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The Impact of *in ovo* Injection of Silver Nanoparticles, Thyme and Savory Extracts in Broiler Breeder eggs on Growth Performance, Lymphoid-Organ Weights, and Blood and Immune Parameters of Broiler Chicks

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Abstract

This study was conducted to evaluate the long-term effects of *in ovo* injection of nano-silver, thyme and savory extracts in broiler breeder eggs on growth performance, lymphoid organ weights, immune and blood parameters and bone mineral concentration of broiler chicks. A total of 560 fertilized broiler breeder eggs were divided into 7 groups. On d 5 of incubation, eggs were treated with the following groups. Group 1 (control, no injection); group 2 (placebo, 1 mL of 0.9% NaCl); group 3 and 4 (placebo + 30 and 45 mg of nano-silver, respectively); group 5 and 6 (placebo + 75 and 100 mg of thyme, respectively) and group 7 (placebo + 75 mg of savory). After hatch, chickens were fed a corn-soybean meal diet under the controlled conditions and slaughtered at 14 and 21 d of age for sample collection and analysis. The lymphoid-organ weights and growth rate were not affected by dietary treatments at 14 and 21 d of age. The results also showed that nano-silver injected into broiler breeder eggs during incubation improved the bone mineral concentration and cell-mediated immunity at 14 and 21 d of age, respectively. Humoral immunity was improved by thyme and savory extracts ($P < 0.05$). Overall, the effect of *in ovo* injection of nano-silver, thyme and savory extracts during embryonic development is a potential means to improve immune activities of broiler chickens, while does not have any detrimental effect on embryo hatchability.

Introduction

Nanotechnology is defined as a technology, enabling us to deal with structures ranging from approximately 1-100 nm in at least one dimension (Ahmadi and Hafsy Kordestany, 2011). Nano-sized metal particles have unique abilities regarding electronic, optical, and catalytic properties which act better than particular bulky ones (Paleo *et al*., 2011). In fact, very high surface area to volume ratio allows nanoparticles to be effective in very small amount (Sawosz *et al*., 2009). Nowadays, nanotechnology has entered in several areas, including skin creams, sunscreens, wound dressings that clean and disinfect burns, food and feed, and agriculture and is rapidly expanding throughout the life of people (Binion. 2008). One of the suitable substances used in nanoformulation is silver (nano-silver). An

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effective killing agent against the broad spectrum of bacteria is nano-silver (Yin *et al*., 1999; Wijnhoven *et al*., 2009), including antibiotic-resistant strains (Wijnhoven *et al*., 2009). In addition, the special structure of nano-silver enables it to sustain atomic oxygen inside the octahedral holes of Ag and probably affects oxygen level in an organism (Studnicka *et al*., 2009).

Among the *Satureja L.* genus of medicinal herb species, thyme and savory have a unique position (Mihajilov-Krstev *et al*., 2009). Thyme (*Satureja hortensis*) is traditionally used for many years in treating diseases. By the reason, extract and essential oil isolated from the aerial parts of this plant (leaf, stalk and flower) have been surveyed in this respect (Mihajilov-Krstev *et al*., 2010). Antimicrobial secondary metabolites of thyme and savory (phenol, thymol and carvacrol) are secreted as a part of their normal program of growth and development or in response to pathogens attack or stress (Mihajilov-Krstev *et al*., 2010).

Hypothetically, antimicrobial and anti-inflammatory properties and possibility to enrich cells with oxygen can improve immune system function of the organism. Furthermore, it is likely that immunity could be maximized and the risk of developing resistant microorganisms can be highly reduced by using of nano-silver, thyme and savory extracts. Although considerable reports have focused on the impact of nano-silver, thyme and savory extracts on animal performance, conflicting results and a few studies have addressed the effect of those on broiler performance, bone and blood parameters and the immune system. Therefore, the objective of this study was to examine potential effects of nano-silver, thyme and savory extracts on broiler with emphasis to above parameters.

Materials and Methods

All procedures used during this study were approved by the University of Bu-Ali Sina Animal Care committee.

