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In ovo injection of vitamin E on post-hatch immunological parameters and broiler chicken performance

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ABSTRACT

Objective: To investigate the *in ovo* injection (IOI) of vitamin E (VE) on serum post-hatch immunological parameters and broiler chicken performance.

Methods: Fertile eggs (n=400) were distributed into four groups of 100 eggs. On 14th day of incubation, two groups were injected with 0.5 mL of 15 or 30 mg VE dissolved in physiology serum. Rest two groups were used as sham control (injected with 0.5 mL physiology serum) and un-injected control. The hatched chickens from each group were randomly assigned to four replications of 12 chickens and reared under standard condition.

Results: Hatchability percentage was apparently increased (P<0.05) by IOI of VE rather controls. Antibodies titer of avian influenza, infectious bronchitis, and the level of IgM were significantly increased (P≤0.043) by IOI of 30 mg/egg VE rather both controls at 21 days of age. Antibodies titer of avian influenza, infectious bronchitis, and newcastle disease were significantly increased (P≤0.033) by IOI of VE (30 mg/egg) rather both controls at 42 days of age. This treatment was significantly increased (P≤0.034) the levels of IgM and IgA relative to sham control at 42 days of age. Higher (P=0.004) level of IgG was obtained by IOI of both levels of VE rather both controls at 42 days of age. Feed intake was increased (P=0.041) by IOI of VE (30 mg/egg) at 1 to 21 days of age.

Conclusions: Broiler chicken performance did not affect with IOI of VE, while hatchability and post-hatch immunity status were boost up by IOI of 30 mg/egg VE.

1. Introduction

The failure of vaccination, infection disease depression of immunity system, and unusual administration of antibiotics leads to impress of immunity responses[1]. The promotion of immunity system is very critical against infection diseases in poultry production. The subsequent development of avian embryos and hatched chickens are influenced by the yolk nutrient status[2]. Egg yolk is the source of lipids which supply energy for early development of embryo by oxidation[3]. During early development, rapid oxidative metabolism could lead to production of large quantities of free radicals and could damage embryo[4,5]. Antioxidants are a critical defense against free radicals[6]. *In ovo* injection (IOI) of antioxidants during incubation may enhance antioxidant status of the chicken embryo[3,7].

Vitamin E (VE) is an excellent biological chain-breaking antioxidant that protects cells and tissue from lipoperoxidative damage induced by free radicals[8], and has multi-effects on fortification of immunity system[9,10]. The feeding of VE reduces oxidative stress and lipid peroxidation[11], and increases immunoglobulin in broiler chicken, turkey, and ducks[12,13]. Owing to the importance of hatchability and post-hatch immunity of broiler chickens, a study was conducted to examine the effect of IOI of VE on hatchability, immunity status, and broiler

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chicken performance.

2. Materials and methods

2.1. VE preparation

VE was prepared as a solution of 1 g of water dispersible VE (E-Viton®; 100 mg alpha tocopheryl acetate per one capsule; Daru Pakhsh Company, Iran) per 10 mL of serum physiology and 0.1 mL from this solution was equivalent to 10 mg of VE.

2.2. Eggs incubation and injection

Fertile eggs (n=400), forming the broiler breeders flock (Ross 308; broiler breeders age, 40 weeks, first cycle of production, average egg weight, 61.5 g, and production percentage, 79%) maintained on the breeders adequate nutritional plan were collected, weighted and distributed into four groups of 100 eggs. Fertility was verified by candling with a hand ultraviolet lamp at 12th day of incubation. On 14th day of incubation, two groups were injected with 15 or 30 mg/egg VE using 25 mm needle as standardized method[14]. Rest two groups were used as sham control (IOI of 0.5 mL sterile physiology serum/egg) and un-injected control. Sterile physiology serum was included as sham control, primarily to rule out a possible negative response caused by the stress of injection and handling. The injections were carried out under laminar flow system, where temperature of the chamber was maintained at 37 °C for avoiding any temperature stress for chicken embryo. Prior to IOI, the site of injection was disinfected with 70% ethanol and the solutions were warmed to 30 °C. The injected eggs were returned to the incubator after injection. Within 20 min, IOI of each treatment was completed of taking out from incubator. Immediately after the injection, the pinhole site was sealed with sterile paraffin wax and eggs were returned to the incubator. On the 19th day of incubation, the eggs were shifted to the hatcher and kept in the respective pedigree hatching boxes. On the day of hatch, chickens were weighted and hatching percentage was recorded.

2.3. Bird management and feeding

The hatched chickens from each group were randomly assigned to four replicates of 12 chickens. All chickens were reared under similar managerial and hygienic conditions. The chickens were raised in clean, well-ventilated, previously disinfected room. The lighting schedule was 23 h light/1 h darkness at 32 °C on the 1st day. This was subsequently reduced 3 °C each week until the end of the third week then maintained at 23 °C. The chickens of different experimental groups were fed on basal diet (Table 1) to meet the nutrient requirement[15]. Mash diet and fresh water were offered *ad libitum*. Weight gain (WG) and feed intake (FI) were measured weekly and cumulatively and feed conversion ratio (FCR) was calculated accordingly.

