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Effects of *in ovo* injection of nanocurcumin and vitamin E on antioxidant status, immune responses, intestinal morphology and growth performance of broiler chickens exposed to heat stress

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Abstract The aim of this study was to investigate the effects of *in ovo* injection of nanocurcumin (NC) and vitamin E (VE) on antioxidant status, intestinal histomorphology and growth performance of the hatched chicks. Fertile eggs (n=352) were allocated to 8 treatments with 4 replicates of 11 eggs each. At d 17.5 of incubation, the eggs were injected with 0.01, 0.03 and 0.05 mL/egg of NC and 0.03, 0.06 and 0.09 mL/egg VE solution into the amniotic cavity, and the rest two groups were used as sham and un-injected controls. The hatchlings from each treatment were randomly assigned to 4 replicates of 10 birds and reared until d 24 of age under heat stress (8 h daily at 35°C). The experimental design used was a completely randomized one. Results showed that superoxide dismutase activity in liver was increased (P<0.01) by IOI of 0.05 mL/egg NC in compare with control and sham groups on d 10. Total antioxidant capacity was decreased (P<0.05) by IOI of 0.03 and 0.06 mL/egg VE in compare with the control group. Liver glutathione peroxidase and malondialdehyde activities, blood heterophil (H) and lymphocyte (L) counts and H/L ratio, serum total cholesterol, HDL, LDL and VLDL levels and also crypt depth (CD) were not significantly affected by treatments on d 10. Villous height (VH) in the birds hatched from 0.01 and 0.03 mL/egg NC treatments were shorter than control group. The VH to CD ratio in NC treatments and 0.03 and 0.06 mL/egg VE were lower than controls (P<0.01). *In ovo* administration of NC and VE had no significant effects on growth performance of broiler chickens during 1-10 days of age; but decreased (P<0.05) feed intake during 1-24 d without affecting body weight gain and feed conversion ratio. Hatchability in 0.03 and 0.05 mL/egg NC treatments were decreased (P<0.05) in compare with the control treatment. In general, *in ovo* administration of NC improved antioxidant status of the hatchlings. *In ovo* injection of NC and VE didn't have a significant effect on posthatch growth performance of the broiler chickens.

Keywords: broiler, heat stress, *in ovo* injection, nanocurcumin, vitamin E

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Introduction

In ovo injection (IOI) with exogenous materials is a method to improve hatchlings performance (Kadam et al., 2013). Many years ago, *in ovo* technology, first became available for vaccination in broiler hatcheries (Ricks et al., 1999). Then it was used to deliver nutrients to embryos; because birds have a limited source of nutrients for development of embryo (Uni et al., 2012). Thus, nutritious pack and antioxidant capacity may be insufficient to supply the embryo requirements leading to poor embryo development, and reduced hatchability and quality of the chicks. This deficiency may be resolved by the provision of additional sources of essential nutrients and antioxidants via *in ovo* administration (Urso et al., 2015).

Heat stress is one of the most challenging circumstances impressing commercial poultry production (Ghazanfarpoor and Talebi, 2013). High ambient temperatures have detrimental effects on thermoregulation, increase mortality and reduce feed consumption and growth rate of the chickens (Bolukbasi et al., 2007). It is well known that high temperatures result in oxidative stress in the body (Rukkumani et al., 2004).

Curcumin is a yellow and hydrophobic polyphenol pigment derived from *Curcuma longa* L. (turmeric) (Anand et al., 2007). Studies on humans (Menon and Sudheer, 2007) and animals (Tayer et al., 2012) have revealed anti-inflammatory, anti-oxidative, anti-carcinogenic, anti-microbial, hypocholesterolemic and cardioprotective properties of curcumin. It has been reported that the curcumin has protective effects against oxidative stress by improving antioxidant capacity and reducing the lipid peroxidation. Thus, it can be considered as an available antioxidant source (Rahmani et al., 2017).

