



## Effects of Dietary Inclusion of the Encapsulated Thyme and Oregano Essential Oils Mixture and Probiotic on Growth Performance, Immune Response and Intestinal Morphology of Broiler Chickens

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### Abstract

This study was conducted to investigate the effects of dietary inclusion of the encapsulated thyme essential oil (TEO), oregano essential oil (OEO) mixture and probiotic on growth performance, immune response and intestinal morphology of broiler chickens. A total of 490 one-day-old Ross 308 broiler chicks were randomly divided into seven treatments consisting of five replicates (n=14). Birds were fed with 1) basal diet (control), and a basal diet containing; 2) 10 mg Avilamicine antibiotic, 3) 200 mg/kg encapsulated TEO+OEO mixture, 4) 200 mg/kg non-capsulated TEO+OEO mixture, 5) Pronigeb<sup>®</sup> (probiotic), 6) Pronigeb<sup>®</sup> with 200 mg/kg non-capsulated TEO+OEO mixture and 7) Pronigeb<sup>®</sup> with 200 mg/kg encapsulated TEO+OEO mixture. Results revealed that birds fed diet containing antibiotic, encapsulated TEO+OEO mixture with and without probiotic had significantly higher body weight gain (BWG) compared with control and non-capsulated TEO+OEO mixture groups on day 42 ( $P < 0.05$ ). Dietary inclusion of TEO+OEO in capsulated form and also in along to probiotic increased humoral immunity in broiler chickens compared with other groups ( $P < 0.05$ ). Birds fed the diet supplemented with different types of additives showed significantly higher dinitrochlorobenzene (DNCB) compared with the control group at 32 days of age ( $P < 0.05$ ). Dietary inclusion of the encapsulated EOs with and without probiotic both significantly increased villus length and villus width in comparison to other groups ( $P < 0.05$ ). The highest and lowest ratios were observed for the control group and probiotic+encapsulated TEO+OEO group respectively. These results indicate that feeding birds with diet containing encapsulated EOs alone or together with probiotic could improve BWG, immune responses and intestinal morphology in broiler chickens.

### Introduction

The use of antibiotics has banned as a growth promoter in the animal breeding industry, because of the potential health risks (Simitzis, 2017). The industry is searching to find the appropriate alternative for antibiotics. Some probiotics have been commercially applied to supply the requirements (Bai *et al.*, 2016). Probiotics have been used to develop and stabilize the intestinal flora (Gaggia *et al.*, 2010; Jahromi *et al.*, 2016). Probiotics have been reported to have beneficial effects on growth performance and

immune response in broiler chickens (Gaggia *et al.*, 2010; Seidavi *et al.*, 2017). Probiotics not only improve the antioxidant levels in the body, but also improve the healthiness in broilers (Tabidi *et al.*, 2013).

It has been suggested the plant derivatives, such as plant essential oils and extracts, as alternatives for antibiotics to improve the productive performance in poultry (Simitzi and Deligeorgis, 2011). Essential oils (EOs) consist of low molecular weight aliphatic

hydrocarbons such as phenols, aromatic aldehydes (Dorman and Deans, 2000). EOs is volatile secondary metabolite which has been broadly applied in the cosmetic and food industry. EOs has been also known to have antioxidant properties that could optimize the antioxidant levels in the feed (Simitzis, 2017).

Thyme (*Thyme Vulgaris L.*) is a medicinal herb that has medical applications and grows in all the Mediterranean regions. Thymolis was known as a dominant compound in thyme essential oil (TEO) (Sengül *et al.*, 2008). The beneficial effects of TEO on growth performance and immune response of laying hens have recently reported (Attia *et al.*, 2017). Oregano (*Origanum vulgare*) is extensively found in the entire Mediterranean region. Oregano essential oil (OEO) has been known to have main phenols such as carvacrol (Mathlouthi *et al.*, 2012). Oral supplementation of OEO improved growth performance as well as humoral and innate immune responses in broiler chicks (Galal *et al.*, 2016).

Despite the beneficial effects of EOs, their uses have faced with major limitations, because of sensitivity to environmental variables, high volatility, easy degradation. It has been suggested to load the EOs into capsules for overwhelming on such problems (Bilia *et al.*, 2014). The present study was developed under the hypothesis that loading the mixture of TEO and OEO into chitosan nanoparticles may efficiently improve the growth performance and immune responses of broilers. Thus, the present study was conducted to evaluate the effects of adding microcapsules containing the mixture of TEO and OEO and probiotic into broiler diet on growth performance and immune responses of broilers.

### Materials and Methods

All the used procedures were in agreement with the Ethical Standard Committee, Islamic Azad University, Shabestar Branch (No. IAUS 2071).

### Birds and housing

A total of 490 one-day-old Ross 308 broiler chicks (mix) were purchased from a commercial hatchery, then weighed and randomly divided into 35 cages (1.2×1.2 m<sup>2</sup>). Broiler chicks had an initial weight of 43±2 g. Broiler chicks were randomly allocated into seven treatments consisting of five replicates with 14 boiler chicks. Birds received one of seven experimental diets from 1 to 42 days of age, including 1) basal diet (control); 2) basal diet containing 10 mg/kg Avilamicine (antibiotic); 3) basal diet containing 200 mg/kg encapsulated TEO+OEO; 4) basal diet containing 200 mg/kg non-capsulated TEO+OEO; 5) basal diet containing 500 mg/kg Pronigeb<sup>®</sup> (probiotic); 6) basal diet containing 500 mg/kg Pronigeb<sup>®</sup> and 200 mg/kg encapsulated TEO+OEO and 7) basal diet containing 500 mg/kg Pronigeb<sup>®</sup> and 200 mg/kg non-capsulated

TEO+OEO). Birds had *ad libitum* access to water and feed in entire the experiment. The temperature for the rearing room was kept at 32±1°C during the first week of the study and then reduced to 23.9°C by the end of the third week and was kept until the end of the trial.

