Journal of Animal & Veterinary Advances
MEDWELL PUBLICATIONS

The Effects of *in ovo* Injection of Nanocurcumin and Vitamin E on Immune Responses and Growth Performance of Broiler Chickens under Heat Stress

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Key words: Nanocurcumin, immunity, *in ovo* administration, production, vitamin E

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Page No.: 124-133
Volume: 20, Issue 5, 2021
ISSN: 1680-5593
Journal of Animal and Veterinary Advances

Abstract: The study aimed to determine the effects of *in ovo* injection of nanocurcumin and vitamin E on the immunity and growth performance of broilers. Six hundred eggs containing live 17.5 day-old embryos were assigned to six treatments. Four groups were inoculated as follows: 0.05 mL corn oil containing 0.015, 0.030 mL/egg nanocurcumin, 0.037 and 0.074 IU/egg vitamin E, whereas two groups remained as un-injected controls and oil-injected sham. The chicks were reared under heat stress. The cellular immunity was significantly increased ($p<0.05$) by *in ovo* administration of vitamin E 0.037 and decreased by *in ovo* injection of vitamin E 0.074 at 8 and 12 h after injection as compared to control. Body weight gain 1-10 day was significantly decreased ($p<0.01$) by *in ovo* injection of nanocurcumin 0.015 and vitamin E 0.074, however, it and feed intake 25-42 day was significantly increased ($p<0.05$) by *in ovo* injection of nanocurcumin 0.030 without affecting feed conversion ratio. This study evidenced the *in ovo* administration of nanocurcumin 0.030 improved growth performance during 1-42 days, however, decreased the hatchability. Solutions did not have a significant effect on humoral immunity but cellular immunity was improved by *in ovo* injection of vitamin E 0.037.

INTRODUCTION

In Ovo Injection (IOI) is a technique to inoculate external materials into the egg during development of embryos to obtain the beneficial effects on hatchability, growth performance, immunity status and meat quality[1]. Sharma and Burmester[2] were pioneers in this technic for the turkey Marek's vaccination. Lately, the *in ovo* technique has been studied for administering ascorbic acid[3], carbohydrates[4,5], amino acids[6,7], vitamins[8,9] and minerals[10,11]. The conclusion of this technique relates to type of diluent and solution inoculated, inoculation area, time of injection and other agents[12]. The yolk nutrient affects development of avian embryos and chickens[13] and provided the avian energy[14]. Turmeric (*Curcuma longa*) is a rhizomatous perennial herb of the ginger family[15,16] that its medicinal properties of turmeric have been known thousands years ago but recognition of the exact

mechanism recently been invented[17]. Ordinary extract of *Curcuma longa* L. contains Diferuloylmethane/ curcumin, Demethoxycurcumin and Bisde-methoxycurcumin, of which type 1 is the most common form[18, 19] that curcumin is the basic matter[20]. Several procedures improve the curcumin bioavailability and enhance cellular permeability include curcumin nanoparticles, curcumin-phospholipid complexes, liposomal curcumin and the adjuvants that block rapid metabolism of curcumin. Curcumin acts as an anti-inflammatory, powerful antioxidant[21, 22] and pro-apoptotic agent in human leukemic cells[23], hepatocytes protective[24] decreasing the aflatoxin in broilers[25]. Vitamin E (VE) is a chain breaking lipid antioxidant and free radical scavenger in the cell membranes and sub-cellular organelle[26] that may affect the immune system via. acting on the immune cells or changing the endocrine factors[27]. Vitamin E enhances T cell production and decreases T-cell suppressive factors in mammals[28] and improves the reproduction performance in breeder chickens[29, 30]. Two type of vitamin E (d-α-tocopherol and dl-α-tocopheryl acetate) were used to evaluate the animal oxidative status[31]. Micellized natural VE is better than the synthetic or natural VE to elevating plasma α-tocopherol E[32] due to high solibility and absorption[33]. One mg of α-tocopherol is equivalent to 1.49 IU of vitamin E[34]. Stress is the most common causes of oxidative stress[35] and the most destructive factor in the animal's lives including birds that has detrimental effects on production and feed efficiency[14]. The acute stress might amplify immunity but chronic stress would be immunosuppressive[36]. In mitochondria due to defective reduction of oxygen to reactive oxygen species, a main cellular source of oxidative stress happens[37]. High environmental temperatures negatively affect the specific immune response development of chicks[38]. Among domestic animals, broiler chickens are the most sensitive to HS[39]. As temperature goes up, growth rate, feed efficiency and survival rate diminish[40]. The H/L ratio was used for evaluating of stress. To our knowledge, there are no published data on the effects of IOI of NC on different traits. Thus, this study was carried out to evaluate the effects of IOI of NC and VE on the immune system of broiler chickens which exposed to chronic HS.