Nanocurcumin is a polymeric nanoparticle-encapsulated curcumin. Physico-chemical characterization of the polymeric nanoparticles confirms a narrow size distribution in the 50 nm range (Bisht et al., 2007). Nanocurcumin has higher stability, bioavailability, target specificity, and lower particle size than curcumin. Nanoparticles show special features like large surface area, quantum effect and ability to bind and carry compounds like drugs (Rajsekhar et al., 2015).

Urso et al. (2015) showed that vitamin E supplementation in broiler breeder diet improved the overall quality of newborn chicks and the egg hatching rate. The embryo uses enzymatic (catalase, superoxide dismutase and glutathione peroxidase) and non-enzymatic (vitamin E, carotenoids, vitamin C, reduced glutathione and coenzyme Q) antioxidants and minerals (Se, Zn, Mn and Fe) to overcome the oxidative stress. Vitamin E, carotenoids and cofactor minerals are provided by the maternal diet, and the others are synthesized in the embryonic tissues (Surai and Fisinin, 2012). Antioxidants are substances that protect cells from the oxidative damages caused by free radicals. So, they are essential for embryo health and survival, especially during the last 3 days of the incubation period (Malheiros et al., 2012). One of the key indicators in poultry industry is production of chickens that bear a variety of environmental situations such as heat stress (Bolukbasi et al., 2007). Some of the beneficial methods to decrease the heat stress of broiler chickens include *in ovo* inoculation of antioxidants such as vitamin E and C (Bolukbasi et al., 2007). Although, there are several reports on *in ovo* administration of VE, there are no data on the effects of *in ovo* feeding of NC on broiler chickens. Therefore, this study was conducted to evaluate the effects of *in ovo* injection of nanocurcumin and vitamin E on the antioxidant status, immune responses, intestinal morphology and growth performance of broiler chickens exposed to heat stress.

Materials and methods

Ethical approval

All procedures were approved by the Animal Care and Use Committee of the Ferdowsi University of Mashhad, Iran.

Preparation of injection solutions

Dark vials with rubber stoppers and caps were used to prepare the solutions of nanocurcumin and vitamin E solutions. At first, the vials were autoclaved at 121 °C for 20 min. The solutions were prepared separately and poured into labelled vials through filtered syringes with pore sizes of 0.22 µm (MS® CA Syringe Filter, China). The vials were placed at 4 °C before the injection. Then, the vials were transferred to an incubator and allowed to reach the level of incubation temperature (37 °C to 38 °C), 15 min prior to the injection. Nanocurcumin (one mL of the solution contained 80 mg nanocurcumin) was provided by Exir Nano Sina Co., Tehran, Iran. Vitamin E solution was a commercial product with 99.99% purity.

Incubation and injection procedures

A total of 352 eggs with an average weight of 59.7 g were purchased from a commercial Ross 308 broiler breeder flock aged 35 wk. The eggs were stored for 2

days before being set in a local commercial hatchery (Dizbad hatchery Inc., Mashhad, Iran) to reach stable condition. The eggs, allotted to eight treatments of 4 replicates with 11 eggs each, were set on the same floor to provide similar incubation conditions. On the third day of incubation, all the eggs were candled and the infertile eggs were replaced with fertile ones. During the first 18 days of incubation period, temperature (T) and relative humidity (RH) were maintained at 37.6 °C and 65%, respectively. At d 17.5 of incubation (Khaligh et al., 2017), all eggs were light-candled to determine the injection site. For this purpose, the blunt end of egg was held upward and the lateral bulge of egg abutted the edge of lighting window. Afterward the egg was turned around its longitudinal axis until the nearest point of the embryonic shadow to the air sac seemed, and 0.5 cm above that point was marked on the eggshell for injection (Khaligh et al., 2017). The injection site was cleaned at the eggshell surface with 70% ethanol prior to piercing. Treatments including 3 doses of NC (0.01, 0.03 and 0.05 mL/egg) and vitamin E (0.03, 0.06 and 0.09 mL/egg) were injected via the egg holes using 1 mL insulin syringes equipped with 15 mm disposable nedles (25 gauge). The control group did not receive any treatment, and the sham control was just pierced (Triplett et al., 2018). The injection holes were immediately covered using paraffin. The injection procedure was performed 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.