Spice extracts preparation

Fifty grams of pulverized and dried spice (thyme or savory) were mixed and extracted by 400 mL of 95% (v/v) ethanol in 250 mL Erlenmeyer flask, then shaken at 100 rpm in a shaker at room temperature for 12 hrs. The spice extracts were separated from plant materials (thyme or savory leaf) by filter paper (Whatman no.2). The residue was also re-extracted with an additional 200 mL of 95% ethanol for an additional 12 hrs and then filtered. The solvent in combined filtrates were evaporated by a rotary evaporator with a vacuum pump and the extracts were freeze-dried. The extracts (thyme or savory) were kept in the sealed vial at 4°C until use (Zhang *et al*., 2009).

Animals, diets and performance

A pre-experiment was designed to determine threshold limit values of savory and thyme extracts. Briefly, a total of 180 broiler fertile eggs were tested using of *ovo* injection according to the hatchability percentage. The levels of 75, 100 and 125 ppm of each plant extract were inspected in this respect. The hatchability percentage in 75 and 100 ppm of thyme and 75 ppm of savory were at least 50 percent and, therefore, the others were relinquishing in this particular case.

Five hundred and sixty broiler fertile eggs (Lohman strain) were obtained from a commercial hatchery and were incubated at 37.7°C, 60% humidity and automatically turned every hour. At 5 days of embryonic age, the small tips of the eggs were disinfected and drilled by frog pins, and randomly allotted to one of the 7 groups, (80 fertile eggs per group); group 1 (control, not treated); group 2 (placebo, 1 mL of 0.9% NaCl); group 3 and 4 (placebo + 30 and 45 mg of nano-silver, respectively); group 5 and 6 (placebo + 75 and 100 mg of thyme, respectively) and group 7 (placebo + 75 mg of savory).

After incubation in the rearing period, chicks were kept in the group cages (pens) for 3 wk at a temperature which was decreased gradually from 33 to 21°C. There was artificial lighting for 24 hrs/d during the experiment. Chicks received water and feed (Table 1) *ad libitum*.

During the experiment, feed intake, body weight gain, mortality and feed conversion ratio were measured weekly. Finally, growth rate and feed intake of each treatment were recorded on d 14 and at the end of the experiment.

Serum biochemical analysis

At 14 and 21 d of age, 3 mL of blood was drawn from the brachial vein of 8 chickens from each group. Blood samples were collected and transferred to vial tubes containing sodium heparin as an anticoagulant. The tubes were centrifuged at 5,000 × g for 20 min, and the

supernatant (plasma) was removed. Total cholesterol, triglyceride, Low-density lipoprotein cholesterol (LDL-C), High-Density Lipoprotein cholesterol (HDL-C), glucose, and alkaline phosphatase in plasma were determined according to the procedures recommended by the manufacturer of the kits (Pars-Azmoon Company, Tehran).

The mineral concentrations of bone were measured by atomic absorption spectrometry (AOAC, 1984). Calcium was also determined by atomic absorption spectrophotometry (AOAC, 1990). Total phosphorus concentration was determined colorimetrically using the vanadomolybdate procedure (AOAC, 1984).

Table 1. Ingredients and chemical composition of diet (%)

Ingredients	Starter (0-14 d)	Grower (14-21 d)
Corn	53.89	54.62
Soybean meal	40.94	38.15
Soybean oil	1.00	3.00
Dicalcium phosphate	1.72	1.70
Oyster shell	1.60	1.60
Salt	0.30	0.30
Mineral premix[1]	0.25	0.25
Vitamin premix[2]	0.25	0.25
DL-Methionine	0.04	0.04
L-Lysine	0.01	0.09
Calculated nutrient composition (as- fed basis)		
ME (kcal/kg)	3000	3100
Crude protein (%)	23.00	21.54
Calcium (%)	1.00	0.93
Available phosphorus (%)	0.45	0.45
Dietary electrolyte balance (mEq/kg diet)	246	259

[1]Each kg contained: Mn, 600 mg; Zn, 480 mg; Fe, 300 mg; Cu, 60 mg; I, 9.00 mg; Se, 0.25 mg; and cobalt, 1. 5 mg.
[2]Each kg contained: Vitamin A, 8,400 IU; vitamin D_3, 18,000 IU; vitamin E, 300 IU; vitamin K, 24 mg; pyridoxine, 18 mg; vitamin B_{12}, 36 mg; niacin, 3600 mg; pantothenic acid, 120 mg; folic acid, 1.2 mg; choline, 900 mg.

Immune organ weight

Two birds of each pen were euthanized. The lymphoid organs, spleen and bursa, were removed on d 14 and at the end of the trial and weighed. The weight of these sections was expressed as a proportion of body weight.

