



Effect of vitamin E and C supplementation on growth and antioxidant status of coloured chicken under hot and humid climatic stress

T K BEURA¹, N PANDA², P K MISHRA³, R C PATRA⁴, P C BEHERA⁵ and B PANIGRAHI⁶

Orissa University of Agriculture and Technology, Bhubaneswar, Odisha 751003 India

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ABSTRACT

The effect of vitamin E and/or vitamin C supplementation on growth and antioxidant status of coloured broiler chicks during summer stress was studied. Coloured broiler chicks (540) were divided into 9 treatment groups and were fed basal diet with supplementation of vitamin E (250 mg or 500 mg) and vitamin C (250 mg or 500 mg) either alone or in combinations from 0–56 days of age. The average weekly highest temperature and relative humidity during the experimental period was 37.98°C and 87.9% respectively. The growth rate, serum total antioxidant activity (TAA), anti oxidant enzymes i.e catalase, super oxide dismutase (SOD), lipid peroxidation in terms of malonaldehyde (MDA) concentration were determined. The body weight and FCR of both the vitamin supplemented groups were better than individual vitamin supplementation. All the vitamin supplemented groups showed significantly higher TAA value, SOD and catalase activities than the control groups and the 2 vitamins combination groups had higher values than the individual supplementation groups. Highest MDA activity was seen in control group and all other supplemented groups had lower value than control. From the results it was found that colored broilers supplementing antioxidant vitamins E @ 250 mg/kg and C @ 250 mg/kg of feed in summer stress condition improved growth performance and increased the antioxidant status of the birds.

Key words: Broiler, Growth, Immunity, Summer, Vitamin C, Vitamin E

High temperature coupled with high humidity influences performance of broiler chickens by reducing feed intake, live weight gain, feed efficiency, mortality and overall a great economic loss to the farmers. The plasma concentrations of antioxidant vitamins such as ascorbic acid (vit C), tocopherol (vit E) and minerals (zinc, copper and chromium) are reduced and the oxidative damage is increased in poultry reared under heat stress (Halliwell and Gutteridge 1989, Sahin *et al.* 2002a). During summer stress, body immune system cells undergo enzymatic or self oxidative peroxidation resulting in destabilizing of cell wall and consequently cell function loss (Puthongsiriporn *et al.* 2001). Studies showed that supplementation of ascorbic acid and tocopherol could be used to attenuate the negative effects of environmental stress (Kafri and Cherry 1984, Sahin *et al.* 2002b). A positive synergistic effect of vitamins E and C was observed on the

immune response. Combination of antioxidant generally showed greater antioxidant activity than each compound singly (Gey 1998). Though enough literature is available on supplementing vitamin E or vitamin C to reduce the heat stress, very scanty literature is available on supplementing either vitamin E or vitamin C or its combination in a high temperature and humid condition in colour broilers, the present study was undertaken to evaluate the influence of vitamin E or C or in combination on the growth performance and antioxidant status of colour broilers during summer.

MATERIALS AND METHODS

Animals, diets and management: Day-old coloured broiler chicks (540) were randomly distributed into 9 groups with 3 replicates having 20 chicks in each. The experiment was conducted for 8 weeks during April to June, the summer of this region. Chicks of each replicate were wing banded, weighed individually and assigned to separate cage of linear battery brooder. Two doses of vit E (250 mg, 500 mg/kg) and vit C (250 mg, 500 mg/kg) were supplemented to the birds either alone or in combinations to 9 groups including a control group. T₁ was treated as control and the groups were fed as follows:

T₁, control diet; T₂, control diet + vit E @ 250 mg/kg

Present address: ^{1,2}Associate Professor (tapanbeuravety@gmail.com, npandaouat@gmail.com, ³pkmishraouat@yahoo.co.in), Department of Animal Nutrition; ⁴ Professor and Head, Department of Medicine (rcpatra@gmail.com), ⁵Associate Professor, Department of Biochemistry (pc_behera65@yahoo.in); ⁶Associate Professor Department of LPM (bpanigrahi65@yahoo.co.in), Faculty of Veterinary Science and Animal Husbandry.