### Preparation of probiotics and encapsulated EOs

TEO and OEO were purchased from Barij Essence Company (Kashan-Iran). Based on the datasheet prepared from the manufacturer, Thymol (49.70%),  $\gamma$ -Terpinene (19.55%),  $\rho$ -Cymene (11.82%), Carvacrol (4.12%),  $\alpha$ -Terpinene (4.11%), Myrcene(3.64%),  $\alpha$ -Pinene (3.16%), Limonene (1.73%) and Linalool (2.92%) were the main compounds in TEO. Based on obtained information, carvacrol (63.11%),  $\rho$ -Cymene (10.72%), Linalool (3.42%) and  $\alpha$ -terpineol (1.73) were the main compounds in OEO. Pronigeb<sup>®</sup> probiotic was obtained from the National Institute of Genetic Engineering and Biotechnology and contaminated *Lactobacillus reuteri*, *Lactobacillus salivarius*, and *Pediococcus ssp* in the amount of 1×10<sup>9</sup> colony forming units (CFU/g) each. Ionic gelation process was performed for encapsulation of the essential oil in chitosan nanoparticles as described by Stoica *et al.* (2013). Chitosan was dissolved in 10 mg/mL of 1% acetic acid. The solution was stirred overnight at room temperature for the dispersion of chitosan completely. Then, the resulting solution was filtered through filter paper (Calbiochem-Novabiochem Corp., San Diego, Calif., U.S.A.) and sterilize at 121°C for 15 min. Sodium tripolyphosphate (TPP, 10 mL) was added to a 25 mL chitosan solution (pH=5) and stirred at room temperature. The solution containing 0.5% TEO and 0.5% Tween 20 was added to the chitosan solution to prepare the chitosan-TPP nanoparticles loaded with essential oil.

The basal diet was prepared as recommended by Ross 308 catalog to satisfy the broilers' requirements (Aviagen, 2014). The feed samples were taken and their chemical composition properties (AOAC, 2004) were investigated based on AOAC (Table 1).

### Growth performance

All birds were weighed at the beginning and the end of 1, 10, 24 and 42 days of age. Feed intake (FI, g/bird) was calculated as the difference between the amount of feed offered and the feed residue at the end of each period. The feed conversion ratio was calculated by dividing FI to WG and corrected for mortality. European production efficiency factors (EPEF) was calculated with the following formula at the end of the experiment (Marcu *et al.*, 2013).

$$EPEF = \frac{\text{Survival rate (\%)} \times BW \text{ (kg)} \times 100}{\text{age (d)} \times FCR \text{ (kg feed/kg gain)}}$$

**Table 1.** The ingredients and composition of basal diet

Ingredients	Starter (1-10d)	Grower (11-24d)	Finisher (25-42d)
Corn grain	51.86	58.23	62.24
Soybean meal (44% CP)	38.35	29.10	30.95
Soybean oil	3.53	4.26	3.22
Fish meal	2.11	5.00	0.00
DCP	0.90	1.23	0.93
Limestone	1.80	0.97	1.43
NaCl	0.25	0.25	0.30
Vitamin premix <sup>a</sup>	0.25	0.25	0.25
Mineral premix <sup>b</sup>	0.25	0.25	0.25
DL-Methionine	0.35	0.31	0.25
L-Lysine mono HCl	0.25	0.15	0.14
L-Threonine	0.10	0.00	0.04
<b>Analyzed composition</b>			
Energy (ME), kcal/kg	3021.4	3152.7	3200.5
Crude Protein, %	23.12	21.30	19.35
Calcium, %	1.05	0.90	0.85
Av. Phosphorus, %	0.50	0.45	0.42
Lysine, %	1.44	1.24	1.09
Threonine, %	0.94	0.83	0.72
Met+Cys, %	1.07	0.95	0.86

a,b -Vitamin & mineral premix supplied (content per kg): vitamin A, 1,800,000 IU; vitamin D<sub>3</sub>, 400,000 IU; vitamin E, 3,600 IU; vitamin K<sub>3</sub>, 400 mg; thiamine, 360 mg; riboflavin, 1,320 mg; niacin, 6,000 mg; vitamin B<sub>6</sub>, 600 mg; vitamin B<sub>5</sub>, 2,000; vitamin B<sub>12</sub>, 3 mg; folic acid, 200 mg; biotin, 20 mg; choline, 80 g; zinc, 17 g; iron, 10 g; copper, 2 g; manganese, 20 g; selenium, 40 mg; iodine, 200 mg.

### Visceral organs

For carcass evaluations, 10 birds per treatment were selected based on the average weight of the experimental unit. The carcasses were manually eviscerated and breast, legs, back, gizzard, liver, heart, spleen, abdominal fat and intestinal separated by hand and weighed individually. The carcass yields were calculated as a percentage of the live weight and the other parts yield was expressed as the percentage of carcass.

