



## Effect of Ascorbic Acid and Alpha-tocopherol Supplementation on Plasma Corticosterone and Production Performance of Commercial Broilers

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### ABSTRACT

The present investigation studies the effect of ascorbic acid (AA) and alpha-tocopherol supplementation on plasma corticosterone and production performance of broilers. Eighty four day-old broiler birds were randomly assigned into seven groups of 12 each, namely G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub>, G<sub>4</sub>, G<sub>5</sub>, G<sub>6</sub> and G<sub>7</sub> maintained at 26±1.0°C. G<sub>1</sub> group was taken as control, whereas G<sub>2</sub>, G<sub>3</sub> and G<sub>4</sub> groups were fed 100 mg/kg feed, 200 mg/kg and 300 mg/kg of ascorbic acid respectively with basal diet. Similarly, basal diet was supplemented with 100, 200 and 300 mg/kg of alpha-tocopherol in groups G<sub>5</sub>, G<sub>6</sub> and G<sub>7</sub> respectively. Weekly record of feed intake was made for six weeks. Body weight, and feed efficiency ratio (FER) was recorded at 1<sup>st</sup>, 3<sup>rd</sup> and 6<sup>th</sup> week. Lower (P<0.05) plasma corticosterone levels were recorded on supplementation of 200 mg and 300 mg of ascorbic acid and alpha-tocopherol respectively. Feed intake was significantly higher (P<0.05) in supplemented groups as compared to control. Body weight gain and FER was significantly higher (P<0.05) in groups supplemented with ascorbic acid at 200 mg/kg feed and alpha-tocopherol at 300 mg/kg. Therefore, it is concluded that supplementation of 200 mg/kg ascorbic acid and 300mg/kg of alpha tocopherol improve performance of commercial broilers.

**Key words:** Alpha-tocopherol, Ascorbic acid, Broilers, Corticosterone, Vitamin-E

### INTRODUCTION

Stressful conditions, such as environmental, pathological and nutritional disorders, may produce conditions that evoke behavioral and physiological chain reactions that normally affect the development, performance and quality of the carcass of poultry. High mortality rate and impaired performance of broiler chickens have been reported in the poultry industry with changes in ambient temperature (Arjona *et al.*, 1988). Therefore, the prevention and relief of heat stress is becoming increasingly necessary. When chickens are exposed to high temperatures, they attempt to reduce their body temperature within a limited range to preserve body homeostasis. In particular, poultry's exposure to high temperature is stressful (Puthongsiriporn *et al.*, 2001). Heat stress adversely affect feed intake, growth rate and mortality in poultry diet, particularly ascorbic acid and vitamin E, as stress boosters have significant positive implications. Reversible oxidation-reduction of ascorbic acid with

dehydroascorbic acid is most important chemical property of vitamin C. Also, scavenging reactive oxygen species, counteracting oxidative stress in keratinocytes and reducing free radicals production promote efficient performance of poultry during heat stress (Bhatti *et al.*, 2016). Vitamin E, on the other hand, acts as potent antioxidant, improving oxidative stability and promoting improved meat quality. Protective action of vitamin C is partially mediated through reduction of circulating levels of glucocorticoids (Nockels, 1990) during stress. Keeping beneficial effects of ascorbic acid (vitamin C) and alpha-tocopherol (vitamin E) in mind, the present study was conducted to investigate effect of ascorbic acid and alpha-tocopherol on plasma corticosterone and production performance in broilers.

### MATERIALS AND METHODS

The research work was conducted in the Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, N.D.V.S.U.

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Jabalpur (M.P.). Feeding trial was conducted in poultry sheds at NDVSU livestock farm, Jabalpur. Total 84 day-old chicks of commercial broiler birds were procured (Phoenix hatchery, Jabalpur). The experimental chicks were reared in battery brooder house. A digital thermo-hygrometer was used to register the temperature and humidity of experimental poultry unit. Day-old chicks were randomly divided into 7 groups (G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub>, G<sub>4</sub>, G<sub>5</sub>, G<sub>6</sub>, G<sub>7</sub>) of 12 birds each. All birds were maintained at 26±1.0°C (comfort zone). G<sub>1</sub> group was taken as control (without supplementation); birds of groups G<sub>2</sub>, G<sub>3</sub> and G<sub>4</sub> were supplemented with 100, 200 and 300 mg of ascorbic acid per kg feed; whereas,

those in G<sub>5</sub>, G<sub>6</sub> and G<sub>7</sub> were supplemented with 100, 200 and 300 mg of alpha-tocopherol per kg feed. Diets were formulated as per NRC (1994) specifications (Table 1). Feed intake was recorded on weekly basis by weighing the feed offered and residue left. Body weight was recorded for each bird at three-week intervals (0, 3 and 6 weeks) by weighing all birds in the morning (9:00) prior to feeding using an electronic scale. Feed efficiency ratio (FER) was calculated on the basis of body weight gain and feed intake.