Table 1
Diets formulation and composition (% or as stated) at 1 to 42 days of age.

Item	Starter (1 to 21 days of age)	Grower (22 to 42 days of age)
Corn grain	53.5	53.8
Soybean meal	41.3	38.7
Soybean oil	1.0	3.0
Calcium carbonate	1.65	1.63
Dicalcium phosphate	1.75	1.72
Mineral and vitamin premix[1]	0.5	0.5
Common salt	0.30	0.44
Methionine	0.44	0.41
Lysine	1.10	1.10
Total	100	100
Metabolizable energy (kcal/kg)	3050	3000
Crude protein	23.00	21.54
Calcium	1.0	0.93
Available phosphorus	0.45	0.45
Calcium: phosphorus	2.22	2.07
Energy: protein	132.61	139.27

[1]Mineral premix: Mn, 80 mg; Zn, 84.5 mg; Fe, 80 mg; Cu, 5 mg; I, 1.0 mg; Co, 0.48 mg; Se, 0.30 mg/kg of diet. Vitamin premix: Vitamin A, 11000 IU (retinol); Vitamin D3, 3000 IU; Vitamin E, 50 mg (DL-α-tocopheryl acetate); Vitamin K3, 5 mg; Tiamin, 2 mg; Riboflavin, 8 mg; Calcium pantotenat, 12.40 mg; Niacin, 50 mg; Pyridoxine, 7 mg; Pholic acid, 2 mg; Vitamin B12, 1.60 mg; Biotin, 5 mg; Choline chloride, 1100 mg; Antioxidant, 100 mg/kg of diet.

2.4. Immunological assay

Two injections of sheep red blood cells (0.1 mm, 5%) antigen were done intramuscularly for the evaluation of humoral system responses at 16 and 37 days of age. Four birds from each replicate were randomly selected and blood samples were taken via wing vein at 21st and 42nd day. Thereafter, serum was separated by centrifugation of coagulated blood at 3000 r/min for 15 min. Immunoglobulin A, M, and G (IgA, IgM, and IgG, respectively) were determined[16].

Antibodies titration against newcastle disease (ND), avian influenza (AI), and infectious bronchitis (IB) diseases were performed using ELISA by specific kits of OVATEC® Plus, SERELISA® Rabies (Synbiotics, USA), and BIA-CK® 121 (Biochek, Netherlands), respectively.

2.5. Statistical analysis

All data were analyzed for normal distribution using the NORMAL option of the UNIVARIATE procedure of GLM procedure of SAS software[17]. Pen was used as the experimental unit and data were analyzed as a completely randomized design by the GLM procedure of SAS software[17]. Statistical differences were established using a Duncan's multiple range test at the level of P<0.05.

3. Results

Hatchability percentage (68%) was apparently increased (P<0.05) by IOI of VE rather un-injected and sham controls

Table 2

Effect of treatments on serum antibody (log_2) of broiler chickens at different ages.

Item	21 days of age			42 days of age		
	ND	AI	IB	ND	AI	IB
Sham control	507.7	347.1^{bc}	158.4^{b}	1365.6^{c}	903.3^{bc}	470.2^{c}
Un-injected control	536.8	331.5^{c}	143.5^{b}	1 530.5^{bc}	790.4^{c}	594. 9^{d}
VE, 15 mg/egg	605.6	388.3^{ab}	170.7^{ab}	1 711.4^{b}	1 022.4^{ab}	731.7^{b}
VE, 30 mg/egg	652.3	423.5^{a}	197.5^{a}	2 011.5^{a}	1 108.4^{a}	911.2^{a}
SEM	23.64	9.51	5.42	46.06	33.86	20.17
P-value	0.11	0.027	0.034	0.008	0.033	0.002

Means with common letters in the same columns are not significantly different ($P>0.05$). SEM: Standard error of the means; ND: Newcastle disease; AI: Avian influenza; IB: Infectious bronchitis; VE: Vitamin E.

Table 3

Effect of treatments on serum immunoglobulin (mg/dL) of broiler chickens at different ages.

Item	21 days of age			42 days of age		
	IgG	IgM	IgA	IgG	IgM	IgA
Sham control	326.5	136.7^{b}	32.2	323.5^{b}	112.8^{c}	25.9^{b}
Un-injected control	380.8	139.8^{b}	36.1	338.2^{b}	120.8^{bc}	32.9^{ab}
VE, 15 mg/egg	401.9	148.3^{ab}	39.1	387.3^{a}	129.9^{ab}	38.5^{a}
VE, 30 mg/egg	411.0	154.9^{a}	44.0	417.6^{a}	136.3^{a}	42.5^{a}
SEM	20.68	4.12	3.31	5.92	2.38	1.76
P-value	0.07	0.043	0.15	0.004	0.028	0.034

Means with common letters in the same columns are not significantly different ($P>0.05$). SEM: Standard error of the means. VE: Vitamin E.