Housing, conditions and diets

Day-old chicks (n=320) were allotted to eight treatments per IOI groups, with four replicates of 10 birds each (five males and five females). The birds were housed in steel battery cages (1 m × 0.4 m × 0.4 m), and fed with standard starter and grower diets during 1-10 d and 11-24 d, respectively (Table 1). The diets were formulated to meet the requirements of Ross 308 broiler chicks (Aviagen, 2014). Feed and water were provided *ad libitum* through a tube feeder and 3 nipple drinkers in each cage. Management practices were performed based on the recommendations (Aviagen, 2014), except that the birds were subjected to heat stress at 35 (±2) ◦C (8 h daily; 09:00 am until 17:00) during the whole experimental

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

Ingredient (%)	Starter (1 to 10 d)	Grower (11 to 24 d)
Corn	40.49	39.40
Soybean meal (44% CP)	40.61	36.13
Wheat	10.00	15.00
Soybean oil	4.37	5.21
Dicalcium phosphate	1.86	1.63
Limestone	1.14	1.06
Common salt	0.44	0.44
DL-Methionine	0.39	0.34
L-Lysine HCl	0.29	0.24
L-Threonine	0.08	0.06
Vitamin premix[a]	0.25	0.25
Mineral premix[b]	0.25	0.25
Calculated analysis (%)		
Metabolizable energy (kcal/kg)	2970	3045
Crude protein	22.29	20.35
Available Phosphorus	0.48	0.435
Calcium	0.96	0.87
Sodium	0.18	0.19
Methionine	0.74	0.66
Methionine + Cystine	1.06	0.99
Lysine	1.45	1.31
Threonine	0.98	0.91

[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 B_{12}, 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.

period (days 1 to 24); otherwise, the room temperature was maintained at the recommended level. Relative hu midity was kept at 60% during the experiment. A lighting program of 23L:1D (light: dark) was used during the trial.

Hepatic antioxidant indices

One male bird from each pen weighing closest to the mean body weight of the pen, was killed by cervical dislocation on d 10. The liver was removed, transferred into a microtube and frozen at −70 °C. The frozen samples were thawed and homogenized in potassium chloride (KCl; ≥ 99% purity) solution (1.15% w/v, pH: 7.4) at 4 °C. The homogenates were centrifuged using a refrigerated centrifuge at 2292 g for 15 min, and supernatants were analyzed for GPX and total superoxide dismutase (TSOD) activities and malondialdehyde (MDA) concentration. The activity of TSOD was determined colorimetrically (auto analyzer, ALCYON 300-Abbott, USA) using a Ransod kit (Randox Laboratories Ltd. UK) according to Habibi et al. (2014). The enzymatic method (auto analyzer, ALCYON 300-Abbott, USA) with a Ransel kit (Randox Laboratories Ltd. UK) was used to measure the GPX activity (Hajati et al., 2018). The concentration of liver MDA was measured spectrophotometrically at 532 nm using thiobarbituric acid reactive substances (TBARS) as described by Satoh (Satoh, 1978). The MDA concentration was expressed as nmol/mg of protein and the TSOD and the GPX activities were reported as unit/mg of protein.

