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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(12): 1470-1473

www.thepharmajournal.com
Received: 25-10-2021
Accepted: 28-11-2021

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Antistress and immunomodulatory response of dietary alpha tocopherol in broiler chicken

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Abstract
Vitamin E is a major cellular antioxidant functioning as a chain-breaker and free radical scavenger. Enzymatic and non-enzymatic antioxidants are the fundamental defense mechanisms and preliminary biomarkers for evaluating oxidative stress at cellular level. Moreover, efficient immunocompetence is a critical determinant for poultry production. Therefore, antioxidant activity and immunological studies were conducted on ninety broiler chicks (Cobb 400), divided into two groups (Group I and II) with three replicates of 15 chicks each. Groups I broilers were fed with basal diet and group II with basal diet along with 100g/tonne feed of α-tocopherol for five weeks. Serum lipid peroxidation level, reduced glutathione (GSH), superoxide dismutase (SOD), glutathione reducatase (GR), glutathione peroxidise (GPx) activity and delayed type hypersensitivity (DTH) reaction, total immunoglobulin concentration were assessed after 5 weeks of the trial. α-tocopherol dietary supplementation significantly (P<0.05) increased serum GSH, SOD activity, DTH response after 48 and 72 hours of initial sensitization, whereas decreased the lipid peroxidation level in chickens. The results suggested the immunomodulatory and antistress properties of α-tocopherol proposing it as a promising antioxidant for maximizing poultry production and performance.

Keywords: α-tocopherol, antioxidant, broiler; immunity, lipid peroxidation

Introduction
Dietary antioxidant supplementation is the salient method for alleviating stress and maximizing production of the most lucrative and highly vulnerable poultry sector. Efficient immunocompetence is a critical determinant of poultry production. Two distinct divisions of immune response are humoral and cell-mediated immunity. Humoral immune response is the adaptive function of the immune system through which antibodies are produced in response to antigenic challenge whereas cell-mediated immunity involves immune mechanism by which cells infected with an antigen are destroyed (Kellie *et al.*, 2017) [5]. Powerful defense mechanism in the form of enzymatic and non-enzymatic antioxidants is present in the body. Endogenous enzymatic antioxidants like superoxide dismutase, glutathione peroxidise, glutathione reductase and catalase play a vital role in scavenging oxidative radicals while non-enzymatic antioxidants such as reduced glutathione (GSH), vitamin A, C, E and flavenoids protect the tissues by stimulating antioxidative enzymatic system. Stress results in the overproduction of oxygen free radicals beyond the protective effect of body's antioxidant defense mechanism, damaging the biomolecules and thus disrupting the normal cell metabolism (Surai, 2018) [12]. Vitamin E is one of the most effective antioxidants for neutralizing the deleterious effects of stress by reducing the generation of reactive oxygen species (ROS) at their initial phase (Rengaraj and Hong, 2015) [8]. The earliest way to ascertain the presence of oxidative stress is by laboratory findings. Serum enzymatic antioxidants are the simplest biomarkers for evaluating fine changes in the body during oxidative stress. The degree of lipid peroxidation is also a useful indicator of ROS mediated damage and the concentration of malonaldehyde (MDA) in blood is used as its biomarker. Hence, the present study was planned with the objective to ascertain the suitability of α-tocopherol on the immune response and antioxidant status in broiler chicken.

Materials and methods
Animals
Initially proposal of the experiment was duly scrutinized by the Institutional Animal Ethical Committee, GBPUAT, Pantnagar, Uttarakhand, India (Vide approval no.: IAEC/VPB/CVASc/443 dt.10.01.21).

Ninety day-old broiler chicks (Cobb Strain) were obtained from M/s AGM Hatcheries Pvt. Ltd., Haldwani, Uttarakhand, India and housed in a Students' Poultry Instructional Farm, Pantnagar located at latitude of $28^0$53'24" North, longitude of $74^0$34'27" with an altitude of 243.84 m above msl and equipped with all poultry care facilities. The experiment was conducted during the months of March to April (spring season) with mean air temperature of 29.20 ± 0.70 to 31.51 ±0.67 ^{0}C and relative humidity of 81.20 ±0.81 %.