feed; T₃, control diet + vit E @ 500 mg/kg feed; T₄, control diet + vit C @ 250 mg/kg feed; T₅, control diet + vit C @ 500 mg/kg feed; T₆, control diet + vit E @ 250 mg/kg feed + vit C @ 250 mg/kg feed; T₇, control diet + vit E @ 500 mg/kg feed + vit C @ 250 mg/kg feed; T₈, control diet + vit E @ 250 mg/kg feed + vit C @ 500 mg/kg feed; T₉, control diet + vit E @ 500 mg/kg feed + vit C @ 500 mg/kg feed.

The supplementation was continued from 0 day to 8 weeks. The chicks were fed with starter ration up to 21 days and finisher ration from 22 to 56 days of age as per BIS (1992) recommendation. Feed and water were provided *ad lib*. The chicks were vaccinated with New Castle disease vaccine on 7 and 21 days of age.

Temperature and humidity: The average maximum and minimum temperature during the 8 weeks experimental period ranged from 36.2 to 40.6°C (average 37.98°C) and 24.2 to 27.5°C (average 26.30°C), respectively. The average maximum and minimum relative humidity during the experimental period ranged from 85.0 to 93.0% (average 87.9%) and 36 to 59% (average 47%) respectively.

Sample collection and analytical procedure: Samples of experimental feeds were analyzed for proximate principles as per AOAC (1995). Weekly group replicate average body weight, feed consumption and FCR were calculated.

Estimation of plasma total antioxidant activity: Blood samples were collected from 15 birds (5 birds from each replicate) in each treatment at fourth week and at eighth week for estimation of plasma total antioxidant activity (TAA) measured by ferric reducing anti oxidant power (FRAP) assay (Benzie and Strain 1999). FRAP assay uses antioxidant as reductants in a redox-linked calorimetric method, employing an easily reduced antioxidant present in stoichiometric excess. At low pH, reduction of ferric tripyridyl-triazine (Fe^{III}-TPTZ) complex to the ferrous form, which has intense blue colour, can be monitored by measuring the change in absorption at 593 nm. The change in absorbance is therefore directly related to the combined or total reducing power of the electron donating antioxidants present in the reaction mixture. Absorbance change (ΔA_{593} nm) is translated into FRAP value (μ M) by comparing the test samples to that of standard solution of known FRAP value as follows:

$$\text{FRAP value of samples} = \frac{\text{0 to 4 min } \Delta A_{593} \text{ nm}}{\text{0 to 4 min } \Delta A_{593} \text{ nm}} \times \frac{\text{test sample}}{\text{standard}} \times \text{FRAP value of standard } (\mu\text{M})$$

Ascorbic acid is taken as standard and the FRAP value of ascorbic acid is 2.

For estimation of antioxidant enzymes and lipid peroxide level blood samples were taken at the 8 week of the experiment aseptically in a clean disposable syringe with addition of anticoagulant EDTA. The blood samples were immediately transferred to laboratory and centrifuged at 2000 rpm for 10 min and packed erythrocytes were collected.

Preparation of RBC hemolysate: The packed red blood cells obtained after centrifugation were mixed with equal quantity of 0.9% NaCl or PBS and shaken gently for proper mixing. It was centrifuged at 2000 rpm for 10 min and this procedure was repeated 3 times. Every time the supernatant was discarded and again the red blood cells were suspended in 0.9% NaCl or PBS. Thus the packed washed RBC was obtained by centrifugation at 2000 rpm for 10 min. 0.1 ml of packed RBC was taken and diluted with 0.9 ml of chilled distilled water to get 10% RBC hemolysate. The hemolysate was immediately processed for estimation of lipid peroxide level and activities of superoxide dismutase (SOD) and catalase.

