



Effect of vitamins E and C supplementation on the immunity status of coloured hens during summer

B P JENA¹, N PANDA², P K MISHRA³, R C PATRA⁴, H K PANDA⁵, B PANIGRAHI⁶ and P C BEHERA⁷

Odisha University of Agriculture and Technology, Bhubaneswar, Orissa 751 003 India

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ABSTRACT

The present investigation was carried out to evaluate effects of vitamins E and C supplementation on immunity status of breeder birds and transfer of passive immunity to day-old chicks during heat stress. Broiler breeder birds (270) were divided into 9 groups with 3 replicates having 10 birds in each and were supplemented with 2 levels of antioxidant vitamins E (250 mg or 500 mg/kg) and C (200 mg or 400 mg/kg) alone and in combinations for 8 weeks during the peak summer. Antibody titre against Newcastle disease vaccine (NDV), cutaneous basophilic hypersensitivity (CBH) test against phytohaemagglutinin- P at fourth and eighth week and passive immunity in chicks were undertaken. At fourth week control group which was not supplemented any vitamins showed the lowest CBH response and at eighth week all the combined vitamins supplemented groups showed significantly higher response than control and individual vitamin supplemented groups. At fourth week antibody titre against NDV did not differ significantly from first day in all the groups and on eighth week there was significant decline in antibody titre in control and individual vitamin supplemental groups compared to first and fourth week but decrease in titre in combined vitamin supplemented groups was not significant. The passive immunity as determined by titre ($\log_2$) against NDV in day-old chicks hatched from the eggs of breeder hens did not differ among the treatments. It can be concluded that the combination of the antioxidant vitamins at lower level i.e. C 200 + E 250mg/kg have higher immunity in the coloured broiler breeder hens compared to individual vitamin supplementation during hot and humid stress.

Key words: Antioxidant vitamins, Hen, Immunity, Summer

Heat stress adversely affects production performance and inhibits immune function. Vitamin E protects cells and tissue from lipoperoxidative damage induced by free radicals. Vitamin C limits the metabolic signs of stress and alleviates physiological consequences of stress, resulting in improved performance, immunological competence and behaviour of chickens. High environmental temperatures suppress circulating white blood cells (WBC) and increase heterophil/lymphocyte ratio (H/L ratio) (Mogenet and Youbicier-Simo 1998), which is an indicator of stress (Gross and Siegel 1983). Heat stress also reduces antibody production in young chickens (Zulkifi *et al.* 2000). Production of HS protein during heat

stress reduces phagocytic activity of chicken macrophages (Miller and Qureshi 1992), decreases antibody responses to vaccination (Gross 1985, Siegel 1985), and increases susceptibility to different diseases (Gross and Siegel 1981, Pamok *et al.* 2009). Climatic stressors can also affect B-cell immunity (antibody production) and T-cell mediated immunity (Regnier and Kelley 1981, Siegel 1985, Freeman 1987).

Response to stress and immunocompetence of chickens are influenced by dietary vitamin E (Tengerdy and Nockels 1973, Leshchinsky and Klasing 2001, and Puthongsiriporn, *et al.* 2001). Vitamin E plays an important role in developing immune response, protecting lymphocytes, macrophages, and plasma cells and enhancing function and proliferation of these cells in chickens in face of oxidative damage (Franchini *et al.* 1991, Meydani and Blumberg 1993). Sahin *et al.* (2001) suggested that vitamin E supplementation @ 250 mg reduced the negative effects of heat stress. Similarly Panda *et al.* (2007) concluded that dietary supplementation of vitamin C (200 mg/kg) improved antioxidant status in White Leghorn layer during summer stress. There is also positive synergistic effect of vitamins E and C on the immune response (Sahin *et*

Present address: ¹Department of Poultry Science (Dbishnu132@gmail.com), ²Associate Professor, Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry (npandaouat@gmail.com), ³pkmishraouat@yahoo.co.in, ⁴Professor and Head Veterinary Medicine (rcpatra@gmail.com), ⁵Associate Professor, Department of Bacteriology and Virology (hkpandaouat@yahoo.com), ⁶Associate Professor, Department of Livestock Production and Management (bpanigrahi65@yahoo.co.in), ⁷Associate Professor, Department of Veterinary Biochemistry (pchandrabehera@yahoo.com).

*al.*2003). The present experiment is planned to see the immunity of the coloured broiler by supplementing both vitamins E and C either alone or in combination during natural hot and humid stress in this tropical climate.