Table 4

Effect of treatments on broiler chicken performance at different ages.

Item	1-21 days of age			22-42 days of age			1-42 days of age		
	FI (g)	WG (g)	FCR	FI (g)	WG (g)	FCR	FI (g)	WG (g)	FCR
Sham control	929.1^{b}	523.4	1.78	3 159.8	1 561.3	2.02	4 088.9	2 084.7	1.96
Un-injected control	941.6^{ab}	525.8	1.79	3 120.8	1 536.3	2.03	4 062.4	2 062.1	1.97
VE, 15 mg/egg	1 041.2^{ab}	642.7	1.64	3 174.6	1 651.0	1.94	4 215.8	2 293.7	1.85
VE, 30 mg/egg	1 080.0^{a}	668.1	1.63	3 179.3	1 626.2	1.96	4 259.4	2 294.3	1.85
SEM	26.48	28.44	0.045	32.90	33.19	0.031	42.76	53.59	0.032
P-value	0.041	0.13	0.44	0.94	0.64	0.71	0.31	0.25	0.47

Means with common letters in the same columns are not significantly different ($P>0.05$). SEM: Standard error of the means; FI: Feed intake; WG: Weight gain; FCR: Feed conversion ratio; VE: Vitamin E.

(57%). Moreover, no significant differences were found in body weight of chickens at hatch (44.22-45.04 g).

IOI of VE (30 mg/egg) led to significant increases ($P\leqslant0.034$) in AI and IB antibodies titer at 21 days of age and significant increases ($P\leqslant0.033$) in AI, IB, and ND antibodies titer as compared to un-injected and sham groups at 42 days of age (Table 2). The level of IgM was significantly increased ($P=0.043$) by IOI of VE (30 mg/egg) in compared with sham and un-injected groups at 21 days of age (Table 3). The level of IgG was significantly higher ($P=0.004$) with IOI of VE at levels of 15 or 30 mg/egg rather both control groups at 42 days of age. Moreover, IOI of VE (30 mg/egg) caused a significant increase ($P\leqslant0.034$) in the levels of IgM and IgA in compared with sham control at 42 days of age. Table 4 shows that IOI of VE (30 mg/egg) led to increases in FI at 1 to 21 days of age ($P=0.041$). No significant differences were found in other performance parameters at different ages.

4. Discussion

The result of hatchability percentage is consistent with other study[13]. The hatchability and embryonic mortality of fertile eggs were influenced by the type of injected substance and site of injection into the eggs. Exogenous VE injection around 14th day of incubation (critical time of fatty acid oxidation) may be beneficial in reducing the production of free radicals that cause a serious damage to the cellular membranes[4,18], and increase lipid utilization for energy production to improve hatchability[3]. Therefore, higher hatchability percentage after IOI of VE may be due to the improvement of the antioxidant status of the eggs[18], or protection effects of the VE against oxidation[19]. It is reported that higher body weight was obtained by IOI of VE due to the prevention of hydro peroxides impacts and more energy production by VE injected eggs which enhance the embryonic growth[3]. This is the opposite of the result of current study. It may be due to differences in VE usage dose.

The results of current study are in agreement with Gore and Qureshi[12], who reported that the hatched chickens and turkeys from VE exposed embryos were high responders compared to the control group for antibody production rather in-feed supplementation of VE. It is reported that the antibody titer to kill ND vaccine was significantly increased by direct injection of VE into eggs[20] and diet[10]. Moreover, significant improvement was observed in antibodies and Ig levels of chickens fed by VE[21,22], and lymphoid organs of turkeys by IOI of VE[13]. Higher improvements in immunity system by IOI of VE in present study are in accordance with Goel *et al*[20]. It is due to antioxidant properties of VE

that protects immunological tissue from destruction[10], influences VE on helper T-cells, phagocytosis activity, and prostaglandin synthesis in lymphoid organelles. The antioxidant status of hatched chick's tissues enhances by VE which protects lipid membranes from radical oxygen species[3]. In addition, VE reduces oxidative stress and increases proliferation of B-cells and thereby leads to higher production of Ig and better antibodies responses[21]. No significant differences were found in WG, FI (except 1 to 21 days of age), and FCR by treatments which are in agreement with other researches[20,23]. They explained no clear details for their observations. However, the degree of response to IOI depends upon genetics, breeder hen age, egg size, and incubation conditions. The development of the neonatal birds is dependent on residual nutrients found in the yolk sacs that have been depleted during the hatching process[24]. It is thought that the residual yolk is sufficient to maintain the bird until feed is offered. However, the initiation of growth may be more dependent on feed consumption than the nutrients found in the yolk post-hatch[25]. Therefore, although IOI of VE increased hatchability and immunity system, offering of similar diets for all treatments led to similar performance.

It could be concluded that broiler chicken performance did not affect with IOI of VE, though oxidative stress protection of embryos through IOI of VE (30 mg/egg) can be regarded as a possible method to improve hatchability and immune status of broiler chickens against challenge of various diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

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