Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2020 / v.22 / n.4 / 001-012

http://dx.doi.org/10.1590/1806-9061-2020-1284

Original Article

Efficacy of Savory Essential Oil Utilization in Conventional and Encapsulated Forms on Performance of Broiler Chickens

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■Keywords

Essential oil, Intestinal health, Nutrition, Savory.

Submitted: 20/May/2020
Approved: 23/August/2020

ABSTRACT

This survey aimed to evaluate the effects of dietary supplementation of different forms of savory essential oil (SEO) on growth performance, intestinal morphology and microbial population in broiler chickens. A total of 360 one-day-old male broilers were randomly allocated to 6 dietary treatment groups with 5 replicates per treatment and 12 birds per pen. The experiment consisted of a 2×3 factorial arrangement including two different forms [encapsulated SEO (ESEO) and nonencapsulated SEO (NSEO)] in three levels (0, 150, and 300 mg/kg diet) of SEO. Growth performance, jejunal morphology and intestine microbial population were examined. Our results revealed that feed intake was not influenced by the dietary treatments in different experimental periods. As well as, the experimental diets did not influence body weight gain (BWG) and feed conversion ratio (FCR) during the starter period. However, at the grower, finisher, and also whole rearing periods, broilers which received 150 mg/kg SEO had significantly higher BWG and lower FCR compared to the birds fed the control diet. The final body weight (FBW) was also higher in chickens fed with diet supplemented with 150 mg/kg SEO in comparison to the others. The results also revealed that 150 mg/kg SEO, significantly increased the concentration of *Lactobacillus* and decreased the intensity of *coliforms* in the ileal digesta in comparison to the control diet. Furthermore, villus height was significantly lower in birds fed the control diet than in the birds that consumed different levels of SEO. Eventually, the findings of this experiment revealed that dietary supplementation of SEO, especially at 150 mg/kg level, was effective in raising the populations of beneficial microorganisms in the gastrointestinal tract as well as improving intestinal morphology and growth performance of broilers.

INTRODUCTION

From previous years, antibiotic growth promoters have been applied in the poultry industry to improve growth and health status in birds' diets. However, the expanding pressure on the poultry industry to clear feed-antibiotics as growth enhancers has commenced exquisite probing to find trusty and efficient alternatives to maintain intestinal health (Salajegheh *et al.,* 2018). This novel generation of feed additives includes the herbs, spices and essential oils (EOs), which exhibits a wide range of attractive properties, such as antimicrobial, antioxidant, immune-modulatory and digestion stimulating properties (Brenes & Roura, 2010; Park & Kim, 2018; Oso *et al.*, 2019).

Summer savory (*Satureja hortensis* L) is an aromatic and medicinal herb belonging to the *Lamiaceae* family. The essential oil of this herb (SEO) has distinct biological properties such as antimicrobial and antioxidant activities, which are associated with the existence of its major chemical compounds such as carvacrol and thymol (Vitanza *et al.*, 2019). These properties aim to illuminate the improved performance observed in poultry. Anecdotally, EOs from various sources have been extensively expedited for their possible antimicrobial capabilities. Numerous studies in this field have stated that savory can also be included in the fowl nutrition as a feed additive to improve growth performance, blood indices, microflora population, intestinal morphology and even health status of birds (Ghalamkari *et al.*, 2011; Zadehamiri *et al.*, 2014; Rajaian *et al.*, 2014; Yeganeparast *et al.*, 2016; Dehghani *et al.*, 2018).

The accomplished researches on the use of summer savory in poultry rations have produced conflicting results. One of the main reasons for this can be attributed to the fact that potency and bioavailability of these additives are mostly influenced by many variables such as intestinal pH, rate of absorption, dosage and chemical nature of the applied substance, animal species as well as the hygienic status of the production environment and buffering characteristics of dietary components (Wang *et al.*, 2009; Yousaf *et al.*, 2017). As well as, Lee *et al.*, (2004) deduced that quality and efficiency of EOs might be reduced due to undesirable reactions, which can reduce palatability and feed consumption particularly when high levels of oil are available in the ration. Therefore, the use of these EOs primarily needs to find a proper method to reduce oxidative stability, control release, and improve the shelf life of these ingredients (Jafari *et al.*, 2008).