Contact hypersensitivity response to dinitrochlorobenzene and phytohemagglutinin

The skin contact sensitivity method was performed according to Verma *et al*. (2004). Briefly, 5 chicks from each treatment were sensitized at 21 d of age by a single percutaneous application of 0.25 mL of 1-chloro-2, 4-dinitrobenzene (DNCB, 10 mg/mL) in a vehicle consisting of an acetone and olive oil (4:1) mixture. A relatively featherless area of approximately 10 cm^2 was chosen on the right side for DNCB application while a similar area on the left side was selected for application of only the vehicle, which served as the control. Ten days later, the right side of each sensitized bird was challenged by application of 0.25 mL of DNCB (1 mg/mL) in the same vehicle. The test was repeated 10 d after the second test. The reaction was assessed by measuring skin thickness before the challenge and 24 hrs post challenge using a constant-tension dial micrometer (Mitutoya Corp., Tokyo, Japan). Each measurement was repeated three times on a constant area and mean skin thickness was considered as the mean of individual birds within the group.

At 21 d of age, cutaneous basophilic hypersensitivity response (to phytohemagglutinin injection) was also determined according to the method of Corrier and DeLoach (1990) using 8 birds per group. In Brief, the right foot was cleaned and the thickness of the toe web between the third and fourth digits was measured, using a micrometer. One hundred microliters of a 100 µg/mL solution of phytohemagglutinin (PHA) in sterile 0.9% saline was injected intradermally. After 24 hrs, the toe webs were cleaned and measured again with the help of micrometer. Cutaneous basophilic hypersensitivity response was determined by subtracting the skin thickness pre and post 24 hrs injections.

Statistical analysis

All experiments were set up as completely randomized designs (CRD). Data were analyzed by one-way analysis of variance (ANOVA) using the General liner Model (GLM) procedure (SAS, 2003). Variables with significant F tests ($P < 0.05$) were compared using Duncan's multiple range test. Variation within treatment was expressed as the standard error of the treatment means (SEM).

Results

Animals, diets and performance

The results of broiler chicken performance are shown in Table 2. Dietary treatments did not affect broiler feed intake, growth rate and feed conversion ratio at 14 and 21 d of age.

Table 2. Effects of *in ovo* injection of colloidal nano-silver, thyme and savory extracts on performance of broiler chickens at 14 and 21 d of age

Treatment		Feed Intake(g/bird)		Weight gain (g)		FCR[1]		Hatchability (%)
Levels		14	21	14	21	14	21	
Control	-	359	462	230	288	1.56	1.79	66.25
	*	345	494	231	277	1.49	1.79	58.12
Nano-silver	30	368	506	239	283	1.54	1.74	69.83
	45	362	498	243	295	1.49	1.72	66.26
Thyme	75	375	536	243	299	1.55	1.67	63.77
	100	393	545	247	296	1.60	1.70	63.50
Savory	75	406	545	262	324	1.54	1.65	62.62
SEM		16	16	10	15	0.089	0.106	4.023
P-value		0.21	0.05	0.35	0.36	0.97	0.08	0.14

*placebo, 1 mL of 0.9% NaCl was injected; [1]Feed conversion ratio.

Table 3. Effects of *in ovo* injection of colloidal nano-silver, thyme and savory extracts on immune parameters of broiler chickens[1]

Treatment		14 d of age			21d of age						
Levels		Bursa[2]	Spleen[2]	DNCB[3]	Bursa[2]	Spleen[2]	DNCB[3]	Phytohemagglutinin[4]	IgA	IgM	IgG
Control	-	0.525	0.123	0.43^{c}	0.363	0.123	0.82	0.20^{b}	29.59	115.47^{b}	357.84^{b}
	*	0.499	0.122	0.37^{c}	0.370	0.108	0.76	0.21^{b}	27.51	125.31^{b}	422.32^{ab}
Nano-silver	30	0.473	0.112	0.64^{ab}	0.363	0.108	1.06	0.38^{a}	40.25	130.31^{b}	420.71^{ab}
	45	0.422	0.109	0.68^{a}	0.300	0.106	1. 14	0.34^{a}	39.73	140.23^{ab}	440.43^{ab}
Thyme	75	0.622	0.173	0.44^{c}	0.406	0.146	0.89	0.27^{ab}	40.99	132.53^{b}	455.97^{ab}
	100	0.521	0.163	0.49^{bc}	0.563	0.141	0.97	0.30^{ab}	40.48	185.67^{a}	546.84^{a}
Savory	75	0.717	0.115	0.45^{c}	0.486	0.124	0.94	0.29^{ab}	43.02	182.12^{a}	559.70^{a}
SEM		0.207	0.022	0.054	0.315	0.073	0.086	0.032	4.481	14.942	42.441
P-value		0.09	0.31	0.012	0.12	0.17	0.054	0.02	0.15	0.03	0.04