Lipid peroxidation, catalase and super oxide dismutase: Membrane peroxidative damage in the erythrocytes was determined in terms of malonaldehyde (MDA) production by modified method of Dormandy and Stock (1971) and Placer *et al.* (1966). The estimation of catalase activity in 10% RBC hemolysate was done as per Cohen *et al.* (1970) and the super oxide dismutase (SOD) activity in 10% RBC hemolysate was measured by using nitroblue tetrazolium as a substrate after suitable dilution following the methods of Marklund and Marklund (1974) with certain modification suggested by Menami and Yoshikawa (1979).

Statistical analysis: The data obtained from the study were statistically analyzed according to Snedecor and Cochran (1980). The data were analyzed for analysis of variance (ANOVA) and DMR test (Duncan 1955) was used to test the difference among treatment means wherever necessary.

RESULTS AND DISCUSSION

Body weight, feed intake and FCR: The initial body weight of the coloured birds was around 40–42 g in all the treatments and up to first week no significant difference of body weight was observed (Table 1). From fourth to seventh week the body weight of both the vitamin supplemented groups (T₆, T₇, T₈, T₉) was higher than the individual supplementations (T₂, T₃, T₄, T₅). The final body weight at 8 weeks of age varied from 1635g to 1782g in all the treatments. The average maximum temperature during the experimental period was 40.6°C and minimum was 36.2°C and average relative humidity was 87.9%. High temperature accompanied with high humidity created a severe stress condition to the birds.

At the end of eighth week no significant difference ($P < 0.05$) of cumulative feed intake was observed among the treatment groups. At fourth week the FCR of T₄, T₇, T₈ and T₉ groups were better than all other groups. Similarly in seventh and eighth week T₇ and T₈ groups have better FCR ($P < 0.05$) than all other groups.

By feeding different doses of vitamin C (Vathana *et al.* 2002), vitamin E up to 500 mg/kg (Shaik *et al.* 2005, Sahin *et al.* 2001, 2002a, 2004) to the commercial broilers and Japanese quails higher body weight and better FCR were obtained during heat stress period. Combination of both the

Table 1. Body weight, feed consumption and FCR of birds supplementing vitamin E and C

Treatments	Vitamin levels (mg/kg)	Body weight (g)			Cumulative feed consumption (g)			FCR		
		0–4 wk	5–8 wk	0–8 wk	0–4 wk	5–8 wk	0–8 wk	0–4 wk	5–8 wk	0–8 wk
T ₁	0	634.54 ^d	1000.46 ^{ab}	1635.00 ^c	1304.17	2468.33	3772.50	2.05 ^a	2.47 ^{ac}	2.30 ^a
T ₂	E 250	725.00 ^c	975.60 ^{bd}	1700.60 ^b	1372.08	2434.99	3807.07	1.89 ^{ac}	2.50 ^a	2.24 ^{ac}
T ₃	E 500	719.07 ^c	996.93 ^{ab}	1716.00 ^{bd}	1384.20	2304.58	3688.78	1.92 ^{ac}	2.31 ^b	2.15 ^{ac}
T ₄	C 250	737.63 ^{bc}	947.71 ^c	1685.34 ^b	1370.28	2372.72	3743.00	1.86 ^b	2.50 ^a	2.22 ^{ac}
T ₅	C 500	733.86 ^{bc}	949.41 ^{cd}	1683.27 ^b	1402.42	2382.68	3785.10	1.91 ^{ac}	2.50 ^a	2.25 ^{ac}
T ₆	E 250+C 250	718.00 ^c	1009.67 ^a	1727.67 ^{ad}	1419.78	2286.09	3705.87	1.98 ^{ac}	2.26 ^b	2.15 ^{ac}
T ₇	E 500+C 250	757.61 ^a	1024.05 ^a	1781.66 ^a	1408.45	2368.80	3777.25	1.86 ^b	2.31 ^b	2.12 ^{bc}
T ₈	E 250+C 500	774.04 ^a	989.96 ^{ab}	1764.00 ^{ad}	1409.40	2256.05	3665.45	1.82 ^b	2.28 ^b	2.08 ^b
T ₉	E 500+C 500	756.10 ^{ab}	986.61 ^{ab}	1742.71 ^{ad}	1403.47	2318.40	3721.87	1.86 ^b	2.35 ^{bc}	2.14 ^{ac}