Encapsulation is the operation of coating fragments or droplets of solid or liquid substances. In this procedure, a continuous film of polymeric material, like gums, chitosan, etc. is applied to produce capsules. Forasmuch as the effect of encapsulated SEO on broiler growth performance has not well been documented, we decided first and foremost to assess the chemical composition, in vitro antimicrobial and also antioxidant activities of SEO. Afterward, the efficacy of essential oil forms [encapsulated savory essential oil (ESEO) and non- encapsulated savory essential oil (NSEO)] on growth performance, intestinal morphology, and microbial population in broiler chicks was examined.

MATERIAL AND METHODS

Composition of the SEO

The savory plant was collected from Kashan, Iran, and air-dried at ambient temperature. The dried materials were distilled using Clevenger distillation (Zaouali *et al.*, 2010). The prepared essential oil was stored at 4° C in dark glass vessels. The preparation process of savory essential oils was done in a pharmaceutical company (Barij Essence Ardehal-Mashhad, Co., Kashan., Iran). The constitution of the oil was analyzed using gas chromatography (GC; Agilent 7890B, Agilent technologies) interfaced with mass spectrometry (MS; Agilent 5977A, Agilent Technologies, USA). The applied capillary column was the HP-5MS (5% phenyl methyl polysiloxane). The carrier gas was helium at a flow rate of 1 ml/min. The temperature of the column was adjusted at 5 ºC /min in the beginning, then programmed to increase up to 280 ºC at 5 ºC /min. the split ratio was 1:50. The mass spectrometer was run in electron- impact (EI) mode, using ionization energy of 70 eV. The essential oil was diluted 1:100 in n-hexane, after that, 0.1 µl was injected into the GC system. The components were recognized by comparing their relative retention times and mass spectra with the standard database NIST 80 (NIST, 2008) ADAMS (Adams, 2007) and Willey MS libraries (Adams & Sparkman, 2007). The GC peak areas were applied to the determination of the relative percentage of the oil constituents.

Encapsulation of SEO

The medium molecular weight chitosan (CS) and sodium triphosphate pentabasic (sTPP) were obtained from Sigma-Aldrich. Other reagents exerted in the trial were of analytical grade. Encapsulation of the savory essential oil was accomplished by ionic gelation based on the procedure of Sawtarie *et al.* (2017). Briefly, after dissolving CS in 1% (w/v) acetic acid, the solution sonicated before it became transparent. The dropwise increase of 10 ml triphosphate pentabasic solution (1 mg/ml) to a 25 ml CS solution (pH equal to 5), under constant shaking at usual temperature, produced the composition of CS-TPP parts by ionic gelation. Before adding the TPP solution, 20 % (w/v) SEO was subjoined to the CS solution due to the procurement of CS-TPP particles loaded with SEO.

In vitro antibacterial activity

Experiments were performed on two different microbial strains, *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 29737), which were obtained from Food Microbiology Laboratory, Veterinary Medicine Faculty, Kerman University (Iran). Afterward, they were grown in sterile Mueller Hinton Broth (Merck, Germany) at 37 ºC for 18-24 h to get a bacterial suspension. A suspension of the organisms was adjusted to the 0.5 McFarland standard turbidity. For the agar dilution method, a series of agar plates containing different concentrations (0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6, and 51.2 mg/ml) of the antimicrobial agent (ESEO and NSEO) was prepared and used 2fold dilution technique. One µl of 0.5 McFarland was placed on each of the series of plates. Then, inoculated plates were incubated at 35 °C for 24 h, and the MIC values were obtained after 24 h. The MIC is the minimum concentration of NSEO that will obstruct the growth of microbes after nocturnal incubation (Wiegand *et al.*, 2008).

