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Effects of Dietary Supplementation of Vitamin E on Growth Performance and Immune System of Broiler Chickens

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ABSTRACT

As a potent antioxidant, Vitamin E may lessen the potentially harmful consequences of such oxidative stress to protect broilers against immune-pathological damage. Broiler chicken growth and viability are enhanced by Vitamin E supplementation. The present study aimed to investigate the effects of Vitamin E dietary supplementation on broiler chickens' growth performance and health status. A total of 48 one-day-old Ross chicks were randomly divided into two groups of control and treatment (supplementation of Vitamin E at a dose of 300 mg/kg diet) with three replicates per group. The study included an equal number of Ross breed chicks and Vitamin E dosage in two trials on two different dates (January and March, 2022). In both trials, the obtained results indicated no significant changes in weight gain in the control and treatment groups. In both trials, there were no significant differences in the spleen weight of the control and treated groups; however, from day 1 to 28 of the second trial, the bursa of Fabricius was heavier in the treated group than in the control group. Additionally, Vitamin E had no significant effects on the mitogenic responses to phytohemagglutinin (PHA) and Concanavalin A (Con A). Dosages of 20 and 10 µl for both PHA and Con A did not significantly affect the rate of pure lymphocyte proliferation in chicks fed 300 mg Vitamin E /kg feed. Cell-mediated immunity did not differ significantly between the two trials. The percentages of CD4, CD8, Bu1, and MHCII molecules in the spleen and cecal tonsil of the chicks that received Vitamin E 300 mg/kg feed did not change significantly. The antibody titers against infectious bronchitis and infectious bursal disease vaccines showed no significant differences. On day 42, there was a trend toward an increase in antibody titer in the case of the Newcastle disease vaccine. In conclusion, 300 mg/kg of Vitamin E added to the diet did not improve growth performance and immunity in broiler chicks.

Keywords: Broiler chicken, Growth performance, Immune system, Vitamin E

INTRODUCTION

The Food and Agriculture Organization (FAO) of the United Nations has recently shown significant growth in the global production of poultry meat (FAO, 2017). To stop lipid oxidation processes in the meat and improve meat quality, Vitamin E should be added to broiler chickens' diet (Vieira et al., 2021). This can be attributed to broiler chickens' ongoing stress from infections, high development rates, and the constantly changing environmental conditions in their housing facilities (Lohakare et al., 2005). A profound change in the productivity of the broiler chicken industry has been achieved via intentional genetic selection through traditional quantitative techniques (Hunton, 2006). Enzymes that eliminate free radicals generated during regular metabolic activity depend on antioxidants to operate appropriately (Niu et al., 2009). Systemic risks caused by free radical damage can be prevented or avoided by antioxidant enzymes, which convert them into

comparatively stable molecules (Niu et al., 2009). Antioxidant dietary supplements increase plasma glutathione peroxidase activity and reduce lipid peroxidation, which is a defensive mechanism affecting liver cells under heat stress (Calik et al., 2022). Vitamin E functions as a cellular enzymatic activity regulator (Schneider, 2005) and shields the cell membranes and tissues from lipoperoxidation damage by free radicals (Gao et al., 2010, Traber and Stevens, 2011). In order to avoid lipid oxidation in the meat and improve meat quality, broiler chicken diets must be supplemented with Vitamin E (Vieira et al., 2021).

Broiler chickens are more susceptible to pathogen infection due to their fast growth rate and the environmental problems associated with modern chicken production methods (McCorkle and Glick, 1980). Tocopherols and tocotrienols, which are fat-soluble and have antioxidant capabilities, are both included in Vitamin E (Packer et al., 2001). Vitamin E is an important fat-soluble nutrient. Its roles in domestic animal production are indispensable since animals cannot synthesize Vitamin E (Zhao et al., 2021). Vitamin E is an excellent biological chain-breaking antioxidant that protects cells and tissue from lipoperoxidation damage by free radicals (Sahin et al., 2006). Dietary supplementation of Vitamin E reduces AST and ALT activity (Amevor et al., 2022), decreases feed consumption, and improves growth parameters in broiler chickens (Dalia, 2018). Various doses of Vitamin E dietary supplementation may enhance the performance of broiler chicks (Calik et al., 2022).