MATERIALS AND METHODS

Preparation of injection solutions: Brown vials with rubber stoppers were used to prepare the sterilized solutions of NC and VE. The vials were autoclaved at 121°C for 20 min. The solutions were prepared using corn oil and were transferred into labelled sterile vials through filtered syringes (0.22 μm) (MS® CA Syringe Filter, China). The vials were kept at 4°C until the injection. The vials were transmitted to an incubator to reach the incubation temperature (37-38°C), 15 min before the injection. SinaCurcumin® 80 (one mL of the solution contained 80 mg nanocurcumin (NC)) was purchased from Exir Nano Sina Co in Tehran, Iran. Vitamin E solution was 99.99% purity product of Merck Company.

Incubation and injection steps: A total of 600 eggs with an average weight of 45.5 g from a commercial Ross 308 broiler breeder flock aged 47-week-old were stored for 2 days before being set in Dizbad hatchery Inc., of Mashhad, Iran to reach stable temperature and humidity. The embryonated eggs, assigned to six treatments of 5 replicates with 20 eggs each and were placed on the same floor to had alike incubation conditions. The infertile eggs were exchanged with fertile ones by candling on the 3th day of incubation. In this study the solutions were inoculated into the amniotic cavity of fertile eggs. To find the injection point, the eggs were candled through exposure to the light source at d 17.5 of incubation. The wide end of egg placed upward and it's lateral bump touched the lighting window. Then, the eggs were turned around longitudinal axis. The nearest place of the embryonic shadow to the air sac was found and 0.5 cm above that point was marked on the eggshell[41]. The injection point was cleaned with 70% ethanol before the piercing. The treatments were injected by 0.015, 0.030 mL/egg of NC, 0.037 and 0.074 IU/egg VE solutions through the holes via insulin syringes, one group were used as un-injected control and another group received just corn oil as sham control. The injection holes were closed using paraffin. The injection steps was carried out in a setter room under standard incubation conditions (T = 37.6°C; RH = 65%). At the end of d 18 of incubation, the eggs were transferred to the hatching cabinet. Hatchlings chicks were divided into 6 treatments. Each treatment included 5 replicates (approximately equal males and females birds).

Rear condition: All of one-d-old hatched chicks were weighed and transferred to a rearing farm. The chicks were divided into 6 treatments with five replicates of 20 chicks each. The birds were reared on floor covered with wood shavings and fed with standard starter, grower and finisher diets during 1-10, 11-24 and 25-42 d, respectively (Table 1). The diets were formulated according to the requirements of Ross 308 broiler chicks. All birds were given ad libitum access to feed and water and they were handled in accordance with therecommendations[42]. But the birds were exposed to chronic HS (35±2)°C for 5 h per day with relative humidity 65%)[43-46] during the trial. Relative humidity was maintained at 65%[44]. A lighting program of 18 L:6 D (light: dark) was performed during the whole experiment (1-42 d).

Table 1: Composition and calculated analysis of experimental diets (as-fed basis) a