Heterophil/lymphocyte ratio and blood serum indices

On d 10, one male bird from each replicate was selected and a blood sample taken from the wing vein using a sterile syringe (2 mL) into 10-mL tube containing ethylenediaminetetraacetic acid (EDTA). Blood smears were prepared on slides and stained by Giemsa method. The white blood cells were enumerated by an improved Neubauer hemocytometer method (Hajati et al., 2018). The heterophil (H) and lymphocyte (L) percentages, H/L ratio, and serum total cholesterol, HDL (high density lipoproteins), LDL (low density lipoproteins) and VLDL (very low density lipoproteins) levels were measured (Hosseini-Vashan et al., 2015).

Intestinal Morphology

One male bird from each pen, weighing closest to the mean body weight, was selected and killed by cervical dislocation on d 10. A one-cm tissue sample from the middle point of the jejunum was taken, and kept in 10% neutral buffered formalin which was replaced after 24 h; then, the samples were immersed in paraffin, fixed on slides and stained with hematoxylin and eosin. The villous height was measured from the tip to the base of the villus, and the villous width was measured at its middle part. The crypt depth was measured from villus-crypt junction to the distal limit of the crypt. Five villi were measured from each sample and their averages were used as the means of the villous height, villous width and crypt depth in each bird (Giannenas et al., 2010).

Hatchability rate

The hatched chicks were counted and weighed on d 1 and transferred to the broiler rearing farm (Poultry Research Center of the Ferdowsi University of Mashhad, Mashhad, Iran). The hatchability rate was calculated as follows (Khaligh et al., 2017):

$$\text{Hatchability rate (\%)} = \frac{\text{number of hatchlings} \times 100}{\text{number of fertile eggs}}$$

The total hatch weight of each replicate was divided to the number of hatchlings of that replicate to calculate the average initial body weight.

Growth performance

The birds were weighed on d 1, 10 and 24. Feed intake was recorded during each phase (1-10 d and 11-24 d), and FCR was calculated after adjusting for the dead birds.

Statistical analysis

Data were analyzed using the General Linear Models procedure of the SAS software (SAS, 2012) in a completely randomized design, and the means compared using the Tukey's test ($P \leq 0.05$).

Results and discussion

Liver antioxidant and blood serum indices

The effects of *in ovo* injection of nanocurcumin and vitamin E on liver antioxidant capacity and blood serum lipids of broiler chickens on 10 days of age are shown in Tables 2 and 3, respectively. *In ovo* administration of 0.05 mL NC/egg, significantly increased liver SOD activity compared with both control groups ($P<0.01$). *In ovo* injection of VE significantly decreased the liver total antioxidant activity compared with the control group

Table 2. The effect of *in ovo* injection of nanocurcumin (NC) and vitamin E (VE) on liver antioxidant indices in broiler chicks (10 d)

Injection solutions (mL/egg)	GPX (U/mg protein)	SOD (U/mg protein)	MDA (nmol/mg protein)	TAC (mmol/l)
NC 0.01	0.133	5.90^{c}	0.0485	1.61^{ab}
NC 0.03	0.150	7.18^{bc}	0.0537	1.57^{ab}
NC 0.05	0.168	10.67^{a}	0.0586	1.41^{abc}
VE 0.03	0.157	8.75^{ab}	0.0418	1.36^{bc}
VE 0.06	0.149	6.85^{bc}	0.0442	1.10^{c}
VE 0.09	0.126	6.51^{c}	0.0397	1.22^{bc}
control	0.132	7.33^{bc}	0.0434	1.80^{a}
Sham control	0.133	7.45^{bc}	0.0411	1.41^{abc}
SEM	0.012	0.72	0.0048	0.13
P-value	0.240	0.007	0.1223	0.044

a,c: Within columns, mean values with common superscripts are not different (P>0.05; Tukey's test).
GPX, Glutathione peroxidase; SOD, Superoxide dismutase; MDA, Malondialdehyde; TAC, Total antioxidant capacity. SEM: standard error of the mean. Each mean represents 4 observations.