Blood samples were collected from each bird on specified day of experiment *i.e.*, on 15<sup>th</sup>, 30<sup>th</sup> and 45<sup>th</sup> day. A 22 gauge needle was used for collection of blood.

**Table 1. Composition of basal diet**

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 rice 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 B<sub>1</sub>-2mg, Vitamin B<sub>2</sub>-4mg, Vitamin B<sub>12</sub>-10mg, Percent of values specified by NRC, 1994, \*\*\*Calculated.

**Table 2. Mean plasma corticosterone concentration (ng/ml) of broilers at different intervals**

Period	Groups							SEM	P value
	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	G <sub>4</sub>	G <sub>5</sub>	G <sub>6</sub>	G <sub>7</sub>		
15 <sup>th</sup> day	6.81 <sup>A</sup> ±0.37	5.34 <sup>A</sup> ±0.25	3.17 <sup>B</sup> ±0.33	4.07 <sup>B</sup> ±0.25	5.39 <sup>A</sup> ±0.4	4.19 <sup>B</sup> ±0.24	3.19 <sup>B</sup> ±0.25	0.25	0.00
30 <sup>th</sup> day	7.21 <sup>A</sup> ±0.37	6.29 <sup>A</sup> ±0.41	3.83 <sup>B</sup> ±0.52	4.44 <sup>B</sup> ±0.29	6.26 <sup>A</sup> ±0.55	4.53 <sup>B</sup> ±0.38	3.92 <sup>B</sup> ±0.48	0.43	0.00
45 <sup>st</sup> day	6.51 <sup>A</sup> ±0.41	5.79 <sup>A</sup> ±0.47	3.48 <sup>B</sup> ±0.30	4.28 <sup>B</sup> ±0.37	5.89 <sup>A</sup> ±0.48	4.39 <sup>B</sup> ±0.40	3.55 <sup>B</sup> ±0.28	0.38	0.00
Mean	6.84 <sup>A</sup> ±0.22	5.81 <sup>B</sup> ±0.23	3.49 <sup>D</sup> ±0.23	4.26 <sup>C</sup> ±0.17	5.85 <sup>B</sup> ±0.28	4.34 <sup>C</sup> ±0.20	3.55 <sup>D</sup> ±0.20	0.22	0.00

<sup>b</sup>Means bearing different superscripts within same row differ significantly (P<0.05); Birds of the G<sub>1</sub> were fed a standard diet (Control). Diets of the birds of groups G<sub>2</sub>, G<sub>3</sub>, G<sub>4</sub> were supplemented with 100, 200 and 300 mg of ascorbic acid per kg diets, whereas those of G<sub>5</sub>, G<sub>6</sub>, and G<sub>7</sub> were supplemented with vitamin A at 100, 200 and 300 mg/kg diets, respectively.

The blood samples 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 corticosterone. Enzyme Linked Immuno Sorbent Assay (ELISA) was done for the estimation of plasma corticosterone as per the protocol prescribed by commercially available kit procured from Cusabio, China. The assay employed for the estimation of corticosterone (CORT) was the competitive inhibition enzyme immunoassay technique. The microtiter plate was pre-coated with the antigen. Standards or samples were added to the appropriate microtiter plate wells with an antibody specific for CORT and HRP conjugated goat-anti-rabbit antibody. The competitive inhibition reaction was launched between with pre-coated CORT and CORT in the samples. A

substrate solution was added to the wells and the color development was inversely proportional to the amount of CORT in the sample. Recorded data were statistically analyzed following procedure for Completely Randomized Design (Snedecor and Cochran, 1994). Various treatment means were compared by Duncan Multiple Range test (DMRT) using SPSS version 10. Significance was declared at P<0.05.