### Humeral immunity

On day 28, 0.5 mL of 10% sheep red blood cells (SRBC) suspension was administrated to two chicks/per replicate through the right-wing vein. On day 31, 3 mL blood samples were collected from left-wing vein puncture. The samples were incubated at 37°C and then centrifuged at 1,500×g for 10 min. The sera were collected and stored at -20°C until assay for assessment of the primary antibody response to SRBC. Seven days after the first and second challenge, birds were blood taken. Serum samples were evaluated for total antibody response including immunoglobulin (Ig) G and M by the 2-mercaptoethanol (ME) procedure as explained previously by Lepage *et al.* (1996). The measured antibody titers against SRBC were expressed as the log<sub>2</sub> of the reciprocal of the highest serum dilution giving complete agglutination. On days 35, the differential counts of heterophils and lymphocytes were measured using two birds per replicate cages as explained by Gross and Siegel (1983).

### Cellular immunity

For evaluating skin hypersensitivity reaction, one area, by 10 cm<sup>2</sup>, was marked for Dinitrochlorobenzene (DNCB) application, at 32 days of age. Before sensitization, skin thickness was evaluated. The birds were sensitized with DNCB at a dose of 0.25 mL per cm<sup>2</sup> area. After two weeks, the birds were challenged with 0.25 mL DNCB and their skin thickness, three parts in this area, was measured 24 and 48 hours after the challenging dose. Also, 0.01 mL Phytohemagglutinin (PHA: 10 mg.mL<sup>-1</sup> acetone and olive oil in 4:1 ratio) was intradermal injected between the third and fourth digits of the right foot and the area thickness was measured 24 and 48 hours after injection.

### Intestinal morphology

On day 42, tissue jejunum samples of broiler chickens (1 male and 1 female per replicate) were separated and fixed in (10%) neutral buffer formalin. The tissue samples were analyzed by the auto-processing apparatus, thereafter the slide sections were prepared by rotary microtome type (Manubeni, Erma-Tokyo, Japan). The prepared slides (n=5) from each jejunal segment each broiler chicks, and five well-oriented villi were measured from each the prepared slide. The average of villi measurements was reported as a mean for each bird. Villus width (VW) was assessed at the base of each villus; villus length (VL) was evaluated from the top of the villus to the villus-crypt junction, and crypt depth (CD) was evaluated from the base of the villus to the sub-mucosa. The VL to CD ratio was also calculated.

### Statistical analyses

The ANOVA procedure from SAS software used to evaluate the studied parameters and data were compared by Duncan multiple range test if were significant. Differences were considered significant for  $P < 0.05$ . The  $\log_2$  transformations were done on antibody titers before statistical analysis.

### Results

#### Growth performance

The effects of dietary treatments on the growth performance of the bird are shown in Table 2. The results showed that dietary inclusion of EOs, antibiotic and probiotic had no significant effects on

FI and FCR of broiler chicks (Table 2). In the grower phase from day 11 to 24, birds fed with encapsulated TEO+OEO and antibiotic had significantly higher BWG compared with those received control group ( $P < 0.05$ ). Dietary inclusion of additives had no significant difference in BWG compared to the control group during the overall rearing period (1-42). Birds receiving the encapsulated PEO+OEO had significantly higher BWG compared to PEO+OEO group ( $P < 0.05$ ). Encapsulated PEO+OEO with or without probiotic increased EPEF in comparison to the control group ( $P < 0.05$ ). SR was significantly higher in birds fed with Eos and probiotic than those received antibiotics ( $P < 0.05$ ).

**Table 2.** Effects of OEO and probiotic on growth performance in broilers at 42 days of age

Treatment	Control	Antibiotic	Encapsulated TEO+OEO	TEO+OEO	Probiotic	Probiotic+TEO+OEO	Probiotic+Encapsulated TEO+OEO	SEM	P-value
Body weight (g)									
1	44.84	44.62	44.68	45.12	44.76	45.04	44.92	0.11	0.150
10	201.98	207.45	204.93	200.30	202.48	198.10	198.81	1.04	0.240
24	780.16 <sup>bc</sup>	819.47 <sup>a</sup>	824.15 <sup>a</sup>	761.19 <sup>bc</sup>	788.40 <sup>bc</sup>	752.87 <sup>c</sup>	791.37 <sup>ab</sup>	5.17	0.000
42	2247.04 <sup>ab</sup>	2366.00 <sup>a</sup>	2377.44 <sup>a</sup>	2214.25 <sup>b</sup>	2308.20 <sup>ab</sup>	2277.30 <sup>ab</sup>	2392.80 <sup>a</sup>	18.22	0.046
Feed intake (g)									
1-10	223.04	209.03	220.12	200.00	209.48	207.70	201.30	2.63	0.15
11-24	782.32	768.60	834.13	709.95	823.13	697.18	761.53	14.32	0.075
25-42	2693.89	2977.84	2692.17	2589.79	2558.43	2644.27	2773.88	43.72	0.068
1-42	3699.26	3955.47	3744.40	3499.75	3591.04	3549.16	3736.69	37.09	0.089
Body weight gain (g)									
1-10	157.14	163.13	160.24	155.18	158.72	153.06	154.89	1.09	0.190
11-24	578.19 <sup>bc</sup>	612.03 <sup>a</sup>	619.21 <sup>a</sup>	560.88 <sup>bc</sup>	585.92 <sup>bc</sup>	554.76 <sup>c</sup>	590.55 <sup>ab</sup>	4.94	0.001
25-42	1466.87	1546.53	1553.29	1453.06	1519.92	1524.43	1601.46	17.38	0.224
1-42	2202.20 <sup>ab</sup>	2321.68 <sup>a</sup>	2332.75 <sup>a</sup>	2169.13 <sup>b</sup>	2264.56 <sup>ab</sup>	2232.26 <sup>ab</sup>	2347.91 <sup>a</sup>	18.24	0.046
Feed conversion ratio									
1-10	1.41	1.27	1.37	1.29	1.32	1.35	1.30	0.015	0.110
11-24	1.34	1.25	1.34	1.26	1.40	1.26	1.28	0.020	0.430
25-42	1.84	1.90	1.74	1.81	1.68	1.74	1.73	0.020	0.116
1-42	1.67	1.69	1.61	1.62	1.58	1.59	1.59	0.014	0.201
SR (%)	95.20 <sup>ab</sup>	92.80 <sup>b</sup>	97.60 <sup>a</sup>	95.20 <sup>ab</sup>	97.60 <sup>a</sup>	95.20 <sup>ab</sup>	98.40 <sup>a</sup>	0.47	0.017
EPEF	304.03 <sup>c</sup>	309.33 <sup>bc</sup>	343.08 <sup>a</sup>	309.77 <sup>bc</sup>	339.45 <sup>ab</sup>	324.60 <sup>abc</sup>	352.46 <sup>a</sup>	4.16	0.003