Means with same superscripts within a column do not differ significantly ($P < 0.05$).

vitamins were supplemented by Patrio and Palcer (2002) and Ciftci *et al.* (2005) and better response of body weight gain were observed. Vathana *et al.* (2002), Sosnowka-Czajka (2005) and Shaik *et al.* (2005) did not find any significant effect on the feed intake of birds with supplementation of either of the vitamins. In the present experiment combination of vitamin E and C at 2 doses (250 and 500 mg/kg feed) found better than the individual supplementation for body weight and FCR. But Sosnowka –Czajka *et al.* (2005) did not observe any significant effect on body weight and FCR of the broilers by supplementing vitamin C 40 mg and E-70 mg/kg of feed for 8 days only where the birds were exposed to 10°C higher temperature than recommended thermal condition. In the present experiment the birds were subjected to environmental stress for the entire period of study for which levels of both the vitamins in combination, helped in reducing the stress of both heat and humidity. Gey (1998) also stated that combination of anti oxidants showed better antioxidant status than alone. Vitamin C plays a major role in synthesis of corticosterone, a primary glucocorticoid hormone involved in gluconeogenesis to enhance energy supply during stress. Vitamin E helps in neutralizing oxidants produced during the stress. The maximum temperature more than 45°C for some days during experiment coupled with high humidity created severe stress in birds which was ameliorated by the supplementing both antioxidant vitamins in these 2 doses.

Total antioxidant activity (TAA): The plasma total antioxidant activity (TAA) of different groups estimated at fourth and eighth week FRAP (ferric reducing antioxidant power) showed that all the vitamins supplemented groups have significantly higher value than the control. No significant difference ($P > 0.05$) was observed among T₄, T₅, T₇, T₈ and T₉ groups. Similarly TAA value of T₇ (E500+C250) was highest in eighth week and differed significantly ($P < 0.05$) from T₁, T₂ and T₄ groups. No significant difference was also observed among T₃, T₅, T₆, T₇, T₈ and T₉ at eighth week.

FRAP, a direct test of total antioxidant power of any

biological fluids, uses antioxidants as reductants in a redox-linked calorimetric method by measuring the reducing power of electron donating antioxidants present in the reaction mixture. The higher the FRAP value the better the antioxidant power in plasma. In this experiment, higher FRAP value in vitamin supplemented groups indicated better antioxidant ability of the plasma. Very limited literature is available to measure antioxidant activity through FRAP assay in the birds but supplementation of vitamin E and C increased antioxidant status of the birds (Panda *et al.* 2007, Puthongsiriporn *et al.* 2001). Ozkan *et al.* (2007) found increased antioxidant status by supplementing vitamin E and selenium to the broilers reared at low and optimum temperature.

Oxidative enzyme activities and MDA concentrations: The antioxidant enzymes like catalase (CAT), super oxide dismutase (SOD) and lipid peroxidase as expressed by malanoaldehyde (MDA) concentration were estimated at 8 weeks of age (Table 2). The activity of catalase enzyme in all the vitamin supplemented groups showed higher values than control, however, T₆, T₇, T₈ and T₉ groups had higher ($P < 0.05$) values than the T₂, T₃ and T₄ groups. The SOD activities of all the vitamin supplemented groups were also higher than the control group. Highest value of 79.86 unit/g Hb was observed in T₉ group. Combinations of vitamins give better SOD status of the birds showing higher value than the individual vitamin supplementation.

