl) and minimum (4.36±0.15 mg/dl) concentration of plasma AA was observed in G4 and control group respectively which differ significantly ($p<0.01$). However, in G3 and G1 non-significant difference was observed. In heat stressed condition, maximum (5.51±0.17 mg/dl) and minimum (5.47±0.14 mg/dl) concentration of plasma AA was observed in G4 and control group respectively which differ non-significantly ($p<0.05$). Further, non- significant difference was observed in G2 and G3 and similar trend was observed in G1 and G2 group.

The data recorded and analyzed for plasma glucose is represented in (Table 3). The overall mean concentration of glucose showed non-significant difference between comfort and heat stressed birds in alpha-tocopherol supplemented groups. In comfort condition, G3 and G4 group differ significantly ($p<0.01$) from G1 but non-significant difference was observed between G2, G3 and G4 group.

Also, G1 and G2 group differed non-significantly. In heat stressed condition non-significant difference was observed between G3 and G4. Similar trend was also observed between G5 and G6. Group G1 control group differ significantly ($p<0.01$) from all alpha-tocopherol treated groups of heat stressed condition showing minimum concentration (216.19±6.79 mg/dl) of glucose. On supplementation of 100 mg, 200 mg and 300 mg vitamin E in feed, it was found that the overall mean concentration of glucose showed non-significant difference between comfort and heat stressed birds in all the groups. In comfort condition, present means of plasma glucose at 45^{th} day, on supplementation of vitamin E (302.05±03.77 mg/dl). The present findings were in accordance to findings reported by Navid *et al.,* (2010). Whereas, Sahin *et al.,* (2001) reported that there was lower glucose concentration in broilers supplemented with vitamin E which were in disagreement to present findings.

Increase in glucose levels at higher ambient temperature would be due to the endocrine mechanism of stress regulation. The hypoglycaemia resulted due to stress conditions led to stimulation of hypothalamus and release of ACTH from anterior pituitary which caused the increase of adrenal cortical steroid secretions. Continuous stimulation to adrenal cortex led chronically high levels of corticosteroid hormones. This hormone is responsible for the formation of glucose from body's reserves of carbohydrates, lipids and proteins. Glucocorticoids also have primary effects on metabolism, stimulating glucogenesis leading to increased blood glucose levels (Sturkie, 1986 and 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).

The data recorded and analyzed for plasma albumin is represented in (Table 4). The overall mean concentration of albumin showed non-significant difference between comfort and heat stressed birds in alpha-tocopherol supplemented groups. In comfort condition, G3 and G4 group differ significantly ($p<0.01$) from G1, but non-significant difference was observed between G2, G3 and G4 groups. Also, G1 and G2 group differ non-significantly. In heat stressed condition significant ($p<0.01$) difference was observed between the G1, G3 and G4 groups. Similar trend was also observed between G2 and G4.

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
Nutrient Composition		
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_3- 1200 IU, Vitamin K-1mg, Vitamin B1-2mg, Vitamin B_2-4mg, Vitamin B_{12}-10mg, Percent of values specified by NRC, 1994, *** Calculated.

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

Period	Condition	G1	G2	G3	G4
15th day	Comfort	$4.89^{B} \pm 0.29$ (12)	$4.53^{B} \pm 0.24$ (12)	$4.53^{B} \pm 0.24$ (12)	$5.93^{A} \pm 0.31$ (12)
	Heat	$5.35^{B} \pm 0.21$ (12)	$4.34^{B} \pm 0.16$ (12)	$4.34^{B} \pm 0.16$ (12)	$5.51^{A} \pm 0.32$ (12)
30th day	Comfort	$4.09^{B} \pm 0.19$ (12)	$4.10^{B} \pm 0.21$ (12)	$4.10^{B} \pm 0.21$ (12)	$5.16^{A} \pm 0.28$ (12)
	Heat	5.87±0.26 (12)	4.81± 0.26 (12)	4.81± 0.26 (12)	5.72 ± 30 (12)
45st day	Comfort	$4.09^{B} \pm 0.20$ (12)	$4.62^{B} \pm 0.17$ (12)	$4.62^{B} \pm 0.17$ (12)	$4.73^{A} \pm 0.26$ (12)
	Heat	$5.19^{B} \pm 0.23$ (12)	$5.22^{B} \pm 0.26$ (12)	$5.22^{B} \pm 0.27$ (12)	$5.30^{B} \pm 0.26$ (12)
Overall mean	Comfort	$4.36^{C} \pm 0.15$ (36)	$4.42^{C} \pm 0.12$ (36)	$4.42^{C} \pm 0.12$ (36)	$5.27^{B} \pm 0.18$ (36)
	Heat	$5.47^{b} \pm 0.14$ (36)	$4.79^{b} \pm 0.14$ (36)	$4.79^{b} \pm 0.14$ (36)	$5.51^{b} \pm 0.17$ (36)