Dietary Treatments

Fourty-five day old chicks were randomly divided into two groups comprising of fifteen chicks in each group. Basal diet (Table 1) as prestarter (1-7 days), starter (8-21 days) and finisher (22-35 days) was offered. Birds in group I (control) were offered basal diet while in group II were offered basal diet supplemented with α-tocopherol (100 g/tonne feed; Evion-400, MERCK Ltd., Goa, India). Feed and water was provided *ad libitum* to both the groups.

Table 1: Parts composition of ingredients of basal diets of Pre-starter, Starter and broiler chicken Finisher.

Ingredients	Pre-starter (0-7 days)	Starter (8-21 days)	Finisher (22-35 days)
Maize	46.197	49	54
Soyabean (De-oiled cake)	43.50	40.55	35.05
Soyabean oil	5.57	6.298	6.92
L-Lysine	0.010	-	-
DL-Methionine	0.185	0.190	0.192
Limestone powder	1.125	1.152	1.1
Dicalcium phosphate	2.013	1.86	1.788
Trace-mineral mixture	0.5	0.30	0.30
Vitamin mixture	0.30	0.15	0.15
Salt	0.30	0.30	0.30
Choline chloride	0.1	0.05	0.05
Coccidiostat	0.10	0.05	0.05
Toxin binder	0.10	0.10	0.10
Total	100	100	100
Crude protein (%)	23	22	20
Metabolizable energy (kcal/kg)	3000	3100	3200

Blood collection and laboratory analysis

The blood samples were collected aseptically from the heart (at 7, 14 days) and from the wing vein (at 21, 35 days) of broiler birds in well labelled blood collecting tubes and serum was collected for the antioxidant and total immunoglobulin analysis.

Serum antioxidant activity was estimated at 5 weeks of age in broilers as per the methods described by Sujatha *et al.*, 2010 and Maini *et al.*, 2007 [10, 7]. Lipid peroxidation (LPO) in the serum was determined in terms of malonaldehyde (MDA) production using thiobarbituric acid method and expressed as nmol MDA/ml. Reduced glutathione (GSH) was expressed as mmol/ml. Serum SOD activity was expressed as U/mg of Hb. Glutathione reductase (GR) was expressed as µmol NADPH oxidized/mg Hb/min. Glutathione peroxidase (GPx) was expressed as EU/mg Hb.

Humoral immunity was measured as in vivo antibody production whereas cell mediated immunity was measured in vivo as cutaneous basophil hypersensitivity (CBH). Blood samples were collected randomly at weekly interval till 5th week, for humoral immune response studies. The total serum immunoglobulin was estimated by the zinc sulphate turbidity test (Sujatha *et al.*, 2010) [10]. A delayed type hypersensitivity reaction to dinitrochloro benzene (DNCB) was carried out by the procedure described by Huynh and Chubb (1987) [4].

Statistical analysis

The data were analysed by one way ANOVA using the SPSS software package version 26.0. The significant mean differences were separated by Duncan's post-hoc analysis with significance level defined at $p< 0.05$.

Results and Discussion

In the study the serum antioxidant activity and humoral, cell-mediated immune response to α-tocopherol dietary supplementation in broilers was assessed. After five weeks of the experimental trial, the serum lipid peroxidation depicted in Table 2 showed that malondialdehyde (MDA) concentration after α-tocopherol dietary supplementation significantly ($P<0.05$) lower (5.41 ±0.87 nmol MDA/ml) from control value of 8.89 ±0.99 nmol MDA/ml in broiler chicken. Similarly, significantly ($P<0.05$) higher levels of GSH were observed in group II (0.34 ±0.06 mmol/ml) as compared to group I (0.23 ±0.04 mmol/ml). The activity of serum SOD was significantly ($P<0.05$) higher (73.65 ±5.78 U/mg of Hb) in group II as compared to group I (61.20 ±3.70 U/mg of Hb). The enzyme GR exhibited non-significant increment of 6.43 ±0.28 µmol NADPH oxidized/mg Hb/min after α-tocopherol dietary supplementation as compared to group I (6.14 ±0.49 µmol NADPH oxidized/mg Hb/min). Similarly, GPx activity recorded non-significant elevation from 1.45 ±0.23 EU/mg Hb in group I to 1.46 ±0.23 EU/mg Hb in group II.