Ingredients %	Starter (1-10 days)	Grower (11-24 days)	Finisher (25-42days)
Corn (8% CP)	47.53	51.63	57.57
Soybean meal (44% CP)	42.35	37.99	32.35
Soybean oil (9000 kcal/kg)	5.54	6.24	6.29
Limestone (38% Ca)	1.2	1.12	1.05
Dicalcium phosphate (21% Ca)	1.79	1.56	1.34
Vitamin premix a	0.25	0.25	0.25
Mineral premix b	0.25	0.25	0.25
NaCl	0.4	0.4	0.4
DL-Methionine (99%)	0.37	0.32	0.28
Lysine (78%)	0.28	0.22	0.22
Threonine (98.5%)	0.05	0.02	0
Calculated values (%)			
Metabolizable energy (kcal/kg)	2990	3082	3218
Crude protein (%)	23	21.3	19.3
Calcium (Ca) (%)	0.96	0.87	0.79
Available phosphorus (%)	0.456	0.409	0.361
Sodium (Na) (%)	0.16	0.16	0.16
Methionine (%)	0.71	0.64	0.58
Methionine+Cystine (%)	1.07	0.89	0.89
Lysine (%)	1.46	1.3	1.17
Arginine (%)	1.56	1.45	1.3
Threonine (%)	0.96	0.87	0.78
Tryptophan (%)	0.35	0.32	0.29

a = The vitamin premix supplied the followings per kilogram of diet: vitamin A, 9,000 IU; vitamin D3, 1,000 IU; vitamin E, 18 IU; vitamin K3, 2 mg; thiamine, 2 mg; riboflavin, 6.5 mg; vitamin B6, 2 mg; vitamin B12, 0.01 mg; niacin, 30 mg; choline chloride, 500 mg; vitamin C, 50 mg; calcium pantothenate, 8 mg; folic acid, 0.5 mg; b = The mineral premix supplied the followings per kilogram of diet: Mn, 100 mg; Fe, 50 mg; Zn, 70 mg; Cu, 10 mg; I, 1 mg, Se, 0.2 mg

Assessed variables

Humoral immunity: On d 28 and 35, two male chicks per replicate were injected intravenously with 1 mL 5 % SRBC suspension and on d 35 and 42, blood samples (2 mL) were collected from the wing jugular and the SRBC suspension again was injected to chicks.

Cell-mediated immunity: Two male birds of each replicate were injected hypodermically between first and second toe of right leg by 0.1 cc phytohemagglutinin (PHA) on d 30 and the toe web thickness was measured in 4, 8, 12 and 24 h later to evaluate the cell-mediated immunity.

Heterophil/lymphocyte ratio: On d 10 two male birds from each replicate were selected and blood samples were taken from the wing vein using a disposable syringe (2 mL). The blood sample were transferred to10-mL tube containing ethylenediaminetetraacetic acid (EDTA) and blood smears were stained by Giemsa. The white blood cell counts were determined based on Neubauer-hemocytometer procedure[47]. The percentages of heterophil (H), lymphocyte (L) and Heterophil/ lymphocyte ratio (H/L) were recorded.

Performance and hatchability rate

Hatchability rate: The hatched birds were counted on d 1. The hatchability percentage was determined through this formula: (number of hatchlings per treatment*100)/ total number of fertile eggs[41]. To calculate BW 1d, total initial weight of each replicate was divided to the number of live chicks of that replicate.

Growth performance: The birds were weighed on d 1, 10, 24 and 42. Feed Intake (FI) and Feed Conversion Ratio (FCR) was calculated during mentioned days with considering the dead birds.

Statistical analysis: Data obtained through this study were analyzed using the General Linear Models method of the SAS software[48] in a Completely Randomized Design (CRD), the means compared by the Duncan's test ($p \leq 0.05$).

Funding: The authors would like to appreciate the president for research at the Ferdowsi University of Mashhad for funding this research (project number 49201) and Dizbad hatchery Co. staff for their cooperation in *in ovo* injections. Also, we thank Mr. Masoud Kamkarhoseizadeh for his assistance throughout the experiment.

RESULTS

Humoral immunity: The effects of different levels of *in ovo* inoculation of NC and VE on humoral immunity on 35 and 42 d of broilers chickens under chronic HS are summarized in Table 2. Humoral immunity 42 d, total immunoglobulin (T Ig) and immunoglobulin G (Ig G) 35 d were not significantly affected by IOI of NC and VE whereas immunoglobulin M (Ig M) 35 d was significantly decreased by IOI of NC 0.015, 0.030 mL/egg and VE 0.074 IU/egg as compared to non-injected ones ($p \leq 0.05$).