Regarding broiler chickens' growth performance and relative organ weights, different Vitamin E sources (lipid soluble and water soluble) and their inclusion amounts from 22.11 to 67 mg/kg in diets show minimal effects (Pitargue et al., 2019). In broiler chickens, oxidative stress causes biological damage that negatively affects growth performance and health (Estevez, 2015). Vitamin E is mainly found close to the membrane interface in the hydrocarbon section of the membrane's lipid bilayer near the oxidase enzymes that initiate the creation of free radicals (Packer, 1991). Additionally, it has been demonstrated that Vitamin E antioxidants can enhance health by increasing humoral and cell-mediated immunity in broiler chickens (Leshchinsky and Klasing, 2001). It has been observed that Vitamin E enhances the survival, proliferation, and functionality of lymphocytes, macrophages, and plasma cells by defending them against oxidative damage (Maggini et al., 2007). Therefore, dietary supplementation of Vitamin E strengthens the immune system under stress conditions (Attia et al., 2016).

The recommended dosage of Vitamin E is 6.7-53.6 mg Vitamin E /Kg of broiler chickens' diet (NRC, 1994), depending on their growth stage (Aviagen, 2014). However, additional antioxidants, such as Vitamin C and Selenium, the kind and quantity of fats consumed, and environmental conditions all impact the necessary dietary amounts (NRC, 1994). Consequently, there is ongoing discussion regarding the appropriate levels of Vitamin E inclusion in broiler diets (Kuttappan et al., 2012).

MagginiGiven the possible advantages of Vitamin E on the health of fast-growing broiler chicks, the immunomodulatory effects of Vitamin E on broiler chickens need to be further investigated. This study aimed to assess how dietary Vitamin E affected the health and growth performance of broiler chickens.

MATERIALS AND METHODS

Ethical approval

This study was performed at Animal Production Department's Poultry Research farm. All experimental procedures involving animals were conducted in accordance with the Institutional Care guidelines of Nangarhar University Nangarhar, Afghanistan. Chickens were cared for using husbandry guidelines and standard operating procedures of broiler chickens and approved by the ethics committee of Nangarhar University, Afghanistan (12.07.2021-06).

Study design

The research was conducted in two trials of 1 and 2 in January and March 2022, respectively. A total of 48 one-day-old mixed Ross strain broiler chicks (50% male) with an average body weight of 45 g were purchased from a local hatchery in Jalalabad, Nangarhar Province, Afghanistan. The chickens were randomly divided into two groups (24 chickens in each control and the Vitamin E supplemented group). Each group had three replicates, and each replicate included eight broiler chicks. The study included an equal number of Ross breed chicks and Vitamin E dosage in two trials on two different dates The cage for each replicate was designed with a 65 cm length, 60 cm width, and 30 cm height. Total mortality for the 6 weeks was 8%. Weekly management of cage temperature and humidity included 33°C and 40% relative humidity for the first week, 30°C and 50% relative humidity for the second week, 27°C and 50% relative humidity for the third week, and 24°C and 50% relative humidity for the fourth

week, and 21°C and 60% relative humidity for the Fifth and sixth weeks. The cage was fully lit during the first 24 hours, then there was one hour of darkness on the following day, and after they reached 100-150-g body weight, the light was turned off for six hours until the experiment's completion. Lighting density was controlled (20-40 Lux).

Feed preparation

Accordingly, starter feed (0-21 days) and grower feed (22-42 days) were produced in mash form, and during both experiment trials, feed and water were always freely available. The basal diet was supplied to meet the nutrient requirements of Ross broiler chicks' as a recommendation of the National Research Council (NRC, 1994). Accordingly, starter feed (0-21 days) and grower feed (22-42 days). Feed was produced in mash form, and during both trials, feed, and water were always in free availability to the chicks (Cinar et al., 2014; Table 1).

Vaccination

The use of particular vaccinations for the specific antibody titer measurement was considered in accordance with the experiment's protocol. In Afghanistan, the production of broilers is affected by the viral illnesses Newcastle disease (ND), infectious bronchitis (IB), and infectious bursal disease (IBD). As a result, IBD, ND, and IB vaccines were administered to chickens (Lin et al., 2005). The first dose of the NB (ND+IB) vaccine (NISSEIKEN company, Japan) was administered to each chick on day 6 as an eye drop (0.03 ml). On days 18 and 32, the vaccine was administered again through the water.

On days 12 and 24, each chicken received a single dosage (0.015 ml/kg) of the IBD vaccine (NISSEIKEN company, Japan) directly using an eye dropper. All vaccinations were preserved at a temperature of 2-5°C.

Immunoproliferation assay

For the immunoproliferation assay, 15 samples of spleen from the control and 15 samples from the treatment groups were randomly obtained under sterilized conditions. The samples were then mixed with about 5 ml of phosphate-buffered saline (PBS). The suspension was prepared following the guideline methodology and centrifuged (Germany) at 300 x g for 5 minutes at room temperature (27°C).