*placebo, 1 mL of 0.9% NaCl was injected.
[1]Five and eight chickens per treatment for DNCB and other variables were used, respectively.
[2]Relative to body weight; [3]dinitrochlorobenzene (mm); [4]Phytohemagglutinin (mm).
[a-c]Means within columns with no common superscripts differ significantly ($P < 0.05$).

Table 4. Effects of *in ovo* injection of colloidal nano-silver, thyme and savory extracts on blood parameters of broiler chickens at 14 d of age (mg/dL)[1]

Treatment	Levels	Glucose	Triglyceride	Total cholesterol	LDL-C[2]	HDL-C[3]	Alkaline phosphatase
Control	-	242.00	92.76	95.94	73.73	43.33	420^{c}
	*	239.00	86.68	107.87	72.80	41.67	414^{c}
Nano-silver	30	245.33	95.08	99.63	78.73	40.33	656^{ab}
	45	242.00	97.13	103.17	77.70	39.33	668^{a}
Thyme	75	248.33	97.66	111.20	79.87	48.00	488^{abc}
	100	255.67	103.48	111.86	80.03	46.67	474^{bc}
Savory	75	264.00	113.81	114.99	82.60	50.33	456^{c}
SEM		9.460	6.013	6.642	3.994	3.387	58.898
P-value		0.53	0.12	0.40	0.60	0.37	0.03

*placebo, 1 mL of 0.9% NaCl was injected.
[1]Mean of eight chickens per treatment.
[2]Low-density lipoprotein cholesterol (LDL-C); [3]High-Density Lipoprotein-cholesterol (HDL-C).
[a-c]Means within columns with no common superscripts differ significantly ($P < 0.05$).

Spleen and bursa measurements

At 14 and 21 d of age, relative weights of the bursa and spleen did not differ ($P < 0.05$) among the control, placebo, nano-silver, thyme and savory groups (Table 3). The relative weights of bursa of fabricius and spleen were numerically decreased as increasing nano-silver in dietary treatments. By the way, no determined trend was observed between weights of spleen and bursa of fabricus with other treatments, thyme and savory.

With attention to mean skin thickness sensitive to phytohemagglutinin, cell-mediated immunity at 21 d of age was significantly ($P < 0.05$) affected by diets. The highest immune response was obtained in nano-silver treatment compared with the control. However, the mean immune response was not statistically different

among the other treatments. No response in cell-mediated immunity was reflected by DNCB at 14 and 21 d of age. Higher levels of IgM and IgG were produced by the birds under the performed treatments, nano-silver, and extracts, but only differences between thyme 100 and savory 75 mg were significant compared with the control ($P < 0.05$).

Serum biochemical analysis

All treatments had no significant effect on concentrations of glucose; triglyceride, total cholesterol, LDL-C and HDL-C at 14 and 21 d of age (Tables 4 and 5). In comparison to the control, the blood concentration of alkaline phosphatase was not affected by thyme and savory treatment, while that was significantly increased ($P < 0.05$) by nano-silver treatments at 14 and 21 d of age. The similar trend was also found in ash, calcium and copper of bone at 14 d of age ($P < 0.05$); but at 21 d of age only copper of bone significantly increased by nano-silver treatments (Tables 6 and 7).