Table 2: The effect of in ovo injection of nanocurcumin (NC) and vitamin E (VE) on humoral immunity (µL) of broiler chicks

Injection solutions (mL/egg)	Total Ig 35d	Ig G 35d	Ig M 35d	Total Ig 42d	Ig G 42d	Ig M 42d
NC 0.015 (mL/egg)	4.13	0.75	3.38b	9.17	7.5	1.75
NC 0.030 (mL/egg)	5.38	1.86	2.88b	8.88	8	0.88
VE 0.037 (IU/egg)	4.13	0.25	3.88ab	9.38	7.88	1.5
VE 0.074 (IU/egg)	4.5	0.88	3.63b	9	7.88	1.13
control	6.63	2.57	3.50b	9.29	8	1.5
Sham control	7.25	2.5	4.75a	9.25	8	2
SEM	0.87	0.7	0.38	0.53	0.5	0.28
p-value	0.054	0.1	0.03	0.98	0.99	0.09
p-value						
Control vs. NC	0.08	0.16	0.43	0.7	0.74	0.59
Control vs. VE	0.03	0.03	0.6	0.88	0.84	0.59
NC vs. VE	0.62	0.29	0.11	0.76	0.76	1

Each mean represents 5 observations; SEM: Standard Error of the Means; Ig: Immunoglobulin; The means within the same row with at least one common letter, do not have significant difference (p>0.05)

Table 3: The effect of *in ovo* injection of nanocurcumin (NC) and vitamin E on the toe thickness (mm) (d 30) of broiler chicks

Injection solutions	PHA pre injection	PHA 4 h	PHA 8 h	PHA 12 h	PHA 24 h
NC 0.015 (mL/egg)	1.10b	1.96	1.70bc	1.60cd	1.81
NC 0.030 (mL/egg)	1.20b	2.46	2.06b	1.89ab	1.98
VE 0.037 (IU/egg)	1.61a	2.46	2.56a	2.07a	2.04
VE 0.074 (IU/egg)	1.10b	1.99	1.47c	1.34d	1.55
Control	1.39ab	2.36	1.93b	1.77bc	1.91
Sham control	1.62a	2.37	1.76bc	1.63bc	1.84
SEM	0.1332	0.17	0.14	0.1	0.15
p-value	0.017	0.11	0.0001	0.0001	0.26
p-value					
Control vs. NC	0.12	0.45	0.74	0.82	0.9
Control vs. VE	0.79	0.52	0.6	0.61	0.51
NC vs. VE	0.14	0.92	0.33	0.72	0.52

Each mean represents 5 observations; NC: Nanocurcumin; VE: Vitamin E; PHA: Phytohemagglutinin; SEM: Standard Error of the Means; The means within the same row with at least one common letter, do not have significant difference (p>0.05)

Table 4: The effect of *in ovo* injection of Nanocurcumin (NC) and Vitamin E (VE) on heterophil/lymphocyte ratio of broiler chicks (10 d)

Injection solutions	Heterophil (%)	Lymphocyte (%)	Heterophil/Lymphocyte
NC 0.015 (mL/egg)	28.00	59.25a	0.506
NC 0.030 (mL/egg)	28.75	56.75ab	0.472
VE 0.037 (IU/egg)	26.75	58a	0.493
VE 0.074 (IU/egg)	28.25	52.75c	0.517
Control	27.75	53.75bc	0.503
Sham control	28.00	52.75c	0.537
SEM	0.96	1.21	0.024
p-value	0.79	0.0032	0.53
p-value			
Control vs. NC	0.60	0.01	0.65
Control vs. VE	0.83	0.29	0.94
NC vs. VE	0.38	0.04	0.52

Each mean represents 5 observations; SEM: Standard Error of the Means; The means within the same row with at least one common letter, do not have significant difference (p>0.05)

Cellular immunity: The effects of IOI of nanocurcumin and vitamin E on cellular immunity are shown in Table 3. The resultant thickness after 8 and 12 h was significantly increased by IOI of VE 0.037 IU/egg and it was significantly decreased by IOI of VE 0.074 IU/egg as compared to control ($p \leq 0.05$). Orthogonal contrast between control group and NC, control group and VE, NC and VE regard to cellular immunity didn't have a significant difference.