Table 3. The effect of *in ovo* injection of nanocurcumin (NC) and vitamin E (VE) on blood serum indices in broiler chicks (10 d)

Injection solutions (mL/egg)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Total Cholesterol (mg/dl)
NC 0.01	51.67	57.67	13.67	123.33
NC 0.03	69.0	53.0	15.33	137.33
NC 0.05	61.67	50.0	18.0	130.0
VE 0.03	59.0	54.67	14.67	128.33
VE 0.06	68.67	70.33	13.67	152.67
VE 0.09	57.0	59.67	17.33	134.0
control	69.33	49.0	14.33	133.0
Sham control	70.33	43.33	17.33	131.33
SEM	5.69	8.71	1.87	12.67
P-value	0.231	0.547	0.540	0.842

Each mean represents 4 observations.
SEM: standard error of the mean.

(P<0.05). The liver GPX activity, and MDA concentration, and serum metabolites of HDL, LDL, VLDL and total cholesterol levels were not significantly affected by *in ovo* injection of nanocurcumin and vitamin E on 10 days of age (Table 3). The first enzyme that participates in the antioxidant defense procedure is the metallo-protein enzyme SOD. The seleno-enzyme GPX reduces glutathione to form glutathione disulfide (GSSG) and catalyzes the reaction of hydroperoxides. Therefore, elevated concentration of these enzymes may improve the antioxidant capacity in broilers. The concentration of plasma MDA is an index of the antioxidant systems (Heckert et al., 2002). Our results are in agreement with the findings of Hosseini-Vashan et al. (2012) who reported that the diets containing turmeric (*Curcuma longa* L.) rhizome powder increased the activity of SOD and GPX and improved the antioxidant status of the heat stressed broilers without affecting the immune system and growth performance. Our data on MDA concentration and liver GPX and SOD activities were in disagreement with Suvanated et al. (2003) who reported that diets containing turmeric rhizome powder reduced the oxidative reactions in broiler chicks and the rate of MDA production and TBARS index; but were in agreement with reports of Hajati et al. (2014) and Sirovina et al. (2013). Dietary supplementation of turmeric rhizome extract at doses of 100, 200 and 300 mg/kg increased the antioxidant capacity (Wang et al., 2015).

Hajati et al. (2014) reported that IOI of vitamin C at a rate of 3 mg per egg increased the GPX activity. Sirovina et al. (2013) found that chrysin and quercetin exhibited high antioxidant activities and strong hepatoprotective effects upon intraperitoneal injection to diabetic mice for 7 days; the treated mice showed significant reductions in hepatic lipid peroxidation determined by measuring MDA production. In the current study, GPX activity

was higher in birds receiving 0.03 and 0.05 mL NC/ egg as well as in the birds treated with vitamin E at the doses of 0.03 and 0.06 mL/egg than the control groups.

Because the antioxidant status of hatching eggs is variable and unpredictable, *in ovo* supplementation of antioxidants may have significant benefits (Malheiros et al., 2012). In agreement with Brenes et al. (2008) who fed the broilers with diets containing grape seed extract, we also found increased antioxidant activity. As found in broilers feeding on diets containing turmeric rhizome powder (Basavaraj et al., 2011; Mehala and Moorthy, 2008), we did not find any significant effect of antioxidant supplementation on total cholesterol, HDL, LDL, TG and total protein in broilers. Inconsistency in published data may be due to the concentration of curcumin in turmeric, or in the method of administration. In disagreement with our results, Hosseini-Vashan et al. (2015) revealed higher blood HDL for broilers fed 8 g/kg dietary turmeric rhizome powder. They also reported that turmeric reduced serum total cholesterol and LDL levels and dehydrogenase activity. Similar findings were reported by Emadi et al. (2007). Nanocurcurmin decreased blood cholesterol by increasing cholesterol 7α-hydroxylase (CYP7A1) as a rate limiting enzyme in the biosynthesis of bile acids from cholesterol (Kim and Kim, 2010).