SEM: Standard error of means. Superscripts (a-c) show significant differences per row ( $P < 0.05$ ). EPEF= European production efficiency factors, SR= Survival rate

**Table 3.** Effects of OEO and probiotic on carcass and cut yields weight (%) in broilers at 42 days of age

Groups	Carcass	Breast	Legs	Back	Liver	Gizzard	Heart	Spleen	Bursa	Fat	Intestine
Control	72.98	24.47	18.46	21.62	1.97	2.54 <sup>bc</sup>	0.465	0.088	0.050	1.13	5.32
Antibiotic	72.84	23.33	18.72	22.67	1.94	2.97 <sup>a</sup>	0.502	0.097	0.063	1.41	5.30
Encapsulated TEO+OEO	74.93	24.75	18.90	22.74	2.01	2.45 <sup>c</sup>	0.467	0.082	0.053	1.68	4.63
TEO+OEO	74.29	24.93	19.37	22.42	1.96	2.61 <sup>bc</sup>	0.486	0.086	0.054	1.34	4.54
Probiotic	73.89	25.05	18.16	22.06	1.98	2.28 <sup>d</sup>	0.491	0.093	0.060	1.41	4.55
Probiotic+TEO+OEO	73.91	24.47	18.84	22.04	1.91	2.63 <sup>b</sup>	0.503	0.090	0.082	1.38	4.71
Probiotic+Encapsulated TEO+OEO	72.34	23.74	18.04	22.93	2.25	2.89 <sup>a</sup>	0.533	0.106	0.086	1.48	5.42
SEM	0.437	0.286	0.152	0.206	0.041	0.055	0.853	0.003	0.003	0.055	0.108
P-value	0.733	0.672	0.256	0.639	0.372	0.008	0.438	0.496	0.274	0.269	0.058

SEM: Standard error of means. Superscripts (a-c) show significant differences per column ( $P < 0.05$ ).

#### Relative weight of visceral tissues

The influences of treatments contain OEO, probiotic and antibiotic on carcass and cut yields relative

weight of broilers at 42 days of age are shown in Tables 3. The birds fed diets containing antibiotics, and probiotic plus encapsulated TEO+OEO had

significantly higher gizzard weight while the bird received probiotic had the lowest gizzard weight compared to other treatments. There was no significant difference between treatments for other segments.

### Cellular immunity

The data for the effect of treatments contain OEO,

probiotic, and antibiotic on cellular immunity are presented in Table 4. Our data showed that birds fed with probiotic plus encapsulated TEO+OEO showed higher DNCB and PHA after 24 h compared to the control group ( $P < 0.05$ ). After 48 h, DNCB was significantly lower in the control group compared with other treatments ( $P < 0.05$ ). It was no significant difference among groups for PHA after 48 h.

**Table 4.** Effects of OEO and probiotic on cellular immunity in broilers at 32 days of age

Groups	24 h post challenge		48 h post challenge	
	DNCB24 (mm)	PHA24 (mm)	DNCB48 (mm)	PHA48 (mm)
Control	1.82 <sup>d</sup>	1.87 <sup>d</sup>	0.26 <sup>b</sup>	0.77
Antibiotic	2.11 <sup>bc</sup>	2.20 <sup>b</sup>	0.56 <sup>a</sup>	0.71
Encapsulated TEO+ OEO	2.11 <sup>bc</sup>	2.07 <sup>bc</sup>	0.57 <sup>a</sup>	0.70
TEO+OEO	1.92 <sup>d</sup>	1.96 <sup>cd</sup>	0.59 <sup>a</sup>	0.63
Probiotic	2.05 <sup>c</sup>	2.12 <sup>b</sup>	0.54 <sup>a</sup>	0.62
Probiotic+TEO+OEO	2.18 <sup>b</sup>	2.20 <sup>b</sup>	0.52 <sup>a</sup>	0.66
Probiotic+Encapsulated TEO+OEO	2.44 <sup>a</sup>	2.56 <sup>a</sup>	0.60 <sup>a</sup>	0.61
SEM	0.071	0.21	0.019	0.061
P-value	0.001	0.001	0.001	0.39