H/L ratio: The effects of IOI of nanocurcumin and vitamin E on H/L ratio of broilers chickens exposed to chronic HS are presented in Table 4. IOI of NC and VE didn't show a significant effect on H/L ratio but Lymphocyte (L) percentage was significantly increased by IOI of NC 0.015 mL/egg and VE 0.037 IU/egg as compared to the control ($p \leq 0.05$). The birds of NC 0.030 mL/egg and VE 0.037 IU/egg had the numerical decrease in H/L ratio as compared to non-injected ones. The H/L ratio is more valuable than heterophil percentage or lymphocyte percentage alone[17].

Performance and hatchability rate: The effects of IOI of nanocurcumin and vitamin E on growth performance of broilers chickens exposed to chronic HS are summarized in Table 5 and 6. In this study Body Weight Gain (BWG)

Table 5: The effect of *in ovo* injection of Nanocurcumin (NC) and vitamin E (VE) on growth performance and hatchability rate of broiler chicks (1-10, 11-24, 25-42 d)

Injection solutions	BWG (g/bird) 1-10d	dFI (g/bird/d)	FCR (g:g)	BWG (g/bird) 11-24d	dFI (g/bird/d)	FCR (g:g)	BWG (g/bird) 25-42d	dFI (g/bird/d)	FCR (g:g)
NC 0.015 (mL/egg)	13.91bc	25.21	1.817	48.59	78.85	1.574	113.06ab	202.52b	1.79
NC 0.030 (mL/egg)	14.59ab	25.03	1.664	50.89	83.78	1.65	121.15a	219.10a	1.782
VE 0.037 (IU/egg)	14.83a	24.1	1.624	50.37	79.05	1.57	111.12bc	199.82b	1.801
VE 0.074 (IU/egg)	13.39c	25.5	1.742	47.57	79.96	1.625	103.69c	185.49c	1.789
control	15.20a	24.24	1.599	50.44	80.32	1.597	107.06bc	195.26bc	1.826
Sham control	13.40c	23	1.712	46.19	76.74	1.704	107.61bc	195.48bc	1.819
SEM	0.31	0.89	0.09	1.36	2.09	0.4	2.95	4.65	0.03
p-value	0.002	0.42	0.56	0.17	0.32	0.18	0.018	0.002	0.87
p-value									
Control vs NC	0.02	0.42	0.21	0.67	0.71	0.75	0.01	0.01	0.29
Control vs VE	0.01	0.61	0.46	0.38	0.75	0.99	0.92	0.65	0.4
NC vs VE	0.67	0.73	0.54	0.57	0.41	0.72	0.005	0.001	0.77

Each mean represents 5 observations; NC, nanocurcumin; VE, vitamin E; Ig, immunoglobulin; SEM: standard error of the means. The means within the same row with at least one common letter, do not have significant difference (p>0.05)

Table 6: The effect of in ovo injection of nanocurcumin (NC) and vitamin E (VE) on growth performance and hatchability rate of broiler chicks (1 to 42 d)

Injection solutions	Hatch (%)	BWG (g/bird/d)	BW (g/bird)	dFI (g/bird/d)	FCR (g:g)
NC 0.015 (mL/egg)	95a	54.68bc	2762.5	93.75b	1.736
NC 0.030 (mL/egg)	84c	58.85a	3036	100.28a	1.692
VE 0.037 (IU/egg)	96a	55.00b	2848	93.35b	1.719
VE 0.074 (IU/egg)	90b	51.27d	2860	89.22b	1.739
control	96a	53.89bcd	2941	92.06b	1.72
Sham control	94ab	51.80cd	2840	90.69b	1.73
SEM	1.39	1.05	99.18	1.88	0.025
p-value	0.0001	0.001	0.51	0.02	0.79
p-value					
Control vs. NC	0.001	0.038	0.74	0.047	0.85
Control vs. VE	0.096	0.57	0.47	0.73	0.77
NC vs. VE	0.022	0.003	0.66	0.008	0.55

Each mean represents 5 observations; BW: Body Weight; BWG: Body Weight Gain; FI: Feed Intake; FCR: Feed Conversion Ratio; SEM: Standard Error of the Mean; The means within the same row with at least one common letter, do not have significant difference (p>0.05)

was significantly decreased (p<0.01) by IOI of NC 0.015 mL/egg and VE 0.074 IU/egg as compared to non-injected ones during d 1-10. BWG (p<0.05) and FI (p<0.01) was significantly increased by IOI of NC 0.030 mL/egg during d 25-42. During whole of experiment (d 1-42) the birds hatched from NC 0.030 mL/egg had the highest BWG, the highest FI and the lowest FCR between all treatments. The hatchability rate was significantly decreased (p<0.01) by IOI of NC 0.030 mL/egg and VE 0.074 IU/egg as compared to the control.