Immune cell counts

The effects of *in ovo* injection of nanocurcumin and vitamin E on heterophil and lymphocyte counts and H/L ratio are summarized in Table 4. The percentage of immune cells percentages at d 10, was not significantly affected by *in ovo* treatments. There are no available data on nanocurcumin *in ovo* administration; but Tayer et al. (2012) reported that heterophil percentage and H/L ratio were increased by dietary green grape leaves in broilers. Hajati et al. (2018) reported that differential and white blood cell counts were not significantly affected by vitamin C or dietary grape seed extract. Grape seed extract supplementation at the levels of 300 and 450 mg/kg diet reduced heterophil percentage and H/L ratio, and increased the percentage of lymphocyte in broilers under heat stress (Hajati et al., 2018). Heckert et al. (2002) observed that the blood leukocyte profile was affected by stress conditions, and H/L ratio is commonly used as an index of stress in poultry. Decreases in lymphocytes and monocytes, increases in heterophil counts, and a higher H/L ratio, have been reported in animals exposed to environmental stress (Heckert et al., 2002).

Intestinal Morphology

The effects of *in ovo* injection of nanocurcumin and vitamin E on intestinal histomorphology are presented in-Table 5. In the present study, villous height in the jejunum was significantly affected on d 10 by *in ovo* treatments ($P<0.001$). Jejunal villous height at the doses of 0.01 and 0.03 mL NC/egg was significantly lower than the control group. The height of villi was not affected by *in ovo* VE treatment. There were no published data on the effect of *in ovo* NC administration in poultry. In contrast to our results, Rajput et al. (2013) reported that jejunal villous height of chicks receiving 200 and 100 mg/kg dietary curcumin, were significantly taller than control group at the ages of 21 and 42 d, respectively. Oso et al. (2019) reported that a dietary phytogenic blend (a mix of *Aerva lanata*, *Piper betle*, *Cynodon dactylon*, and *Piper nigrum*) improved the intestinal morphology and the chicks fed the diet supplemented with phy-

Table 4. The effect of *in ovo* injection of nanocurcumin (NC) and vitamin E (VE) on immune responses of broiler chicks (10 d)

Injection solutions (mL/egg)	Heterophil (%)	Lymphocyte (%)	Heterophil/ Lymphocyte
NC 0.01	28.33	54.33	0.520
NC 0.03	28.0	57.67	0.487
NC 0.05	28.67	58.67	0.493
VE 0.03	28.67	58.33	0.490
VE 0.06	28.67	56.33	0.503
VE 0.09	29.67	56.33	0.530
control	28.0	55.67	0.507
Sham control	29.33	59.67	0.487
SEM	0.71	1.22	0.015
P-value	0.67	0.10	0.408

Each mean represents 4 observations.
SEM: standard error of the mean.

Table 5. The effect of *in ovo* injection of nanocurcumin (NC) and vitamin E (VE) on jejunal histomorphology in broiler chicks (10 d)

Injection solutions (mL/egg)	Villous height (µm)	Villous width (µm)	Crypt depth (µm)	Villous height / crypt depth
NC 0.01	770.7^{d}	146.7^{c}	217.3	4.12^{e}
NC 0.03	950.0^{c}	178.0^{b}	220.0	4.37^{de}
NC 0.05	1048.0^{ab}	148.0^{c}	186.0	4.87^{bc}
VE 0.03	1035.3^{ab}	182.7^{b}	198.0	4.72^{cd}
VE 0.06	1022.0^{abc}	160.7^{bc}	216.0	4.77^{cd}
VE 0.09	1074.0^{ab}	138.0^{c}	234.0	5.34^{ab}
control	1077.3^{a}	149.3^{c}	192.7	5.61^{a}
Sham control	996.7^{bc}	249.3^{a}	183.3	5.49^{a}
SEM	28.0	10.07	6.95	0.17
P-value	0.0001	0.0001	0.732	0.0001

a,e: Within columns, mean values with common superscripts are not different (P>0.05; Tukey's test).
Each mean represents 4 observations.
SEM: standard error of the mean.

togenic blend had taller villous height in the jejunum than the antibiotic group. As shown in Table 5, the jejunal villous width in nanocurcumin and vitamin E treatments at doses of 0.03 mL/egg was significantly higher than in the control group (P<0.001); but all doses of NC and VE significantly decreased the villous height compared to the sham group (P<0.001).