Means bearing different superscripts within same row differ significantly (^{ABC}; $p<0.01$, ^{ab}; $p<0.05$).
Comfort (26±1°C), Heat (37±5°C)
G1 (Control), G2 (100 mg vitamin E), G3 (200 mg vitamin E), G4 (300 mg vitamin E),

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

Period	Condition	G1	G2	G3	G4
15th day	Comfort	266.5^{B} ±11.6 (12)	286.7^{AB} ±11.9 (12)	302.08^{AB} ±8.99 (12)	314.13^{A} ±6.57 (12)
	Heat	$243.1^{B} \pm 10.8$ (12)	270.00^{AB} ±7.22 (12)	283.3^{A}±19.4 (12)	309.25^{A}±5.75 (12)
30th day	Comfort	252.51^{B}±7.78 (12)	281.8^{AB}±15.7 (12)	292.00^{AB}±10.6 (12)	310.50^{A}±12.4 (12)
	Heat	223.50^{C}±7.74 (12)	270.9^{B}±12.9 (12)	291.50^{AB}±18.4 (12)	310.53^{A}±4.87 (12)
45st day	Comfort	238.83^{B}±7.55 (12)	273.00^{A}±8.79 (12)	306.45^{A}±9.33 (12)	306.97^{A}±4.07 (12)
	Heat	182.02^{B}±9.33 (12)	269.97^{A}± 7.38 (12)	286.08^{A}±9.81 (12)	302.50^{A}±3.77 (12)
Overall mean	Comfort	252.61^{B}±5.48 (36)	280.50^{AB}±7.06 (36)	300.18^{A}±5.51 (36)	310.37^{A}±4.76 (36)
	Heat	216.19^{C}±6.79 (36)	270.30^{B}± 5.35 (36)	286.97^{AB}±9.24 (36)	307.43^{A}±2.79 (36)

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

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

Period	Condition	G1	G2	G3	G4
15thday	Comfort	1.65^{B}±0.08 (12)	2.40^{AB}±0.10 (12)	3.16^{A}±0.20 (12)	3.49^{A}±0.14 (12)
	Heat	1.33^{b}±0.87 (12)	1.80^{ab}±0.12 (12)	3.12^{ab}±0.14 (12)	3.37^{a}±0.16 (12)
30thday	Comfort	1.76^{b}±0.14 (12)	2.90^{ab}±0.22 (12)	3.80^{A}±0.18 (12)	4.17^{A}±0.1.4 (12)
	Heat	1.60^{B}±0.06 (12)	2.54^{AB}±0.15 (12)	3.48^{A}±0.13 (12)	4.00^{A}±0.19 (12)
45stday	Comfort	1.83^{b}±0.12 (12)	3.53^{ab}±0.14 (12)	4.43^{ab}±0.11 (12)	4.90^{a}±0.18 (12)
	Heat	1.60^{C}±0.09 (12)	2.64^{BC}±0.15 (12)	3.76^{AB}±0.66 (12)	4.70^{A}±.0.15 (12)
Overall mean	Comfort	1.74^{B}± 0.07 (36)	2.94^{AB}±0.12 (36)	3.97^{A}±0.13 (36)	4.19^{A}±0.13 (36)
	Heat	1.51^{C}± 0.05 (36)	2.33^{BC}±0.10 (36)	3.45^{B} ±0.09 (36)	4.02^{A}± 0.13 (36)

Means bearing different superscripts within same row differ significantly (^{ABC}; p<0.01, ^{abc}; p<0.05).
Comfort (26±1°C), Heat (37±5°C), G1 (Control), G2 (100 mg vitamin E), G3 (200 mg vitamin E), G4 (300 mg vitamin E)

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

Birds	Condition	G1	G2	G3	G4
Breast Muscle pH	Comfort	6.8	6.6	6.7	6.5
	Heat	6.6	6.5	6.6	6.3
TBA (nmol MDA equivalent/mg wet tissue)	Comfort	5.01	2.93	3.46	2.88
	Heat	5.74	3.48	2.44	2.20