SEM: Standard error of means. Superscripts (a-d) show significant differences per column ( $P < 0.05$ ). DNCB= Dinitrochlorobenzene, PHA= Phytohemagglutinin

### Humoral immunity

Our findings showed that dietary inclusion of TEO+OEO in capsulated form plus probiotic increased IgG<sub>1</sub> in broiler chicks compared with other groups (Table 5,  $P < 0.05$ ). The addition of

TEO+OEO and its encapsulated form with probiotic resulted in an increase in IgM<sub>1</sub>, IgG<sub>2</sub>, and IgM<sub>2</sub> compared with other treatments. Heterophil, lymphocyte and their ratio were not influenced by experimental treatments.

**Table 5.** Effects of OEO and probiotic on antibody response to SRBC (log<sub>2</sub>) and white blood cells differential count

Groups	7 Day after 1 <sup>st</sup> SRBC injection		7 Day after 2 <sup>nd</sup> SRBC injection		Heterophil	Lymphocyte	Heterophil/ Lymphocyte
	IgG <sub>1</sub>	IgM <sub>1</sub>	IgG <sub>2</sub>	IgM <sub>2</sub>			
Control	1.73 <sup>c</sup>	2.50 <sup>b</sup>	2.51 <sup>b</sup>	2.26 <sup>b</sup>	17.90	77.70	0.23
Antibiotic	1.63 <sup>c</sup>	2.49 <sup>b</sup>	2.47 <sup>b</sup>	2.35 <sup>b</sup>	18.00	80.10	0.22
Encapsulated TEO+OEO	1.96 <sup>b</sup>	3.22 <sup>a</sup>	3.05 <sup>a</sup>	2.77 <sup>a</sup>	17.10	79.60	0.22
TEO+OEO	1.67 <sup>c</sup>	2.77 <sup>b</sup>	2.58 <sup>b</sup>	2.21 <sup>b</sup>	16.40	78.00	0.23
Probiotic	1.73 <sup>c</sup>	2.59 <sup>b</sup>	2.48 <sup>b</sup>	2.24 <sup>b</sup>	16.60	76.40	0.23
Probiotic+TEO+OEO	2.03 <sup>b</sup>	3.08 <sup>a</sup>	3.18 <sup>a</sup>	2.81 <sup>a</sup>	16.00	78.00	0.22
Probiotic+Encapsulated TEO+OEO	2.19 <sup>a</sup>	3.03 <sup>a</sup>	3.32 <sup>a</sup>	2.84 <sup>a</sup>	16.40	76.40	0.23
SEM	0.12	0.15	0.24	0.15	0.37	1.07	0.013
P-value	0.001	0.001	0.001	0.001	0.61	0.102	0.091

SEM: Standard error of means. Superscripts (a-d) show significant differences per column ( $P < 0.05$ ).

**Table 6.** Effects of OEO and probiotic on jejunum morphology ( $\mu$ m) in broiler chickens

Groups	Villus length ( $\mu$ m)	Villus width ( $\mu$ m)	Crypt depth ( $\mu$ m)	Villus length/ Crypt depth
Control	1489.00 <sup>b</sup>	185.00 <sup>b</sup>	145.30 <sup>b</sup>	10.26 <sup>a</sup>
Antibiotic	1467.00 <sup>b</sup>	181.30 <sup>b</sup>	145.80 <sup>b</sup>	10.06 <sup>a</sup>
Encapsulated TEO+OEO	1767.00 <sup>a</sup>	204.60 <sup>a</sup>	183.80 <sup>a</sup>	9.65 <sup>b</sup>
TEO+OEO	1457.00 <sup>b</sup>	182.80 <sup>b</sup>	145.20 <sup>a</sup>	10.03 <sup>a</sup>
Probiotic	1458.00 <sup>b</sup>	183.50 <sup>b</sup>	144.60 <sup>b</sup>	10.08 <sup>a</sup>
Probiotic+TEO+OEO	1434.00 <sup>b</sup>	183.00 <sup>b</sup>	144.40 <sup>b</sup>	9.93 <sup>a</sup>
Probiotic+Encapsulated TEO+OEO	1775.00 <sup>a</sup>	205.80 <sup>a</sup>	188.70 <sup>a</sup>	9.41 <sup>b</sup>
SEM	87.56	5.24	8.12	0.18
P-value	0.001	0.001	0.001	0.043

SEM: Standard error of means. Superscripts (a-b) show significant differences per column ( $P < 0.05$ ).

### Intestinal morphology

Our findings showed that dietary inclusion of encapsulated EOs with and without probiotic significantly increased villus length, villus width and crypt depth in comparison to other groups ( $P < 0.05$ ) (Table 6). The results also showed that the villus length/Crypt depth ratio was significantly lower in the encapsulated TEO+OEO and probiotic+encapsulated TEO+OEO groups compared to other groups ( $P < 0.05$ ). The highest and lowest ratios were observed for the control group (10.26) and probiotic+encapsulated TEO+OEO group (9.41) respectively.