In ovo injection of NC and VE had no significant effects on the crypt depth (Table 5). All NC doses as well as VE at doses of 0.03 and 0.06 mL/egg significantly decreased the villous width/crypt depth ratio compared to both control groups (P<0.001). Rajput et al. (2013) reported that crypt depth throughout the small intestine in birds fed diets containing curcumin was significantly lower than in the control group. Also, the villous height to crypt depth ratio in small intestine of birds fed 150 and 200 mg of curcumin per kg diet was higher than the control and 100 mg/kg dietary curcumin.

Hatchability and growth performance

The effects of *in ovo* injection of nanocurcumin and vitamin E on hatchability rate and growth performance are shown in Tables 6 and 7, respectively. *In ovo* administration of NC at doses of 0.03 and 0.05 mL/egg significantly decreased the hatchability compared to the control groups (Table 6). This may be due to the high NC doses in the current study. Therefore, lower NC doses are recommended for future investigations. In agreement with our results, Aygun (2016) found that hatchability of the eggs injected with 2% and 3% propolis was significantly lower than the control group; but there were no significant differences between the 1% propolis and control groups. In contrast to our findings, Hajati et al. (2014) observed higher hatchability in eggs injected with grape seed extract and vitamin C compared to uninjected group. However, some authors reported that

Table 6. The effect of *in ovo* injection of nanocurcumin (NC) and vitamin E (VE) on hatchability rate and growth performance in broiler chicks (1 to 10 d)

Injection solutions (mL/egg)	Hatchability (%)	BW (g/b)	BWG (g/bird/d)	FI (g/bird/d)	FCR (g:g)
NC 0.01	100.0^{a}	162.67	13.56	15.89	1.007
NC 0.03	86.67^{b}	148.50	11.89	15.89	1.033
NC 0.05	86.67^{b}	149.17	12.09	17.43	1.125
VE 0.03	100.0^{a}	163.50	13.72	17.45	1.020
VE 0.06	100.0^{a}	158.23	13.17	16.52	0.982
VE 0.09	100.0^{a}	155.75	12.71	15.66	0.958
control	100.0^{a}	151.63	12.29	18.62	1.219
Sham control	100.0^{a}	162.17	13.57	17.89	1.089
SEM	3.33	6.90	0.77	1.01	0.083
P-value	0.0195	0.605	0.527	0.380	0.420

a,b: Within columns, mean values with common superscripts are not different (P > 0.05; Tukey's test).
BW, body weight; BWG, body weight gain, FI, feed intake; FCR, feed conversion ratio.
SEM: standard error of the mean.

injection of 100 µL of sterile saline (0.85%) alone or containing 0.5, 1.5, 4.5, or 13.5 mg L-ascorbic acid (Zhang et al., 2018) and different doses of vitamin C (Nowaczewski et al., 2012) did not significantly affect the egg hatchability rate (above 92% in all groups). The findings in regard to hatchability in this study were in disagreement with Hajati et al. (2014) who reported that IOI of plant extract (grape seed at a rate of 4.5 mg/egg) improved hatchability rate of broilers and Khaligh et al. (2017) who reported that *in ovo* injection of plant extracts did not significantly affect the broiler hatchability.