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

However, non-significant difference was observed between G1 and G2. Similar trend was also observed between G3 and G4 groups. The overall mean concentration of albumin showed non-significant difference between comfort and heat stressed birds in all the groups supplemented with vitamin E. In both heat stressed and comfort condition, a significant difference was observed in 200 and 300 mg vitamin E supplemented group of broilers as compared to that of control. In the present findings, the vitamin E supplemented birds had higher serum albumin level as compared to control group of broilers. Sahin *et al.,* (2002) found that albumin concentrations increased linearly with dietary vitamin E supplementations which were in agreement to present findings. Also Ismail *et al.,* (2014) reported that the was significant increase in the plasma albumin concentrations with dietary 300 mg kg^{-1} vitamin E supplementation, which were in agreement with the present findings.

The increase of serum albumin concentrations observed in experimental groups of present

investigation could be partially explained by the fact that this increase might because of the reduction of synthesis (lipids and protein through non-carbohydrate source) and secretion of corticoids in birds supplemented with vitamin E. The probable reason for such increase in concentrations of albumin might be due to the fact that, at temperatures above thermoneutral zone, corticoid secretion increases as a response to stress.

The data recorded and analyzed for breast muscle pH and TBA value is presented in table 5. On day 45, in the comfort condition, the higher breast muscle pH of 6.8 and 6.7 was observed in control and G2 group of sacrificed broilers, whereas, the lower pH of 6.5 was recorded in G4, supplemented with 300 mg alpha-tocopherol. On day 45, in heat stressed condition, the higher breast muscle pH of 6.6 was observed in control group of sacrificed broilers, whereas, the lower pH of 6.3 was recorded in G4, supplemented with 300 mg alpha-tocopherol**.** On day 45, in both comfort and heat stressed condition, supplementation with 300 mg vitamin E recorded numerically lower breast muscle pH as compared to control sacrificed broilers. The pH of the sacrificed birds is comparatively higher as compared to the ultimate pH obtained after few hours of sacrifice, which suggests the beneficial effect of vitamin E supplementation in broilers. Zaferino *et al.,* (2015) found that beast muscle pH decrease numerically with dietary vitamin E supplementation and these findings are in agreement to present findings. The possible justification for present findings might be the fact that high ambient temperatures reduce the bird's feed intake and impose physiological stresses, which activate glycogenolysis in skeletal muscle (Kreikemeier *et al.,* 1998). Physiologically stressed birds use glucose and gluconeogenic precursors as their major oxidative fuel. Low muscle glycogen content resulting from exhaustion or chronic stress before death results in high pH values and minimal rigor shortening, which could be one of the significant factors causing deterioration of meat quality characteristics.

In the comfort condition, the higher TBA value of sacrificed broilers on day 45 was 5.01 nmol MDA equivalent / mg wet tissue in control group whereas, the lower TBA value was recorded 2.88 nmol MDA equivalent / mg wet tissue in G4, which was supplemented with 300 mg alpha-tocopherol. In the heat stressed condition, the higher TBA value of sacrificed broilers on day 45 was 5.74 nmol MDA equivalent / mg wet tissue in control group whereas, the lower TBA value was recorded 2.20 nmol MDA equivalent / mg wet tissue in G4, which was supplemented with 300 mg alpha-tocopherol.

The thiobarbituric acid value (nmol MDA equivalent / mg wet tissue) of sacrificed broilers was numerically lower in 300 mg vitamin E supplemented birds as compared control birds during comfort and heat stresses conditions. Ismail *et al.,* (2014) reported that supplementation of vitamin E in feed has decreasing effect of MDA. These findings were in agreement to present findings.

High environmental temperature is a major stress factor for poultry and induces adverse effects on performance as well as on anatomical, physiological and behavioural parameters and may lead to exhaustion or even death. Hence, there is a need to explore the efficient means to improve the thermo tolerance of broiler chickens in hot climates without affecting their productivity. Hence nutritional manipulation with inclusion of anti-stress compounds like vitamins, make a practical alternative in alleviating the effects of high ambient temperature in poultry. The present results found that supplementation of the diets with antioxidants like vitamin E, is essential to overcome the deleterious effects

of heat stress conditions on the oxidative status and performance of broilers.

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