Experimental animals and design

Broiler breeder hens (IBI -91 strain; 270) of 40 weeks of age were procured from the University Farm. The birds were randomly distributed into 9 treatment groups with 3 replicates having 10 birds in each. The birds were kept in individual cages with floor space of 1,125 cm²/hen. Two levels of vitamin C (L- ascorbic acid) i.e. 200 mg, 400 mg and vitamin E (DL- α -tocopheryl acetate), 250 mg, 500 mg were supplemented either alone or in combinations with breeder basal diet to eight groups besides a control group. The composition of basal diet is given in Table 1. The supplementation was continued for 8 weeks. Water was provided *ad lib*. Daily feed given was weighed and supplied @ 150g/bird/day. In-house temperature and relative humidity was recorded daily during the experimental period and average values were calculated by standard method.

Feed analysis: Samples of experimental feeds were analysed for moisture content, crude protein, ether extract, crude fibre, total ash, and acid insoluble ash (Table 2) as per AOAC (1995).

Temperature and humidity: The daily maximum and minimum temperature along with relative humidity inside the farm was recorded during the experimental period and average was calculated for each week. The maximum temperature during the experimental period ranged from 35.69 to 40.36°C and the average maximum temperature during that period was 38.05°C. The minimum temperature during the experimental period inside the shed was ranged from 25.09 to 28.46°C and the average being 26.12°C. The average maximum and minimum relative humidity was 87.73 and 54.19% respectively during the 8 weeks experimental period.

Table 1. Experimental design and feeding levels

Group	Feeding level of vitamins
T ₁	Basal diet (without supplementation)
T ₂	Basal diet + vitamin C 200mg/kg feed (lower)
T ₃	Basal diet + vitamin C 400mg/kg feed (higher)
T ₄	Basal diet + vitamin E 250mg/kg feed (lower)
T ₅	Basal diet + vitamin E 500mg/kg feed (higher)
T ₆	Basal diet + vitamin C 200mg/kg feed + vitamin E 250mg/kg feed
T ₇	Basal diet + vitamin C 200mg/kg feed + vitamin E 500mg/kg feed
T ₈	Basal diet + vitamin C 400mg/kg feed + vitamin E 250mg/kg feed
T ₉	Basal diet + vitamin E 400mg/kg feed + vitamin E 500mg/kg feed

Table 2. Composition of basal diet

Ingredients	Composition (g/kg)	Proximate Composition	Percentage on DM basis
Maize	520	Moisture	9.24
Soybean meal	290	Crude protein	18.01
De-oiled rice bran	110	Ether extract	4.16
Mineral mixture ¹	30	Crude fibre	4.92
Oyster shell meal	45	Total ash	10.54
NaCl	03	Acid insoluble ash	2.66
L-lysine	0.5	Nitrogen free extract*	53.82
DL- methionine	0.5	Calcium	2.97
Tracemin ²	01	Available phosphorus	0.57
Feed additives used (g/kg diet)		Metabolisable energy*	2805
Biobloom (probiotic)	0.5	(Kcal/kg)	
Vilocym-z (Toxin binder)	1.0	Lysine*	1.39
Ayufytase 5000 (Phytase enzyme)	0.1	Methionine*	0.64
K-live (liver stimulant)	1.0		
Ventrimix ³ (vitamin premix)	0.1		

*Calculated value

¹ Mineral mixture composition-(g/kg) Ca,320; P,60; Mg,10;

²Trace min composition (g/kg): Mn,120; Zinc,110; Fe,45; Cu,5; I,5; cobalt,2.5. ³Vitamin composition (each g contains): vitamin A, 82,500 IU; B₂, 50 mg; D₃, 12,000 IU; K, 10 mg.

Immunological tests: Blood samples were collected from wing vein in sterile test tubes from 12 birds (4 birds from each replicate) of each treatment on 0, fourth and eighth week of experiment for serological tests and were kept for 2 h in slanting position. Samples were centrifuged at 3,000 rpm for 10 min. Serum was collected by Pasteur pipette attached to a rubber teat. Sera samples were stored at -20°C till they were utilized for analysis.