### Discussion

Our findings showed that dietary inclusion of EOs (encapsulated and non-capsulated) and probiotics did not have significant effects on FI and FCR. Pournazari *et al.* (2017) reported that dietary inclusion of TEO and probiotic, singly form, increased FI but only dietary inclusion of probiotic decreased BWG in broiler chicks. It has been reported that oral supplementation of OEO did not improve FI and FCR in broiler chicks (Galal *et al.*, 2016). It seems that encapsulation of EOs cannot improve the FI and FCR in broiler chicks. Encapsulation of the EOs could increase BWG in comparison with non-capsulated form on days 24 and 42; suggesting that encapsulation can efficiently increase BWG. Encapsulation of EOs also caused to increase in the BWG compared with the control group. It was no observed significant differences among TEO+OEO (non-capsulated) compared with the control group. Ragga *et al.* (2016) showed that dietary inclusion of TEO could increase BWG in broiler chicks. Increased BWG can be attributed to antioxidant properties and phenolic properties of EOs which decreases the harmful effects of bacteria on the intestinal system and help to more absorption amino acids (Lee *et al.*, 2004). EOs compounds not only help more absorption amino acids but also promote more digestive enzymes secretion which subsequently increases nutrient absorption for more growth (Lee *et al.*, 2004). Also, intestinal morphology was significantly improved in the encapsulated group which can confirm improved BWG by increasing nutrient absorption. The intestinal villi are known to have essential roles in promoting nutrient digestion and absorption because villi greatly enhance small intestine surface area and are known as initial tissues in the intestine which cause contact with nutrients (Gartner and Hiatt, 2001). It seems that encapsulation could increase the survivability of the EOs during processing and in the digestive tract. Dietary inclusion of probiotics could not improve the growth performance compared with the control group. Pournazari *et al.* (2017) reported that dietary inclusion of TEO and probiotic increased BWG. It has been reported that probiotics improve

broiler performance by increasing the immune modulation capacity of broilers (Yang *et al.*, 2012). The conflicts between our findings and others can be due to strains of probiotics, dosage, procedures of preparation, bird age, diet compounds and hygiene conditions (Zhang *et al.*, 2012). The SR and EPEF were also better in encapsulated groups which implicates on better efficiency of encapsulation.

Table 3 shows the effect of EOs, probiotic and antibiotic on broiler carcass traits. The results achieved here agreed with other studies that reported no significant effect of phytochemical additives and probiotic on the relative weight of carcass and cut yields of broilers (Toghyani *et al.*, 2010; Falaki *et al.*, 2010). On the contrary, Jamroz and Kamel (2002) found higher slaughter percentages of breast muscle in broilers fed with essential oil.

Our findings showed that all the experimental treatments had better cellular immunity in comparison with the control group. Also, encapsulated groups and TEO+OEO+probiotic (non-capsulated) had better humoral immunity in comparison with other groups. Hashemipour *et al.* (2013) reported that dietary inclusion of thymol+carvacrol increased the cellular and humoral immune responses in broilers. Flavonoids and other phenolic components, present in essential oils, increase the activity of vitamin C as an immune stimulator (Manach *et al.*, 1996). Amresh *et al.* (2007) have also reported flavonoids and polyphenolic compounds help the immune system by their antioxidant activity. It seems that capsulation help to maintain the active compounds in EOs and improve the immune responses. A combination of EOs and probiotic improved humoral immunity; showing synergism interaction effects between EOs and probiotic. Probiotics have been known to have immune-modulatory activities in birds (Paturi *et al.*, 2007). Probiotics improve the immune system by modulating in the intestinal system. Thus, a combination of EOs and probiotics can improve the immune system. Heterophil, lymphocyte and their ratio were not influenced by experimental treatments which were similar to those reported by Attia *et al.* (2017).

Our findings showed that capsulation of the EOs could improve intestinal morphology compared to other groups. The VL and CD are known as a good indicator of intestinal health and digestive tract maintenance (Pluske *et al.*, 1996). It has been accepted the role of some nutrients as promoter the morphological development of the small intestine (Kadam *et al.*, 2009; Yadav *et al.*, 2010). Several *in vivo* studies have reported the role of EOs as promoters for growth in the intestine (Yadav *et al.*, 2010; Amad *et al.*, 2011). The improved intestinal morphology can be attributed to the alleviating effects of EOs on toxins. Bacterial toxins are known to have negative effects on intestinal morphology

(Samadian *et al.*, 2013). It has been reported that EOs reduces the production of toxic compounds and damage to intestinal epithelial cells of broiler chicks (Yakhkeshi *et al.*, 2011; Samadian *et al.*, 2013). With regards to probiotics, it has been shown the positive role of probiotics in increasing the villus length (Awad *et al.*, 2009; Tsirtsikos *et al.*, 2012). The addition of encapsulated EOs and probiotic improved intestinal morphology; however, it was not observed significant differences with capsulated groups without probiotic; showing inefficiency the probiotic.

### Conclusion

In conclusion, the encapsulation of TEO and OEO

improved growth performance, immune response and intestinal morphology in comparison with non-capsulated status. It can be advised to use the encapsulation form of EOs for increasing the production efficiency in broiler chickens.