In vitro antioxidant activity

The evaluation of the antioxidants activity of SEO based on the free radical-scavenging activity of the oils was performed based on DPPH (2, 2-diphenyl-1-picryl-hydrazyl) radical method. This activity was assessed according to the procedure of Burits & Bucar (2000). A volume of 1ml of the dilution of each sample in methanol was mixed with 2.5 ml of DPPH methanolic solution (0.004%, *w/v*). Then 200 µl of each sample (at various levels) were added to the microplate cells. A DPPH solution was used as a blank sample. After that, the microplate was kept for half-hour at lab temperature, in the dark. The absorbance of the samples was determined at 517 nm using a Power Wave™ HT Microplate Spectrophotometer (Bio Tek Instruments, Winooski, VT). This trial was carried out in 3 replicates. The antioxidant activity of the samples, which were described as percentage inhibition of the DPPH free radicals was estimated using the following equation:

I % = (A blank- A sample / A blank) × 100

Where I symbol is the DPPH radical inhibition %; A blank and A sample are the absorbance values of the control (blank) and test sample, respectively. The IC50 value is distinguished as the level of the antioxidant needed for the 50% inhibition of the DPPH activity. The synthetic antioxidant reagent BHT was applied as a positive control.

Animals and experimental design

The animal care and use protocol was approved by the Research Ethics Committee of the Shahid Bahonar University of Kerman, Iran (IR.UK.VETMED.REC.1398.020). A total of 360 one-day-old (Ross 308) male chicks were purchased from a commercial hatchery and subjected to 6 dietary groups. Then, all birds were weighed and randomly housed into 30 cages (each cage of dimension 120 × 120 cm), consisted of 6 treatments, 5 replicates and 12 birds in each replicate. During the whole feeding period, birds were allowed *ad libitum* access to feed and water. The experiment was conducted in a completely randomized design, using a 2×3 factorial arrangement with two different forms of SEO (ESEO and NESO) at three levels (0, 150, and 300 mg/kg diet), which were added to a corn-soybean meal-based diet as a basal diet. Also, a 3-phase feeding scheme was utilized with a starter (1- 10 d), grower (11-24 d), and finisher (25-42 d) phases. The gross composition of the basal diets is presented in Table 1. Diets were adjusted according to the standards described in Ross 308 broiler nutrition specification (Aviagen, 2014).

Table 1 – Ingredients and nutrient compositions of basal diets in different periods.

Item	Starter (d 1–10)	Grower (d 11–24)	Finisher (d 25–42)
Ingredients (%)			
Corn	48.42	52.30	58.22
Soybean meal (44%)	42.00	37.84	32.20
Vegetable oil	5.00	5.70	5.70
Limestone	1.15	1.05	1.00
Dicalcium phosphate	1.75	1.60	1.40
Common salt	0.43	0.43	0.43
DL-methionine	0.40	0.32	0.30
Lysine HCl	0.25	0.18	0.19
L-threonine	0.10	0.08	0.06
Vitamin & mineral premix1	0.50	0.50	0.50
Calculated chemical composition			
Metabolisable energy (kcal kg^{-1})	3000	3100	3200
Crude protein (%)	23	21.5	19.5
Methionine (%)	0.740	0.643	0.599
Methionine+ cysteine (%)	1.08	0.99	0.91
Lysine (%)	1.44	1.29	1.16
Threonine (%)	0.8	0.69	0.6
Calcium (%)	0.96	0.87	0.79
Available phosphorous (%)	0.480	0.435	0.395
Sodium (%)	0.17	0.16	0.16

[1]Mineral and vitamin premix provided per kilogram of diets: A: 10 000 IU, D3: 5000, E: 50 IU, K: 3 mg, B1: 2 mg, B2: 6 mg, niacin: 60 mg, pantothenic acid: 15 mg, B6: 3 mg, biotin: 0.1 mg, folic acid: 1.75 mg, B12: 0.016; Cu: 16 mg, I: 1.26, Fe: 40 mg, Mn: 120 mg, Se: 0.3 mg, and Zn: 100 mg.