## RESULTS AND DISCUSSION

The data on plasma corticosterone (CORT) at different supplementations of ascorbic acid and alpha-tocopherol is shown in Table 2. Significant (P<0.01) decrease in CORT concentration was observed in G<sub>3</sub> and G<sub>7</sub> in comparison to G<sub>1</sub>. The present findings are in accordance with Pardue and Thaxton (1986) who reported that plasma corticosterone levels in control group of chickens increased significantly in comparison to the ascorbic acid supplemented groups. Also, Ismail *et al.* (2014) reported that supplementation of 300 mg of vitamin E per kg of feed decreased plasma

**Table 3. Feed intake (g/bird/day) of broilers at weekly intervals**

Week	Groups							SEM	P value
	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	G <sub>4</sub>	G <sub>5</sub>	G <sub>6</sub>	G <sub>7</sub>		
1 <sup>st</sup>	18.20±0.52	18.20±0.47	19.00±0.58	18.63±0.29	18.43±0.50	18.93±0.29	19.17±0.58	0.46	0.46
2 <sup>nd</sup>	32.95±0.65	32.43±0.74	34.93±0.80	33.19±0.98	32.66±0.76	33.59±0.95	32.43±0.78	0.81	0.38
3 <sup>rd</sup>	40.95±1.33	41.61±1.03	43.76±0.89	42.91±1.06	42.81±1.07	42.96±1.06	43.16±0.93	1.08	0.57
4 <sup>th</sup>	48.20±1.69	48.52±1.62	51.19±1.18	49.67±1.49	48.72±1.63	49.96±1.39	49.19±1.20	1.45	0.43
5 <sup>th</sup>	69.60±1.26	72.92±1.32	73.58±1.30	73.16±1.30	72.82±1.34	74.16±1.33	76.38±1.37	1.31	0.47
CFI(kg)	2.90 <sup>b</sup> ±0.61	2.99 <sup>b</sup> ±0.67	3.09 <sup>a</sup> ±0.65	3.03 <sup>ab</sup> ±0.61	3.00 <sup>b</sup> ±0.67	3.05 <sup>ab</sup> ±0.61	3.08 <sup>a</sup> ±0.64	0.64	0.04

<sup>a,b</sup>Means bearing different superscripts within same row differ significantly (P<0.05); Birds of the G<sub>1</sub> were fed a standard diet (Control). Diets of the birds of groups G<sub>2</sub>, G<sub>3</sub>, G<sub>4</sub> were supplemented with 100, 200 and 300 mg of ascorbic acid per kg diets, whereas those of G<sub>5</sub>, G<sub>6</sub>, and G<sub>7</sub> were supplemented with vitamin A at 100, 200 and 300 mg/kg diets, respectively.

**Table 4. Mean body weight (g) of broilers at different intervals**

Week	Groups							SEM	P value
	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	G <sub>4</sub>	G <sub>5</sub>	G <sub>6</sub>	G <sub>7</sub>		
0	63.94 ±1.84	62.78 ±1.26	65.87 ±1.61	64.51 ±1.26	60.75 ±1.03	62.48 ±1.06	63.75 ±1.45	1.35	0.62
3	778.20 ±08.72	799.44 ±15.62	864.55 ±19.78	883.15 ±10.64	794.40 ±14.60	878.10 ±10.56	858 ±18.76	14.09	0.46
6	1844.09 <sup>b</sup> ±21.41	2043.00 <sup>a</sup> ±14.53	2184.83 <sup>a</sup> ±32.26	2109.25 <sup>a</sup> ±26.60	2034.10 <sup>a</sup> ±14.28	2112.20 <sup>a</sup> ±24.21	2158 <sup>a</sup> ±30.20	23.56	0.05
BWG (g)	1780.15 <sup>b</sup> ±13.43	1980.22 <sup>b</sup> ±15.58	2118.40 <sup>a</sup> ±32.40	2044.74 <sup>a</sup> ±27.29	1973.35 <sup>b</sup> ±13.58	2049.72 <sup>a</sup> ±27.47	2095 <sup>a</sup> ±31.70	23.06	0.03

<sup>a,b</sup>Means bearing different superscripts within same row differ significantly (P<0.05); Birds of the G<sub>1</sub> were fed a standard diet (Control). Diets of the birds of groups G<sub>2</sub>, G<sub>3</sub>, G<sub>4</sub> were supplemented with 100, 200 and 300 mg of ascorbic acid per kg diets, whereas those of G<sub>5</sub>, G<sub>6</sub>, and G<sub>7</sub> were supplemented with vitamin A at 100, 200 and 300 mg/kg diets, respectively; BWG, body weight gain

corticosterone level in broiler chickens. Vitamins C and vitamin E are involved in reducing free radical formation and thus causing less secretion of corticosterone, a catabolic hormone.