Concanavalin (Con A) was used in this test in doses of 20 µl (6.25 µg) and 10 µl (3.125 µg) for T-cell activation, and 20 µl (37.5 µg) and 10 µl (18.75 µg) for B-cell activation. T-cell activation also utilized phytohemagglutinin (PHA), which was also used at 20 and 10 µl. The specific microplate containing the samples was incubated for 72 hours at 40°C before being thoroughly examined (Tay et al., 2020).

Flow cytometry assay

In the case of the flow cytometry assessment (McKinnon, 2018), 15 spleen and 15 cecal tonsil samples from the control and treatment groups, respectively, were randomly collected under sterile conditions. The samples were added to specific wells, such as CD4, CD8, and Bu1, and washed twice. Each well received 100 µl of PBS, including the control, and was centrifuged (Germany) at 300 x g for 5 minutes at room temperature (27°C). The PBS was discarded after centrifuging, and 100 µl of the antibody solution (1-time antibody and 99-times PBS) was added to each well except for the control. Centrifuging was repeated for 3 minutes, and the mixture was incubated for an hour. Each well received a 100 µl 10 x solution of propidium iodide (PI) after the plates were cleaned three times (one time PI and nine times PBS). In the next step, 100µl of each well content was transferred to relevant tubes for flow cytometry analysis (BD Facs canto II, United State). All of the CD4 (CT-4), CD8 (CT-8), BU1 (L22), and MHC class II (2Gll) antibodies utilized in this investigation were from Southern Biotech in Birmingham, Alabama, USA. A flow cytometer was used to evaluate the samples. About 1-2 ml of blood was drawn from the wing vein for a particular antibody titer. No heparinized blood collection tubes were used. The blood was centrifuged at 120 x 100 rpm for 20 minutes. The resultant serum was then placed in a special tube and maintained at -20°C for further analysis (Kaspers et al. 1993).

Statistical analysis

All data were analyzed by R statistical software version 2.14.2. T-test was used to compare the means of the Vitamin E supplemented group with those of the control group. Means and standard errors were used to represent the values for the observed results. The results were considered significantly different at $p < 0.05$.

Table 1. The ingredients and nutrient levels of the broiler chicken basal diet for a period of 42 days

Ingredients (%)	Starter (Day 1-21)	Grower (Day 22-42)
Corn seed	64.60	69.94
Soybean meal, *CP 48%	30.25	24.19
Soybean oil	0.00	0.50
Fish meal	1.00	1.00
Dicalcium phosphate	1.60	1.57
Calcium carbonate	1.36	1.30
Salt	0.46	0.40
Mineral-vitamin premix[a]	0.50	0.50
DL-methionine	0.17	0.15
HCL-lysine	0.00	0.44
Nutrients composition		
Metabolizable energy (kcal/kg)	2,988	3,083
Crude protein (%)	(23)	(20)
Calcium (%)	1.01	0.96
Phosphorus (%)	0.50	0.48
Vitamin E (mg/kg)	(6.7)	(6.7)
Selenium (mg/kg)	(0.15)	(0.15)

Source: NRC (1994), *CP: Crude protein; [a] Mineral-vitamin premix provided the following per kilogram of diet: vitamin A, 5,500 IU; vitamin D3, 1,100 IU; vitamin E, 10 IU; riboflavin, 4.4 mg; vitamin B12, 12 mg; nicotinic acid, 44 mg; menadione, 1.1 mg; biotin, 0.11 mg; thiamine, 2.2 mg; and ethoxyquin, 125 mg, Mn, 120 mg; Zn, 100 mg; Fe, 60 mg; Cu, 10 mg; Se, 0.17 mg; I, 0.46 mg; and Ca, minimum:150 mg, maximum: 180 mg

RESULTS

Growth performance

The findings showed that adding Vitamin E at 300 mg/kg to the feed had no significant effect on the growth performance of broiler chicks from 1 to 42 days of age ($p > 0.05$, Table 2).

The obtained results indicated no significant difference in spleen weight between the control and Vitamin E -supplemented groups ($p > 0.05$, Table 3). However, there was a significant difference in the bursa of Fabricius between the control and treatment groups in the first trial ($p < 0.05$).

Immunoproliferation assay

Dietary supplementation of 300 mg Vitamin E /kg feed did not suppress mitogenic responses significantly to PHA and Con A ($p > 0.05$). Pure lymphocyte proliferation rates were not significantly different between control, and Vitamin E supplemented groups ($p > 0.05$), where the PHA was used at 20 and 10 µl as well as Con A at 20 and 10 µl (Table 4).