As shown in Table 6, *in ovo* administration of NC and VE did not significantly affect the body weight (BW) on d 10 and daily weight gain, feed intake and FCR during d 1-10. Among these parameters, only feed intake, was significantly affected (P<0.05) by treatments during d 1-24; so that the average feed intake in all groups receiving NC or VE was significantly lower than control or sham control groups (Table 7). Similarly, Khaligh et al. (2017) reported that *in ovo* injection of chrysin, quercetin or ascorbic acid, as antioxidants, had no remarkable effect on the initial and d 10 body weight in broiler chicks. Hosseini-Vashan et al. (2012) observed that dietary turmeric rhizome powder did not affect feed intake, BW, FCR, production index and protein and energy efficiency ratio in broilers. Rahmani et al. (2017) reported that different levels of curcumin and nanocurcumin (200 and 400 mg/kg diet) did not have any significant effect on feed intake. Also, they found that the chicks fed with 200 mg/kg dietary curcumin or NC, showed more weight gain than control group, and body weight gain of the birds fed diet supplemented with 200 mg/kg dietary curcumin was significantly lower than 400 mg/kg group. Supplementation of the diet with curcumin or NC, significantly improved FCR compared to the control birds. Other authors have found that the growth performance of broiler chickens was improved with supplementation of turmeric powder at 5 g/kg diet (Burke, 1992) and 7 g/kg diet (Durrani et al., 2006). Zakaria et al. (1998) and Ghonim et al. (2009) reported that IOI of ascorbic acid on d 15 of incubation resulted in higher body weight in broiler chickens. There is currently little information on the effect of *in ovo* injection of curcumin and nanocurcumin. Most researchers have added curcumin to the diets. It has been reported that dietary supplementation of curcumin (200 mg/kg diet) resulted in significant improvement in live body weight and FCR (Rajput et al., 2013); growth performance and breast yield (Wang et al., 2015); but, feed intake was not markedly influenced (Rajput et al., 2013). Nevertheless, Emadi and Kermanshahi (2007) showed that turmeric powder (100, 150, 200 mg/kg diet) improved the FCR in broiler chickens. Wang et al. (2015) reported that dietary supplementation of turmeric rhizome extract reduced the abdominal fat in broilers. Itallo et al. (2019) reported that the *in ovo* administration of vitamin E improved the chick growth performance and meat quality. *In ovo* administration of VE (30 mg/egg diet) during d 1 to 21 of age increased the feed intake without affecting the weight gain (Salary et al., 2014).

Conclusions

It is concluded that *in ovo* administration of NC improved the antioxidant status of the hatchlings. The NC and VE *in ovo* injection did not impact on the cellular immunity and blood serum cholesterol level. *In ovo* administration of NC at doses of 0.03 and 0.05 mL/egg, but not 0.01 mL/egg, decreased the egg hatchability rate.

Table 7. The effect of *in ovo* injection of nanocurcumin (NC) and vitamin E (VE) on hatchability rate and growth performance in broiler chicks (1 to 24 d)

Injection solutions (mL/egg)	BW (g/b)	BWG (g/bird/d)	FI (g/bird/d)	FCR (g:g)
NC 0.01	888.67	35.33	51.88^{c}	1.28
NC 0.03	808.33	31.95	52.73^{c}	1.31
NC 0.05	819.05	32.44	58.18^{bc}	1.49
VE 0.03	859.75	34.16	51.84^{c}	1.28
VE 0.06	828.67	32.88	52.87^{c}	1.34
VE 0.09	854.50	33.88	52.05^{c}	1.30
control	819.17	32.42	71.45^{a}	1.66
Sham control	864.17	34.34	66.01^{ab}	1.44
SEM	45.75	1.91	3.96	0.11
P-value	0.904	0.901	0.015	0.193

a,c: Within columns, mean values with common superscripts are not different (P>0.05; Tukey's test).
BW, body weight; BWG, body weight gain, FI, feed intake; FCR, feed conversion ratio.
SEM: standard error of the mean.

Vitamin E and NC decreased the feed intake without affecting the feed conversion ratio and weigh gain.

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