All the sera samples were processed to detect the HI titer as per Allan *et al.* (1978). For cutaneous basophilic hypersensitivity test 3 birds from each replicate i.e. 9 birds per treatment were taken at fourth and eighth week of feeding of vitamins. The mitogen, phytohaemagglutinin- P (SD-fine) was dissolved in normal saline. The right wattle of each bird was injected with 100 µg of phytohaemagglutinin-P (PHA-P) in 0.1ml of normal saline solution to assess the CBH response (Corrider and DeLoach 1990). The left wattle of each bird was injected with 0.1ml NSS to serve as a control. Measurement of skin thickness was made using digital slide calipers just prior to and 24h after the injection with PHA-P. For determination of passive immunity in day old chicks blood samples were collected directly from heart from 9-day-old chicks of each group (3 from each replicate) hatched from the eggs of breeder hens after fourth and eighth week of experiment and sera samples were prepared.

Statistical analysis: The data obtained from the study were statistically analyzed according to Snedecor and Cochran (1994) and tabulated. The data were also analyzed for analysis of variance (ANOVA) and DMR test (Duncan, 1955) was used to test the difference between treatment means wherever necessary.

RESULTS AND DISCUSSION

CBH response: Among treatment groups control showed the lowest CBH response to PHA-P at fourth week, but at eighth week all the combined vitamin supplemented groups showed significantly ($P<0.05$) higher response than control and individual vitamin supplemented groups (Table 3). CBH response at eighth week showed significantly ($P<0.05$) higher values than at fourth week for each vitamin supplemented groups except in control.

Lymphocyte proliferation in response to mitogens is correlated with the ability of the host to mount a cellular immune response. It is suggested that differential reactivity to mitogens reflects either maturational or functional differences in the responsive lymphocytes. Cell mediated immune responses, as measured by CBH response to phytohaemagglutinin-P differed significantly among the groups at eighth week but not at fourth week. Antioxidant vitamin supplementation showed higher cell mediated immune response which is agreed with the findings of Lohakare *et al.* (2005) and Panda *et al.* (2008) who found higher CMI response by addition of vitamin C @ 200 ppm. Contrary to this, Leshchinsky and Klasing (2001) found vitamin E levels did not affect CBH response under normal environmental conditions.

HI antibody titre against NDV: The mean antibody titre ($\log_2$) against NDV in the hens on first day of the experiment ranged from 4.15 (T_6) to 4.22 (T_2), Table 3. On fourth week the antibody titre did not differ significantly from first day in all the groups and then on eighth week there was significant

($P<0.05$) decline in antibody titre in control and individual vitamin supplemental groups compared to first day and fourth week but the decrease in titre in combined vitamin supplemented groups was not significant.

Humoral immunity is a type of immunity which is conferred through the release of antibodies which are used to target cells for destruction. This type of immunity is a complement to cellular immunity. In the present study dietary levels of vitamins C and E did not affect antibody titre (IgG) against NDV during heat stress. Similar result was also observed by Lohakare *et al.* (2005), Shaik *et al.* (2005) and Andi *et al.* (2006) whereas higher HI titre against NDV was observed in broiler chicks by supplementation of both vitamin E @ 200 mg and Selenium @ 0.2 mg by Singh *et al.* (2006). Contrary to these findings Panda *et al.* (2008) reported that depression of humoral immunity can be alleviated by vitamins C and E supplementation alone but not in combination. High titre was observed at fourth week in all the groups as birds were vaccinated with R₂B strain against ND at 20 days prior to start of the experiment. At eighth week the titre showed declining trend but was protective. Combined supplementation of antioxidant vitamins helped to prevent the fall of antibody titre at eighth week.

Transfer of passive immunity to chicks: Antibody titre ($\log_2$) against NDV in day old chicks hatched from the eggs of breeder hens after fourth week varied between 1.85 (control) to 2.19 (T_6) and after eighth week it was between 1.79 (control) and 2.20 (T_6) among the groups but there was no significant ($p>0.05$) variation in antibody titre among the groups at both the weeks (Table 3). Though the titre started declining at eighth week in comparison to fourth week, it was not significant for each treatment group.