The MDA concentration of different groups followed the reverse trend of antioxidant enzymes. Highest MDA activity of 2.91nmole/ml was seen in control group and all other supplemented groups had lower value than control. No significant difference of MDA value was seen among the vitamin supplemented groups ($P > 0.05$).

In heat stress there is increased concentration of MDA due to increased lipid peroxidation (Panda *et al.* 2007). The concentration of MDA in the blood is an indication of lipid peroxidation in the body. There should be enough of antioxidant enzymes in the blood to neutralize above reactive oxygen species (ROS). Enzymes like SOD and catalase play

Table 2. Effect of vitamin supplementation on total antioxidant, oxidative enzyme activities and MDA concentration of heat stressed broilers

Groups	Vitamin/dose	Total antioxidant activity		Catalase (K/g Hb)	SOD (unit/g Hb)	MDA (n mole/ml)
		Fourth week	Eighth week			
T ₁	Control	1442.67 ^c ±32.16	1568.31 ^c ±57.29	259.13 ^c ±22.56	53.25 ^c ±2.83	2.91 ^a ±0.11
T ₂	E250	1515.26 ^{bc} ±41.54	1723.06 ^{ab} ±62.32	302.56 ^{cd} ±18.35	60.18 ^{de} ±3.59	2.32 ^b ±0.09
T ₃	E500	1524.27 ^{bc} ±53.26	1787.37 ^{ab} ±70.08	296.67 ^d ±24.48	62.29 ^{cd} ±4.01	2.36 ^b ±0.12
T ₄	C250	1588.52 ^{ab} ±50.18	1658.53 ^{bc} ±68.24	292.75 ^d ±18.45	65.18 ^{bcd} ±3.35	2.19 ^b ±0.12
T ₅	C500	1628.36 ^{ab} ±49.66	1765.43 ^{ab} ±56.22	307.86 ^{bcd} ±20.86	64.56 ^{bcd} ±3.98	2.07 ^b ±0.10
T ₆	E250+C250	1584.37 ^{ab} ±44.32	1855.54 ^a ±62.75	332.92 ^a ±21.35	72.53 ^{abcd} ±2.85	2.15 ^b ±0.09
T ₇	E500+C250	1692.35 ^a ±51.69	1868.32 ^a ±63.19	328.34 ^a ±17.54	78.44 ^{ab} ±2.77	1.96 ^b ±0.12
T ₈	E250+C500	1684.63 ^a ±52.11	1856.43 ^a ±59.44	318.19 ^{abc} ±16.28	75.79 ^{abc} ±2.50	2.03 ^b ±0.11
T ₉	E500+C500	1705.17 ^a ±47.23	1835.51 ^a ±60.48	320.86 ^{ab} ±17.09	79.86 ^a ±3.18	1.98 ^b ±0.13
SE _m		37.41	47.08	5.12	4.20	0.16

a major role to neutralize the above free radicals. The activities of these enzymes increased with the stress condition in broilers as reported by Altan *et al.* (2003). Sahin *et al.* (2004) also found increased concentration of MDA in serum and liver of Japanese quail under heat stress. They reported that by feeding ascorbic acid, the concentration of MDA decreased both in thermo neutral and heat stressed quails. In the present experiment there was significant increase of both the enzymes and decrease of MDA in the broilers supplemented with either of vitamins or in combination indicating better oxidative status. Panda *et al.* (2007) reported that vitamin C (200–600 mg/kg) supplementation increased the catalase activity of the blood and decreased the MDA concentration in White Leghorn. Belge *et al.* (2003) also found that the MDA value of Leghorn chickens decreased with injection of vitamin C to the birds and increased the activity of super oxide dismutase (SOD).

Our results revealed that combination of both the antioxidant vitamin E and C showed improved growth performance and increased the antioxidant status in coloured broilers during hot and humid stress. As both the doses of vitamins have shown equal performance so lower doses i.e. vitamin E @ 250 mg/kg with vitamin C @ 250 mg/kg of feed may be recommended for ameliorating the summer stress in these birds.

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