Growth performance

The growth performance parameters were obtained in the starter, grower, finisher, and overall (1 to 42 d) periods. On days 1, 10, 24, and 42 of age, the birds were weighed, and the weight of feed residuals per pen was registered. These data were applied to measure BWG and FI. FCR was also determined by BWG and FI. The mortality rate was registered daily.

Intestinal microflora population

On day 42, two male chicks per replicate were randomly opted and slaughtered for intestinal microflora analyzes. The ileal contents of the birds were attentively depleted into a sterilized bottle and immediately transferred to the laboratory. After that, serial dilutions were performed during one hour of the collection (10^{-1} dilution as the primary dilution up to 10^{-9} as the ultimate dilution). Optional agar media was employed for the enumeration of bacterial target populations: *Lactobacillus* bacteria (MRS agar-Merck, Darmstadt, Germany); and total *coliform* bacteria (Mac Conkey Agar-Merck, Darmstadt, Germany) (Yang *et al.*, 2012). Incubated samples under anaerobic and aerobic conditions (37 °C for 72 h), were applied for the determination of the total numbers of *Lactobacillus* and *coliform* respectively (Adaszynska-Skwirzynska & Szczerbinska, 2018). Obtained findings were expressed as log10 CFU/g ileal digesta.

Intestinal morphology

For intestinal morphology evaluation, two male birds per pen were killed by cervical dislocation at the end of the trial. The 2-cm segments of the jejunum were obtained and fixed in 10 % buffered formalin for 24 h, and then the 10 % buffered formalin was renewed. After dehydration, samples were placed into xylol and embedded in paraffin. A microtome was applied to make five cuts that were stained with hematoxylin-eosin. Villus height was determined from the apex of the villus to the valley in the middle of individual villi, and the crypt depth was measured from the valley between individual villi to the basolateral membrane. The values of the villus height (VH), crypt depth (CD), and villus width (VW) were determined 5 times from various villus and crypts per slide (Thompson & Applegate, 2006).

Statistical Analysis

The experimental design was a completely randomized design, using a 2×3 factorial arrangement with two forms of SEO and three levels of SEO. The data were analyzed by ANOVA using the GLM procedures of SAS statistical software (SAS, 2001). Treatments were compared applying the Tukey's test, and the differences were judged statistically significant at $p \leq 0.05$.

RESULTS

SEO Chemical characterization

The chemical composition of SEO was determined by a GC-MS instrument, in which the retention times and the mass spectra of oil ingredients accompanied by the data library, as exhibited in Table 2. According to the data, eighteen compounds identified by GC–MS, so that carvacrol (50.46%), γ-terpinene (16.91%), thymol (11.74%), and p-cymene (8.14%) were obtained as the major constituents of the SEO.

Table 2 – Major composition (%) of SEO.

Number	RT (min)	Components	%
1	7.78	α-Thujene	1.140
2	8.39	α-Pinene	0.580
3	9.00	Camphene	0.250
4	10.98	β-Pinene	0.420
5	11.53	Myrcene	1.230
6	11.97	α-Phellandrene	0.230
7	13.73	γ-3-Carene	0.171
8	13.93	α-Terpinene	1.600
9	14.78	P-Cymene	8.140
10	15.19	Limonene	0.320
11	17.21	γ-Terpinene	19.910
12	19.47	Linalool	0.210
13	23.95	Thymol	11.74
14	32.38	Carvacrol	50.46
15	34.99	Carvacrol acetate	0.100
16	35.62	Bisabolene	0.800
17	37.28	Spathulenol	0.180
18	42.27	Isopropyl palmitte	0.116

RT = Retention time (min)

Antibacterial activity

Minimum inhibitory concentration (MIC)

The results of the MIC method (as shown in figure 1) indicated that SEO has antibacterial activity against pathogenic bacteria, including *E. coli* and *S. aureus.* Both of them were more sensitive to the ESEO in comparison to the NSEO. The MIC value of ESEO against *E. coli* and *S. aureus* were 3.2 and 0.2 mg/ml, and for NSEO were 0.8 and 0.2 mg/ml, respectively. Among pathogenic bacteria, *E. coli* was the most sensitive to NSEO and ESEO, while *S. aureus* was the most resistant.