DISCUSSION

Humoral immunity: The birds of VE groups had lower T Ig and Ig G 35 d than birds of control (p≤0.05). This decrease may be due to amount of VE was decreased in heat stress (HS)[49], in other word the stress of oxidants and injection alleviated antioxidant effect of VE. In addition when chickens were exposed to high temperatures (32.2-43°C), the resulting humoralimmunity[50], primary and secondary antibody responses[51, 52] was significantly declined. Also, Qureshi and Miller[50] reported the antibody responses to SRBC in chickens exposed to 32.2-43°C were significantly reduced. Chickens exposed to stress showed decrease in the phagocytic capability of macrophages. Bakyaraj *et al*.[53] mentioned IOI of vitamins didn't effect on the humoral immunity in broilers and Lin and Chang[54] demonstrated that vitamin E treatment groups didn't show any significant difference in antibody titer responses to SRBC and newcastle. Results obtained in current study are contrary to Singh *et al*.[55], Leshchinsky and Klasing[56] and Niu *et al*.[57] that reported dietary vitamin E led to significant enhancement of antibody to SRBC in broilers exposed to HS and Emadi and Kermanshahi[58, 59] that reported addition of turmeric to broiler chickens diets led to improvement of the immune system. Inconsistency in published informations may be due to the method of administration, purity of vitamin E, or the concentration of curcuminin turmeric.

Cell-mediated immunity: The thickness difference of toe is as a criteria for the cell-mediated immunity[60]. Its increase by IOI of VE 0.037 IU/egg and decrease by IOI of VE 0.074 IU/egg after 8 and 12 h might demonstrate that the lower dose of VE could amplify the cellular immunity whereas higher dose vitamin E might have inhibitory effect on cellular immunity[53, 50]. In agreement with our findings Bakyaraj *et al*.[53] showed the higher

dose of VE (0.50 mg) had lower anti SRBC response than the lower dose (0.5 IU). Some scientists reported that phagocytic potential of macrophages[53, 50] and it's phagocytic ability[52, 50, 57] in chicken exposed to HS was decreased. Although, there are scarce published information on the effect of HS on the cell-mediated immune responses. Orthogonal contrast didn't have a significant difference in all comparison.

H/L ratio: As shown in Table 4, the results of current study showed that the ratio of H/L was not significantly affected by injected solutions but it was numerically decreased ($p \leq 0.05$) by IOIof NC 0.030 mL/egg and VE 0.037 IU/egg. This may be due to the numbers of monocytes and lymphocytes are reduced and numbers heterophils and the H/L ratio are increased in stress[61]. Because cortisone production in stress can affect the number of lymphocyts, granulocytes and the granulocyte: lymphocyte ratio[62]. This numerical decrease of H/L ratio probably was due to antioxidant effect of NC[63] and VE[64] escalating of lymphocyte number, improving of immunity status and subsequently diminishing of the H/L ratio. Orthogonal contrast of lymphocyte was significantly increased by IOI of NC groups as compared to VE groups and control group. It probably conclude that nanocurcumin is more effective than VE on lymphocyte percentage. Two of the most ordinary physiological indices of stress are the H/L ratio and concentrations of CORT[65, 66]. H/L ratio acts as a proper indicator of evaluation of stress in poultry[17]. The stressors increase plasma concentrations of CORT followed by elevated secretion of ACTH and corticotropin releasing hormone. Glucocorticoids and notably cortisol enhance H/Lratio[14]. Supplementation of curcumin/nanocurcumin (200 or 400 mg kg^{-1}) to diet significantly increased the number of lymphocyte and decreased the number of heterophil and H/L ratio as compared to control diet[44]. Also, Hosseini-Vashan[63] concluded that the supplementation of TRP (0.4 or 0.8%) to diets, decreased the stressor index. There are very scarce data on nanocurcumin *in ovo* administration but Tayer *et al*.[60] resulted that H/L ratio and heterophil percentage were increased by Dietary supplementation of green grape leaves in broilers. It should be noted that physiological reaction to stress is a multifactorial phenomenon, thus just one parameter (whether the H/L ratio or a behavioral response plasma concentrations of corticosteroid (CORT)) should be considered to evaluate the stress[14].