Flow cytometry

After 4 and 6 weeks of age, there was no significant difference between control and Vitamin E supplemented groups in both trials in terms of immune molecules percentage ($p > 0.05$). Dietary supplementation of Vitamin E at 300 mg/kg feed in Ross broiler chicks had no significant effect on the percentages of CD4, CD8, Bu1, and MHC II molecules in the spleen and cecal tonsil ($p > 0.05$).

Specific antibody titers

In the Vitamin E 300 mg/kg supplemented group, total antibody titers for IBD and IB after days 14, 28, and 42 did not change significantly ($p > 0.05$). After days 14 and 28, there was no significant difference between the control and Vitamin E supplemented groups in both trials for Newcastle disease ($p > 0.05$); however, there was an increase after day 42 ($p > 0.05$).

Table 2. Feed intake, feed efficiency, and mean body weight differences in broiler chickens

Group	Trial 1			
	BWG (g/chick)	BWG (g/chick)	Feed intake (g/chick)	FCE
	day 14	days 14-28	days 14-28	
Control	363 ± 25	634 ± 53	982 ± 50	1.59 ± 0.12
Treatment	309 ± 27	565 ± 44	824 ± 45	1.48 ± 0.09
	day 42	days 28-42	days 28-42	
Control	1536 ± 76	902 ± 121	1354 ± 21	1.62 ± 0.21
Treatment	1476 ± 118	910 ± 99	1478 ± 43	1.71 ± 0.20
	Trial 2			
	day 14	days 14-28	days 14-28	
Control	261 ± 4	568 ± 46	867 ± 42	1.63 ± 0.12
Treatment	269 ± 13	650 ± 51	1091 ± 0.5	1.75 ± 0.14
	day 42	days 28-42	days 28-42	
Control	1259 ± 119	691 ± 161	1530 ± 62	3 ± 0.32
Treatment	1421 ± 85	771 ± 116	1561 ± 15	2.5 ± 0.44

FCE: Feed conversion efficiency, BWG: Body weight gain, Trial 1: Experimental exploratory-1 conducted on January 2022, Trial 2: Experimental exploratory-2 conducted on March 2022.

Table 3. Effects of Vitamin E dietary supplementation on some internal organs of broiler chickens

Organ (g/100gw) body	Age (day)	Trial 1		Trial 2	
		Control	Treatment	Control	Treatment
Spleen	28	0.136 ± 0.02	0.139 ± 0.02	0.117 ± 0.01	0.105 ± 0.01
	42	0.224 ± 0.05	0.329 ± 0.04	0.146 ± 0.03	0.100 ± 0.02
Bursa of Fabricius	28	0.193 ± 0.04[a]	0.311 ± 0.02[b]	0.219 ± 0.03[a]	0.205 ± 0.01[a]
	42	0.206 ± 0.02	0.182 ± 0.04	0.147 ± 0.01	0.172 ± 0.01

[a, b] different values in the same row are significantly different ($p < 0.05$). Trial 1: Experimental exploratory-1 conducted on January 2022, Trial 2: Experimental exploratory-2 conducted on March 2022.

Table 4. Effect of dietary Vitamin E and mitogen concentration on the proliferation of mononuclear cells

Vitamin E (mg/kg)	Number of chickens	Age	Con A		PHA	
			6.25 (µg/ml)	3.12 (µg/ml)	37.5 (µg/ml)	18.75 (µg/ml)
0	4	Second week	0.56 ± 0.04	1.54 ± 0.24	1.95 ± 0.17	1.17 ± 0.07
300	4		0.78 ± 0.09	1.22 ± 0.12	1.64 ± 0.12	0.94 ± 0.06
0	4	Fourth week	1.09 ± 0.08	1.06 ± 0.03	1.02 ± 0.04	0.96 ± 0.03
300	4		0.9 ± 0.23	1.17 ± 0.04	1.42 ± 0.24	1.02 ± 0.04
0	4	Sixth week	1.09 ± 0.03	1.055 ± 0.03	1.23 ± 0.04	0.96 ± 0.03
300	4		1.25 ± 0.06	0.999 ± 0.02	0.97 ± 0.03	0.96 ± 0.05

Con A: Concanavalin A, PHA: Phytohemagglutinin A

DISCUSSION

Numerous dietary components, such as vitamins A and C, help animals incite the best immunological responses. Vitamin E is well known as an efficient chain-breaking antioxidant preventing oxidative damage to body tissues (Voljc et al., 2011). Moreover, it was demonstrated that the antioxidant properties of Vitamin E can improve animal health by improving cell-mediated and humoral immunity in broiler chickens (Leshchinsky and Klasing, 2001). The findings of the current study indicated that 300 mg/kg of Vitamin E as a dietary supplement did not affect broiler chickens' performance.