Table 5. Effects of *in ovo* injection of colloidal nano-silver, thyme and savory extracts on blood parameters of broiler chickens at 21 d of age (mg/dL)[1]

Treatment	Levels	Glucose	Triglyceride	Total cholesterol	LDL-C[2]	HDL-C[3]	Alkaline phosphatase	Calcium	Phosphorus
Control	-	230	114	129	64	40.67	288.57^{cd}	11.50^{c}	3.26
	*	222	112	126	65	37.67	285.30^{d}	12.14^{bc}	3.21
Nano-silver	30	221	117	130	67	37.00	305.79^{ab}	18.36^{ab}	4.90
	45	219	121	134	69	36.00	310.17^{a}	21.50^{a}	4.98
Thyme	75	231	123	136	75	49.33	303.81^{abc}	13.50^{bc}	4.13
	100	233	126	137	72	48.67	301.50^{abc}	14.63^{bc}	4.15
Savory	75	237	130	139	79	52.33	292.10^{bcd}	13.94^{bc}	4.34
SEM		5.755	4.569	4.127	4.217	4.081	5.322	2.117	0.651
P-value		0.29	0.28	0.35	0.20	0.05	0.04	0.03	0.38

*placebo, 1 mL of 0.9% NaCl was injected.

[1]Mean of eight chickens per treatment; [2]Low density lipoprotein cholesterol (LDL-C); [3]High density lipoprotein cholesterol (HDL-C).

[a-d]Means within columns with no common superscripts differ significantly ($P < 0.05$).

Table 6. Effects of *in ovo* injection of colloidal nano-silver, thyme and savory extracts on bone mineral contents of broiler chickens at 14 d of age (percentage of bone ash)[1]

Treatments	level	Ash	Calcium	Phosphorus	Copper	Nano-silver
Control	-	44^{bc}	15.31^{bc}	8.57	1.11^{c}	0
	*	42^{c}	12.33^{c}	7.39	0.93^{c}	0
Nano-silver	30	53^{a}	20.18^{a}	9.87	1.89^{ab}	0
	45	54^{a}	20.58^{a}	10.70	2.31^{a}	0
Thyme	75	47^{abc}	17.05^{ab}	8.15	1.54^{bc}	0
	100	50^{abc}	17.20^{ab}	8.77	1.17^{c}	0
Savory	75	49^{ab}	17.08^{ab}	8.76	1.12^{c}	0
SEM		2.368	1.387	0.852	0.182	0
P-value		0.03	0.01	0.21	0.001	0

*placebo, 1 ml of 0.9% NaCl was injected.

[1]Mean of eight chickens per treatment.

[a-c]Means within columns with no common superscripts differ significantly ($P < 0.05$).

Table 7. Effects of *in ovo* injection of colloidal nano-silver, thyme and savory extracts on bone mineral contents of broiler chickens at 21 d of age (percentage of bone ash)[1]

Treatments	level	Ash	Calcium	Phosphorus	Copper	Nano-silver
Control	-	48.00	15.99	8.38	1.10^{b}	0
	*	46.67	15.95	7.24	1.07^{b}	0
Nano-silver	30	60.33	21.05	9.74	1.79^{ab}	0
	45	64.00	21.79	10.00	2.09^{a}	0
Thyme	75	48.67	16.20	7.86	1.69^{ab}	0
	100	52.33	17.32	7.61	1.15^{b}	0
Savory	75	50.00	17.12	7.74	1.11^{b}	0
SEM		4.038	1.436	0.785	0.231	0
P-value		0.06	0.07	0.11	0.03	0

[a,b]Means within columns with no common superscripts differ significantly ($P < 0.05$).

[1]Mean of eight chickens per treatment.

Discussion

Performance and serum biochemical analysis

In the present study, nano-silver injected into the albumen did not influence biochemical analysis of serum, indicating no effect on metabolic rate and consequently growth rate. Sawosz *et al*. (2009) reported that biochemical indices and growth rate of broiler chickens were not affected by *in ovo* injection of nano-silver. In addition, in rat studies, body and blood parameters in animals exposed to different doses of nano-silver were not also altered (Kim *et al*., 2008), which are consistent with this study. Grodzik and Sawosz (2006) also showed that silver nanoparticle concentration in 10 ppm treatment had no effect on the growth of chicken embryos. No effects of thyme and savory treatment may be explained by their natural effects on biochemical analysis of serum, metabolic rate and consequently growth rate. In the earlier studies, it has also been shown that biochemical parameters and performance of broiler chicken was not significantly affected by savory and thyme (Ghalamkari *et al*., 2011; Hosseini Mansoub & Mohammad Nezhady, 2011).

Compared with the control, numerical growth rate by performed treatments may be related to antibacterial characteristic of nano-silver, thyme and savory extracts. In addition, another factor of thyme and savory extracts which could has a beneficial effect on the growth performance of broilers is probably due to their antioxidant property (Vincent, 2002; Gulluce *et al*., 2003).