Hatchability rate and performance: The decrease of hatchability rate ($p<0.01$) by IOI of NC 0.030 mL/egg and VE 0.074 IU/egg may be due to the high NC and VE doses in the current study. In other words high dose of VE and NC may have inverse effect on different traits[50]. Thus, the recommended dose of NC and VE are 0.015 mL/egg and VE 0.074 IU/egg, respectively. Orthogonal contrast showed that NC groups had lower hatchability rate than VE groups ($p<0.05$) and control group ($p<0.01$). However, Surai[67] mentioned that when high amount of reactive oxygen species are formed in bird cells, VE fights them in membrane, thus, the oxidative stress of birds is minimized and hatchability is improved[67] and when chicks peck to internal shell, concentration of oxygen in embryo tissues goes up, therefore, formation of free radicals is possible[68] VE protects tissues of embryos, against lipid oxidation[67]. According to Salary *et al*.[9] hatchability percentage was increased ($p<0.05$) by IOI of VE (15 or 30 mg) as compared to non-injected ones. VE supplementation (60.4 IU) showed the highest hatching rate ($p<0.05$)[12]. To our knowledge, there are limited published data on the effects of IOI of curcumin on hatchability. In this study, the highest average of daily BWG (d 11-24, 25-42, 1-42) and the lowest FCR (d25-42 and 1-42) was found by IOI of NC 0.030 mL/egg. It might be due to NC regarding to their antioxidant role in biological systems, decreased the oxidant load from body[64] resultant improved growth performance. Results obtained in our study are in agreement with the observations of Xie *et al*.[69] who found the BW, Average Daily Gain (ADG) were significantly decreased and FCR were significantly increased in curcumin groups (1000 and 2000 mg kg^{-1}) as compared to control. Araujo *et al*.[12] concluded that *in ovo* administration VE supplementation (49.5 and 60.4 IU) on 17.5 d of embryonic improved the chick quality and performance results. On the contrary, Bhanja *et al*.[70], Salary *et al*.[9] and Rajkumar *et al*.[71] reported that *in ovo* administration of VE (after 17.5 d of embryonic development) didn't ameliorate performance of chicks. However, some scientists reported that heat stress increases the release of catecholamines and corticosterone[72, 73], subsequently glucocorticoids enhances energy consumption[74] and body weight is declined[75]. Hosseini-Vashan *et al*.[63] demonstrated that supplementation of TRP to diet didn't have a significant effect on body weight, FI, FCR, antibody production against sheep red blood cell, IgG and IgM in broilers but it led to decrease of blood cholesterol and low density lipoprotein and increase of blood high density lipoprotein. Daneshyar *et al*.[76] concluded that when dietary TRP increased from 0.0 or 2.5 to 5 g kg^{-1}, serum total tocopherol was linearly increased and serum MDA content decreased quadratically at week 6. Unfortunately, to date, rare information exists on role of IOI of curcumin on different traits.

CONCLUSION

The IOI of NC 0.030 mL/egg despite the hatchability decrease had the best FCR. The cellular immunity was improved by IOI of VE 0.037 IU/egg.

ACKNOWLEDGMENTS

The authors would like to appreciate the president for research at the Ferdowsi University of Mashhad for funding this research (project#49201) and Dizbad hatchery Co. staff for their cooperation in *in ovo* injections. Also, we thank Mr. Masoud Kamkarhoseizadeh for his assistance throughout the experiment.

Bioethical standards: The authors confirm that they have followed Animal Care and Use Committee of the Ferdowsi University of Mashhad, Iran at the Ferdowsi University of Mashhad research farm between September and October 2019.

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