The inclusion of Vitamin E did not significantly affect broiler chicken development and relative organ weights when comparing 22.11 to 67 mg/kg feed (Pitargue et al., 2019).

According to the findings of the current investigation, broiler chicks' live body weight was not improved by a 300 mg/kg Vitamin E -supplemented diet. According to Dalia (2018), the addition of Vitamin E at 100 mg/kg feed considerably reduced feed consumption and enhanced growth parameters, which is contrary to the findings of the current study.

The results of the present study supported those of Goni et al. (2007), who found that broiler chicks' average daily gain, average daily feed intake, and feed conversion ratio were unaffected by Vitamin E supplementation up to 400 mg/kg. The current study findings are opposed to those of Swain et al. (2000), indicating that dietary supplementation of α-tocopherol acetate at 300 mg/kg feed could considerably increase average daily gain and significantly decrease average daily feed intake. At the dose of 300 mg/kg, Vitamin E supplemented diet revealed a significant increase in the relative weight of the bursa of Fabricius ($p < 0.05$). However, the dietary supplementation of Vitamin E had no impact on the relative weights of the spleen.

Dietary supplementation of 300 mg Vitamin E /kg feed did not suppress mitogenic responses significantly to PHA and Con A ($p > 0.05$). Pure lymphocyte proliferation rates were not significantly different where the PHA was used at 20 and 10 µl as well as Con A at 20 and 10 µl. The results were in contrast to those of Leshchinsky and Klasing (2001), who found that 16.75-33.5 mg Vitamin E /kg feed of broiler chicks significantly changed mitogenic response to PHA and Con A.

After 4 and 6 weeks of age, there were no significant differences between control and Vitamin E supplemented groups in both trials in the current study regarding immune molecules percentage ($p > 0.05$). Vitamin E at a dosage of 300 g/Kg had no significant effect on the proportions of CD4, CD8, Bu1, and MHCII molecules in the spleen or in the cecal tonsil ($p > 0.05$).

On days 14, 28, and 42 after IBD and IB vaccinations, there were no significant differences between the control and treatments in terms of humoral antibody titer ($p > 0.05$). On days 14 and 28 of the

experiment, the antibody titer of the chicks supplemented with 300 mg Vitamin E /kg feed did not increase significantly for the ND vaccine ($p > 0.05$); meanwhile, on day 42 of the experiment, the results showed that there was the tendency for titer rising for ND vaccine ($p < 0.05$). Overall, the results of the present study are in contrast to those of Niu et al. (2009), who demonstrated that broiler raised under heat stress produced considerably more immunoglobulin G (IgG) and immunoglobulin M (IgM) in response to 200 mg/kg dietary Vitamin E.

In the current study, there was no increase in antibody titer against the IB virus in the treated group supplemented with 300mg Vitamin E /kg feed, which was in contrast to the findings of Leshchinsky and Klasing (2001), indicating that supplementation of Vitamin E between 0-16.75 mg/kg feed could increase antibody production to attenuated IB vaccine.

Immunocompetence is not considered in the NRC's suggested Vitamin E level (NRC, 1994), despite the fact that this level (0.67mg/kg feed) is sufficient to stop oxidative damage. However, few research studies outline the exact requirements of Vitamin E for a certain immunological function.

CONCLUSION

The current study indicated that dietary supplementation of 300 mg Vitamin E /kg feed did not improve broiler chicks' live body weight, feed intake, and feed consumption ratio throughout 6 weeks. The study also revealed that Vitamin E at 300 mg/kg feed did not raise immunological responses of broiler chicks. In conclusion, a high-level dose (300 mg/kg) intake of dietary Vitamin E alone is not advised as a growth promoter and immunological enhancer. As a result, it is difficult to recommend the ideal dietary Vitamin E concentration for growth improvement and immunocompetence, which calls for more research in the area.

DECLARATION

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Authors' contributions

Rozi Khan, Mohammad Azim, and Najibullah created the idea and designed the study. Rozi Khan and Sayed Ziaul Haq collected data. Sayed Attaul Haq performed the statistical analysis and wrote the paper. All authors checked and confirmed the final analysis data and the last revised manuscript before publication in the journal.

Competing interests

The authors declare that there is no conflict of interest in this research work.

Ethical consideration

Before submitting, the authors verified for ethical concerns such as plagiarism, consent to publish, misconduct, data fabrication and/or falsification, multiple publishing and/or submission, and redundancy.

Availability of data and materials

The presented data of this study will be sent by the authors upon to a reasonable request.

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