Our result supports previous observations of Timur and Utlu (2020) [13] and Ebrahimzadeh *et al.* (2018) [2] reporting decreased lipid peroxidation and increased SOD, GSH, GPx activity in broilers after vitamin E dietary supplementation. Vitamin E (α-tocopherol) functions as the primary chain-breaking antioxidant in the avian body, by scavenging free radicals and thus inhibiting the propagation of lipid oxidation and elevating GSH level, enhances SOD, GR and GPx activity (Surai, *et al.*, 2019) [11].

Delayed type hypersensitivity test depicted in Table 3, was carried out in four birds from each group, 14 days post New Castle Disease vaccination. Non-significant changes in skin thickness were observed 24 hours after DNCB insertion. After 48 and 72 hours the foot web skin thickness in group II was significantly ($P<0.05$) increased as 2.61 ±0.08 mm and 1.87 ±0.02 mm, respectively as compared to group I.

Vitamin E modulates inflammatory signaling, regulates the production of prostaglandins and leukotrienes, protects lymphocytes, macrophages and plasma cells against oxidative

damage by inhibiting protein kinase C in cells and improves the phagocytic activity of macrophages in broiler chickens (Leshchinsky and Klasing, 2001) [6]. It also reduces the secretion of immunosuppressive factors, including hydrogen peroxide. Vitamin E additives improve the cell mediated immunity of broilers supporting the previous observations in broilers (Hridoy *et al.*, 2021 and Azimi *et al.*, 2020) [3, 1] reporting increased toe web swelling response after α-tocopherol dietary supplementation in broiler chicken.

Total immunoglobulin concentration depicted in Table 4, non-significantly increased in group II on weekly basis till 35 days. These findings are in agreement with the previous observations of Hridoy *et al.* (2021) and Rostami *et al.* (2018) [3, 9] in broiler chicken.

Therefore, α-tocopherol antioxidant was found to significantly improve the cell-mediated immune response at 48 and 72 hours after DNCB injection. Moreover, the activity of serum GSH, SOD was significantly enhanced and the level of lipid peroxidation was reduced after α-tocopherol dietary supplementation.

Table 2: Mean ±S.E. of serum antioxidant activity in broilers (n=4) supplemented with α-tocopherol

Parameters/ Group	LPO (nmol MDA/ml)	GSH (mmol/ml)	SOD (U/mg of Hb)	GR (µmol NADPH oxidized/mg Hb/min)	GPx (EU/mg Hb)
I	8.89^{a} ±0.99	0.23^{a} ±0.04	61.20^{a} ±3.70	6.14 ±0.49	1.45 ±0.23
II	5.41^{b} ±0.87	0.34^{b} ±0.06	73.65^{b} ±5.78	6.43 ±0.28	1.46 ±0.23

Figures with atleast one common alphabetic superscript are non-significant and different alphabetic superscripts along columns differed significantly (P<0.05).

Table 3: Mean ±S.E. of Delayed Type Hypersensitivity Test (DTH) (Skin thickness in mm) in broilers (n=4) at different hours supplemented with α-tocopherol

Hours/Group	0	24	48	72
I	1.18 ±0.01	1.93 ±0.07	2.46^{a} ±0.05	1.65^{a} ±0.11
II	1.28 ±0.06	1.96 ±0.05	2.61^{b} ±0.08	1.87^{b} ±0.02

Figures with atleast one common alphabetic superscript are non-significant and different alphabetic superscripts along columns differed significantly (P<0.05).

Table 4: Mean ±S.E. of Total Immunoglobulin (g/L) in broilers (n=4) at different days supplemented with α-tocopherol

Days/Group	7	14	21	28	35
I	1.07 ±0.09	2.57 ±0.10	3.43 ±0.13	3.77 ±0.13	3.32 ±0.08
II	1.31 ±0.07	2.67 ±0.09	3.51 ±0.11	4.04 ±0.12	3.37 ±0.10

Figures with atleast one common alphabetic superscript are non-significant and different alphabetic superscripts along columns differed significantly (P<0.05).

Conclusion

α-tocopherol antioxidant improved the antioxidant balance and the indices of cell mediated immune response. α-tocopherol can be widely used as an antioxidant to maximize poultry performance and production.

Acknowledgments

The expenses of this work were supported by M/S Ayurvet Limited, Baddi, Himachal Pradesh.

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