Antibody titre to NDV in day old chicks of all groups did not show difference either after fourth week or eighth week contrary to the findings of Andi *et al.* (2006) who found chicks from hens supplemented with 60 IU of vitamin E had

Table 3. CBH response, HI antibody titre and transfer of passive immunity to chicks in different groups

Groups	Vitamin/ dose (mg/kg)	CBH response to PHA-P (mm)		HI antibody titre against NDV ($\log_2$)			Transfer of passive immunity to day old chicks ($\log_2$)	
		fourth week	eighth week	first day	fourth week	eighth week	fourth week	eighth week
T ₁	Control	2.06 ± 0.03	2.19 ^b ± 0.02	4.16 ^A ± 0.17	4.22 ^A ± 0.14	3.57 ^B ± 0.07	1.85 ± 0.37	1.79 ± 0.33
T ₂	C200	2.14 ^B ± 0.02	2.43 ^{bA} ± 0.04	4.22 ^A ± 0.12	4.31 ^A ± 0.27	3.51 ^B ± 0.18	2.02 ± 0.24	1.97 ± 0.42
T ₃	C400	2.20 ^B ± 0.09	2.58 ^{bA} ± 0.13	4.21 ^A ± 0.18	4.33 ^A ± 0.21	3.59 ^B ± 0.20	1.90 ± 0.25	1.89 ± 0.22
T ₄	E250	2.19 ^B ± 0.13	2.53 ^{bA} ± 0.07	4.19 ^A ± 0.13	4.25 ^A ± 0.23	3.51 ^B ± 0.14	1.87 ± 0.38	1.80 ± 0.39
T ₅	E500	2.22 ^B ± 0.08	2.76 ^{bA} ± 0.18	4.18 ^A ± 0.02	4.27 ^A ± 0.20	3.55 ^B ± 0.44	1.89 ± 0.22	1.80 ± 0.18
T ₆	C200+E250	2.61 ^B ± 0.22	3.33 ^{aA} ± 0.12	4.15 ± 0.08	4.27 ± 0.25	3.65 ± 0.21	2.19 ± 0.26	2.20 ± 0.32
T ₇	C200+E500	2.49 ^B ± 0.08	3.23 ^{aA} ± 0.14	4.20 ± 0.12	4.25 ± 0.02	3.59 ± 0.12	1.99 ± 0.38	2.08 ± 0.14
T ₈	C400+E250	2.48 ^B ± 0.21	3.19 ^{aA} ± 0.03	4.21 ± 0.11	4.26 ± 0.12	3.54 ± 0.16	2.09 ± 0.27	2.11 ± 0.22
T ₉	C400+E500	2.53 ^B ± 0.21	3.25 ^{aA} ± 0.26	4.17 ± 0.22	4.22 ± 0.38	3.63 ± 0.11	2.06 ± 0.06	2.11 ± 0.11
				NS	NS	NS	NS	NS

a, b,...Means with no common superscript within a column vary significantly ($P<0.05$). NS, not significant; A, B Means with no common superscript within a row vary significantly. ($P<0.05$)

significantly higher antibody titre at first day of the age than chicks from the control and 20 IU/kg group and attributed this phenomenon to selective antibody absorption by ovarian tissue. In this experiment the breeder birds were vaccinated against ND (R₂B vaccine) before 20 days of start of experiment which is a live virus vaccine. It is known that there is about 50% transfer of titre from the breeder hen to the chick (Leeson and Summers, 2000). Therefore the transfer of passive immunity to progeny was not influenced by supplementation of vitamins E and vitamin C.

Egg production performance: The egg production performance of breeder hens per week was divided into 2 phases i.e. from 0–4 weeks to 5–8 weeks. The average egg production performance of these coloured broiler breeders is less than the commercial broiler breeders. All the 2 doses of vitamin supplementation and combinations showed significantly ($P < 0.01$) higher egg production than the control in both the phases but no difference was observed among the supplemented groups.

Significant improvement in egg production in all the vitamin supplemented groups over control, as was reported from this experiment, is well supported by the findings of Sahin *et al.* (2002), Bollengier-Lee *et al.* (1998) and Panda *et al.* (2008). The increased egg production in all groups during second phase might be due to lower ambient temperature than that of first phase. During the entire period highest egg production was found in T₆ (C200+ E250) group over individual vitamin supplemented groups and combination groups which is in agreement with the findings of Cifti *et al.* (2005), that combination of both vitamin C and E (C 200 + E 125) is more effective than individual supplementation to maintain high egg production during summer.

It may be concluded that combination of vitamins E and at both the levels showed beneficial effect on both cellular and humoral immunity status of coloured broiler breeder hens during natural hot and humid stress. As the higher level of the combination of both the vitamins E and C did not impart any significant improvement over the lower level so, vitamin C 200 + vitamin E 250 mg/kg of feed may be recommended in this tropical humid climate for better immunity during natural summer.

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