Data pertaining to feed intake are shown in Table 3. Among ascorbic acid (AA) supplemented groups, the average feed consumption differed significantly (P<0.05) in G<sub>3</sub> as compared to G<sub>1</sub> and G<sub>2</sub> group. However, non-significant difference was observed between G<sub>1</sub>, G<sub>2</sub>; G<sub>3</sub> and G<sub>4</sub> groups. The maximum feed intake (3.09±0.65 kg) was observed in G<sub>3</sub> followed by G<sub>4</sub> group (3.03±0.61 kg). Among alpha-tocopherol supplemented groups, maximum feed intake (3.08±0.64 kg) was observed in G<sub>7</sub> group followed by G<sub>6</sub> group (3.05±0.61 kg). Supplementation of alpha-tocopherol tended to increase feed intake. These findings are in

accordance to findings of a previous study (Sahin *et al.*, 2001). These results were also in agreement with the findings of another study (Faluyi *et al.*, 2014).

Data on body weight is shown in Table 4. On 3<sup>rd</sup> week, the maximum body weight was found in G<sub>4</sub> group followed G<sub>3</sub> group. On 6<sup>th</sup> week, body weight of groups G<sub>1</sub> and G<sub>2</sub> differ significantly (P<0.05) from G<sub>3</sub> and G<sub>4</sub>. The maximum body weight was found in G<sub>3</sub> supplemented with 200 mg of ascorbic acid, followed by G<sub>4</sub> supplemented with 300 mg of ascorbic acid per kg of feed. Raja and Qureshi (2000) reported a significant improvement in body weight of birds supplemented with ascorbic acid in the feed, which is similar to present findings. Ascorbic acid supplementation increased body weight gain in broilers exposed to high ambient temperature (Kutlu, 2003). Diets

**Table 5. Production performance of broilers**

Parameters	Groups							SEM	P value
	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	G <sub>4</sub>	G <sub>5</sub>	G <sub>6</sub>	G <sub>7</sub>		
BWG (g)	1780.15 <sup>b</sup> ±13.43	1980.22 <sup>b</sup> ±15.58	2118.40 <sup>a</sup> ±32.40	2044.74 <sup>a</sup> ±27.29	1973.35 <sup>b</sup> ±13.58	2049.72 <sup>a</sup> ±27.47	2095.05 <sup>a</sup> ±31.70	23.06	0.02
FI (g)	2908.85 <sup>b</sup> ±6.60	2994.65 <sup>b</sup> ±7.96	3099.50 <sup>b</sup> ±5.96	3034.80 <sup>a</sup> ±5.22	3004.27 <sup>b</sup> ±5.38	3055.20 <sup>ab</sup> ±4.92	3085.06 <sup>a</sup> ±6.16	6.03	0.03
FCE	0.61 <sup>d</sup> ±0.01	0.66 <sup>c</sup> ±0.02	0.68 <sup>a</sup> ±0.03	0.67 <sup>b</sup> ±0.01	0.66 <sup>c</sup> ±0.02	0.67 <sup>b</sup> ±0.01	0.68 <sup>a</sup> ±0.03	0.02	0.01

<sup>a,b</sup>Means bearing different superscripts within same row differ significantly (P<0.05); Birds of the G<sub>1</sub> were fed a standard diet (Control). Diets of the birds of groups G<sub>2</sub>, G<sub>3</sub>, G<sub>4</sub> were supplemented with 100, 200 and 300 mg of ascorbic acid per kg diets, whereas those of G<sub>5</sub>, G<sub>6</sub>, and G<sub>7</sub> were supplemented with vitamin A at 100, 200 and 300 mg/kg diets, respectively; BWG, body weight gain; FI, feed intake, FCE, feed conversion efficiency

enriched with vitamin E also resulted in increased body weight (Navid *et al.*, 2010).

The data on production performance of broilers is presented in Table 5. The body weight differed significantly ( $P < 0.05$ ) in  $G_1$  and  $G_2$  groups from  $G_3$  and  $G_4$ . The maximum body weight gain was attained in  $G_3$  group and minimum body weight gain was attained in control group. Total feed intake differed significantly ( $P < 0.05$ ) in  $G_1$  and  $G_2$  groups as compared to  $G_3$ . Significant ( $P < 0.05$ ) difference was observed between all groups. The maximum FER was found in  $G_3$  group and minimum FER was found in control. Similarly, Navid *et al.* (2010) reported that diets enriched with the vitamin C and vitamin E resulted in the better performance of the birds.

## CONCLUSION

Nutritional manipulation with inclusion of ascorbic acid and alpha-tocopherol, make a practical alternative in reduction of plasma corticosterone levels and improvement in production parameters.

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