It is well documented that alkaline phosphatase is necessary for bone mineralization by hydrolyzing organic phosphates to enhance the local phosphate concentration to a level which is required for the initiation of hydroxyapatite crystal formation (Wennberg *et al*., 2000). It has been demonstrated that nano-silver increases levels of blood alkaline phosphatase which is associated with bone formation (Kim *et al*., 2008). Copper is a vital mineral for vesicle matrix in the bone cell, preventing its premature crystallization and also implements an important role in the crosslinked network of collagen and elastin, which causes bone tensile strength and elasticity (Dibner *et al*., 2007). Based on the statistical analysis, therefore, the greater levels of ash, calcium and copper of bone in nano-silver treatments compared with the control could be justified by the greater activity of alkaline phosphatase.

Immune organs weight

In the present study, lymphoid organs weight was not affected by dietary treatments at 14 and 21 d of age. Nonetheless, lymphoid organs were decreased as increasing nano-silver, thyme and savory levels in diets. Ahmadi and Hafsi Kurdestany (2010) reported that the bursa weight was significantly decreased as nano-silver levels increased in diets. In addition, they showed that other lymphoid organs weight (spleen and thymus) coincides with rising concentration of nano-silver in the treatments, which is analogous to lymphoid organ weights of the current study. Grodzik and Sawosz (2006) also showed that the number and size of the lymph follicles were decreased by 10 ppm of silver nanoparticles in the diet. The lower weights of lymphoid organs in nano-silver, thyme and savory treatments may be explained by the antimicrobial properties of these ingredients which modulate microbial population in poultry caecum, and consequently lymphoid organs. It is established that microorganisms in the gastrointestinal tract have a key role in growth and development of lymphoid organs (Ahmadi and Hafsy Kurdestany, 2010). In addition to the antimicrobial characteristic, nano-silver also can act as an agent that make oxygen available and reduce strictly anaerobic microorganisms and consequently lymphoid organ growth. Matsumura *et al*. (2003) reported that the action of silver might be due to the intake of silver ions by bacterial cells and production of reactive oxygen molecules that finally inhibit the bacterial respiration.

Cell-mediated immunity, in terms of mean skin thickness sensitive to phytohemagglutinin, was significantly enhanced in chicks given the nano-silver treatment compared with the control. Nonetheless, cell-mediated immune responses were similar for the two dietary levels of nano-silver. Cell-mediated immunity does not relate to body antibodies, in the better words, it is an immune response emerged from the action of phagocytes, natural killer cells, antigen-specific cytotoxic T-lymphocytes, and various cytokines against antigen activation (Anonymous, 2015). Previous studies have shown that cell-mediated immunity of chickens was also improved by nano-silver diets. For instance, effects on the immune system, especially cytokine excretion, have been noted *in vitro* and *in vivo*, where application of a 1%

nano-silver cream with <50 nm particles, inhibited contact allergic dermatitis in rats (Bhol and Schechter, 2005). The present study indicates that cell-mediated immunity is properly affected by nano-silver, even at low concentration. No response in cell-mediated immunity was shown by DNCB at the end of the experiment. Few researchers have looked at the effect of nano-silver, and especially, thyme and savory extracts on avian cell-mediated immunity. In addition to the numerically higher cell-mediated immunity in broilers of savory treatment, humoral immune, namely IgM and IgA, also was significantly higher in these animals compared with the control. Humoral immunity, IgM and IgA, was also significantly increased by higher concentration of thyme. Besides the antibacterial and antioxidant activities of savory and thyme that have beneficial effects on immune responses of chicks, higher immunity in broilers received savory treatment may be explained by the content of vitamins in savory. In the other words, higher levels of vitamins especially vitamin A and E in the savory plays a positive role in the antibody production, improve serum antibody titers and phagocytic activity of immune cells of medicated broilers (Elwinger *et al*., 1998; Tampieri *et al*., 2005). Zamani Moghaddam *et al*. (2007) and Ghalamkari *et al*. (2011) also observed immune stimulating effects in broilers by application of savory which is in agreement with our findings.

Conclusion

These results are encouraging because the growth potential of broilers was improved by improvement in cell-mediated and humoral immunity emerged from performed treatments. Finally, our results suggest that the *in ovo* injection of colloidal nano-silver, thyme and savory extracts during early incubation period enhances marginally the growth rate of the body.

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