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### References

- Amad AA, Männer K, Wendler KR, Neumann K & Zentek J. 2011. Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. *Poultry Science*, 90: 2811-2816. DOI: 10.3382/ps.2011-01515
- Amresh G, Reddy GD, Rao CV & Singh PN. 2007. Evaluation of anti-inflammatory activity of *Cissampelospareira* root in rats. *Journal of Ethnopharmacology*, 110: 526-531. DOI: 10.1016/j.jep.2006.10.009
- AOAC. 2004. Official methods of analysis. 18<sup>th</sup> ed. Washington, DC.
- Attia YA, Bakhshwain AA & Bertu NK. 2017. Thyme oil (*Thyme vulgaris L.*) as a natural growth promoter for broiler chickens reared under hot climate. *Italian Journal of Animal Science*, 16: 275-282. DOI: 10.1080/1828051X.2016.1245594
- Aviagen. 2014. Ross 308 Broiler Nutrition specification manual. Midhlotian (UK): Aviagen Ltd.
- Awad WA, Ghareeb K, Abdel-Raheem S, & Böhm J. 2009. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. *Poultry Science*, 88: 49-56. DOI: 10.3382/ps.2008-00244
- Bai K, Huang Q, Zhang J, He J, Zhang L & Wang T. 2016. Supplemental effects of probiotic *Bacillus subtilis* on growth performance, antioxidant capacity, and meat quality of broiler chickens. *Poultry Science*, 96: 74-82. DOI: 10.3382/ps/pew246
- Bilia AR, Guccione C, Isacchi B, Righeschi C, Firenzuoli F & Bergonzi MC. 2014. Essential oils loaded in nanosystems: a developing strategy for a successful therapeutic approach. *Evidence-Based Complementary and Alternative Medicine*, 2014: 593-651. DOI: 10.1155/2014/651593
- Dorman HJ & Deans SG. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, 88: 308-316. DOI: 10.1046/j.1365-2672.2000.00969.x
- Falaki M, Shams Shargh M, Dastar B & Zrehdaran S. 2010. Effects of different levels of probiotic and prebiotic on performance and carcass characteristics of broiler chickens. *Journal of Animal and Veterinary Advances*, 9: 2390-2395. DOI: 10.3923/javaa.2010.2390.2395
- Gaggia F, Mattarelli P & Biavati B. 2010. Probiotics and prebiotics in animal feeding for safe food production. *International Journal of Food Microbiology*, 14: S15-28. DOI: 10.1016/j.ijfoodmicro.2010.02.031
- Galal AA, El-Araby IE, Hassanin O & El-Said Omar A. 2016. Positive impact of oregano essential oil on growth performance, humoral immune responses and chicken interferon alpha signaling pathway in broilers. *Advance Animal Veterinary Science*, 4: 57-65. DOI: 10.14737/journal.aavs/2016/4.1.57.65
- Gartner LP & Hiatt JL. 2001. *Color Textbook of Histology*. 2<sup>nd</sup> ed. W. B. Saunders, Baltimore, MD.
- Gross WB & Siegel HS. 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. *Avian Diseases*, 1: 972-979. DOI: 10.2307/1590198.
- Hashemipour H, Kermanshahi H, Golian A & Veldkamp T. 2013. Effect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens. *Poultry Science*, 92: 2059-2069. DOI: 10.3382/ps.2012-02685
- Jahromi MF, Altaher YW, Shokryazdan P, Ebrahimi R, Ebrahimi M, Idrus Z, Tufarelli V & Liang JB. 2016. Dietary supplementation of a mixture of *Lactobacillus* strains enhances performance of broiler chickens raised under heat stress conditions. *International Journal of Biometeorology*, 60: 1099-1110. DOI: 10.1007/s00484-015-1103-x
- Jamroz D & Kamel C. 2002. Plant extracts enhance broiler performance. In non-ruminant nutrition:

- Antimicrobial agents and plant extracts on immunity, health and performance. *Journal of Animal Science*, 80: 41-46.
- Kadam AS, Nikam MG, Patodkar VR, Muglikar DM, Lonkar VD, Yadav GB, Maini S, Ravikanth K & Meshram MD. 2009. Influence of herbal early chick nutritional supplement on the growth performance, serum biochemicals and immune response of broiler chicken. *International Journal of Poultry Science*, 8: 349-354. DOI: 10.3923/ijps.2009.349.354
- Lee KW, Everts H, Kappert HJ, Van Der Kuilen J, Lemmens AG, Frehner M & Beynen AC. 2004. Growth performance, intestinal viscosity, fat digestibility and plasma cholesterol in broiler chickens fed a rye-containing diet without or with essential oil components. *International Journal of Poultry Science*, 3: 613-618. DOI: 10.3923/ijps.2004.613.618
- Lepage KT, Bloom SE & Taylor RL. 1996. Antibody response to sheep red blood cells in a major histocompatibility (B) complex aneuploid line of chickens. *Poultry Science*, 75: 346-350. DOI: 10.3382/ps.0750346
- Manach C, Regerat F, Texier O, Agullo G, Demigne C & Remesy C. 1996. Bioavailability, metabolism and physiological impact of 4-oxo-flavonoids. *Nutrition Research*, 16: 517-544. DOI: 10.1016/0271-5317(96)00032-2
- Marcu A, Vacaru-Opriş I, Dumitrescu G, Ciochina LP, Marcu A, Nicula M, Peţl, Dronca D & Kelciov B. 2013. The influence of the genotype on economic efficiency of broiler chicken's growth. *Scientific Papers Animal Science and Biotechnologies*, 46: 339-346.
- Mathlouthi N, Bouzaïenne T, Oueslati I, Recoquillay F, Hamdi M, Urdaci M & Bergaoui R. 2012. Use of rosemary, oregano, and a commercial blend of essential oils in broiler chickens: *in vitro* antimicrobial activities and effects on growth performance. *Journal of Animal Science*, 90: 813-823. DOI: 10.2527/jas.2010-3646
- Paturi G, Phillips M, Jones M & Kailasapathy K. 2007. Immune enhancing effects of *Lactobacillus acidophilus* LAFTI L10 and *Lactobacillus paracasei* LAFTI L26 in mice. *International Journal of Food Microbiology*, 115: 115-118. DOI: 10.1016/j.ijfoodmicro.2006.10.007
- Pluske JR, Thompson MJ, Atwood CS, Bird PH, Williams IH & Hartmann PE. 1996. Maintenance of villus height and crypt depth, and enhancement of disaccharide digestion and monosaccharide absorption, in piglets fed on cows' whole milk after weaning. *British Journal of Nutrition*, 76: 409-22. DOI: 10.1079/bjn19960046
- Pournazari M, AA-Qotbi A, Seidavi A & Corazzin M. 2017. Prebiotics, probiotics and thyme (*Thymus vulgaris*) for broilers: performance, carcass traits and blood variables. *Revista Colombiana de Ciencias Pecuarias*, 30: 3-10. DOI: 10.17533/udea.rccp.v30n1a01
- Ragga NM, Kornay RS & Mohamad F. 2016. Effects of thyme/or formic acid dietary supplementation on broiler performance and immunity. *Agriculture and Agricultural Science Procedia*, 10: 270-279. DOI: 10.1016/j.aaspro.2016.09.064
- Samadian F, Zeinoaldini S, Towhidi A, Torshizi MA, Pirasaraei ZA & Gholamzadeh P. 2013. Evaluation of some phyto-genic feed additives in growing chicks' diet. *International Journal of Agriculture*, 3: 35-43. DOI: 10.1399/EPS.2014.55
- Seidavi A, Dadashbeiki M, Alimohammadi-Saraei MH, van den Hoven R, Payan-Carreira R, Laudadio V & Tufarelli V. 2017. Effects of dietary inclusion level of a mixture of probiotic cultures and enzymes on broiler chicken's immunity response. *Environmental Science and Pollution Research*, 24: 4637-4644. DOI: 10.1007/s11356-016-8206-8
- Sengül T, Yurtseven S, Cetin M, Kocyigit A & Sögüt B. 2008. Effect of thyme (*T. vulgaris*) extracts on fattening performance, some blood parameters, oxidative stress and DNA damage in Japanese quails. *Journal of Animal and Feed Sciences*, 17: 608-620. DOI: 10.22358/jafs/66689/2008
- Simitzi PE & Deligeorgis SG. 2011. The effects of natural antioxidants dietary supplementation on the properties of farm animal products. In *Animal Feed: Types, Nutrition, Safety*; Nova Science Publishers, Inc.: New York, NY, USA; 155-168.
- Simitzis PE. 2017. Enrichment of animal diets with essential oils—a great perspective on improving animal performance and quality characteristics of the derived products. *Medicines*, 4: E35. DOI: 10.3390/medicines4020035
- Stoica R, Şomoghi R & Ion RM. 2013. Preparation of chitosan-tripolyphosphate nanoparticles for the encapsulation of polyphenols extracted from rose hips. *Digest Journal of Nanomaterials and Biostructures (DJNB)*, 8: 955-963.
- Tabidi MH, Mukhtar AM & Mohammed HI. 2013. Effects of probiotic and antibiotic on performance and growth attributes of broiler chicks. *Global Journal of Medicinal Plants Research*, 1: 136-142.
- Toghyani M, Tohidi M, Gheisari AA & Tabeidian SA. 2010. Performance, immunity, serum biochemical and hematological parameters in broiler chicks fed dietary thyme as alternative for an antibiotic growth promoter. *African Journal of Biotechnology*, 9: 6819-6825. DOI: 10.5897/AJB09.1998
- Tsirtsikos P, Fegeros K, Balaskas C, Kominakis A & Mountzouris KC. 2012. Dietary probiotic inclusion level modulates intestinal mucin composition and mucosal morphology in broilers.



- Poultry Science, 91: 1860-1868. DOI: 10.3382/ps.2011-02005
- Yadav GB, Kadam AS, Pachpande AM, Lambate SB, Lonkar VD, Maini S & Ravikanth K. 2010. Post hatch histo-morphological studies of small intestinal development in chicks fed with herbal early chick nutritional supplement. International Journal of Poultry Science, 9: 851-855. DOI: 10.3923/ijps.2010.851.855
- Yakhkeshi S, Rahimi S & GharibNaseri K. 2011. The effects of comparison of herbal extracts, antibiotic, probiotic and organic acid on serum lipids, immune response, GIT microbial population, intestinal morphology and performance of broilers. Journal of Medicinal Plants Research, 10: 80-95.
- Yang CM, Cao GT, Ferket PR, Liu TT, Zhou L, Zhang L, Xiao YP & Chen AG. 2012. Effects of probiotic, *Clostridium butyricum*, on growth performance, immune function, and cecal microflora in broiler chickens. Poultry Science, 91: 2121-2129. DOI: 10.3382/ps.2011-02131
- Zhang ZF, Zhou TX, Ao X & Kim IH. 2012. Effects of  $\beta$ -glucan and *Bacillus subtilis* on growth performance, blood profiles, relative organ weight and meat quality in broilers fed maize–soybean meal based diets. Livestock Science, 150: 419-424. DOI: 10.1016/j.livsci.2012.10.003