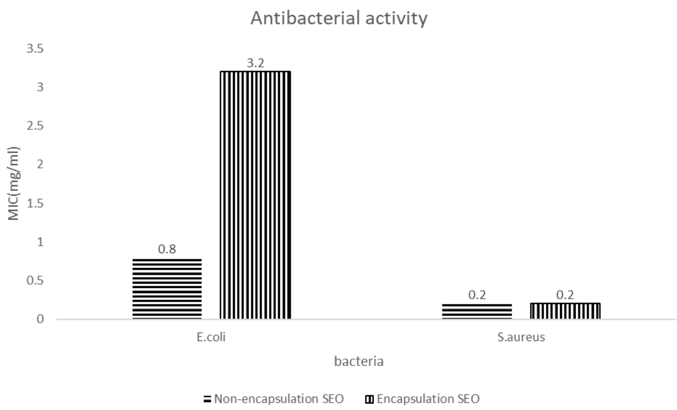

Figure 1 – Minimum inhibitory concentration (MIC values) of SEO and its encapsulated form against bacterial strains.

Antioxidant activity analysis

Antioxidants characteristics of SEO based on free radical-scavenging activity are depicted in figure 2. This activity was assessed according to the method of Burits & Bucar (2000). The values of IC50 for NSEO and ESEO and BHT were 68.895, 384.085, and 20.30 µg/ml, respectively. The findings show that the antioxidant activity of ESEO was significantly higher than the NSEO.

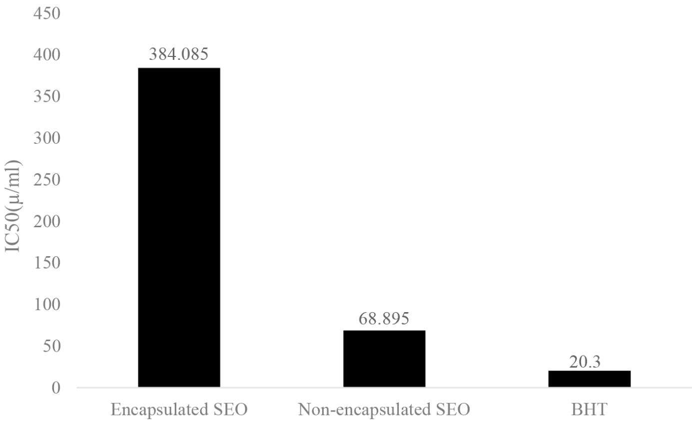

Figure 2 – Comparison of antioxidant activity (IC50 values) of SEO and its encapsulated form with BHT.

Growth performance

The birds were healthy throughout the experiment, with mortality of <2% that was uncorrelated to the dietary treatments. The effects of the experimental treatments on broilers performance are demonstrated in Table 3. Feed consumption was not influenced by the dietary treatments in any of the various phases of the rearing ($p>0.05$). As well as, BWG and FCR were not affected by the experimental diets during the starter period ($p>0.05$). However, at the grower ($p<0.05$), finisher ($p<0.01$), and also the whole rearing period ($p<0.01$), the broilers which received 150 mg/kg SEO had significantly higher BWG and lower FCR compared to the birds fed the control diet. Similarly, the birds which received 150 mg/kg SEO had a higher FBW in comparison to other birds ($p<0.01$). As reverse, throughout the different phases of the experiment, there were no differences between chicks fed 300 mg/kg SEO with those fed the control diet ($p>0.05$). Furthermore, the interaction between the SEO level and SEO form was not significant ($p>0.05$) except for BWG in the overall growing period and FBW ($p<0.05$). Regarding these interaction effects, the highest values were observed in birds fed 150 mg/kg NSEO and the lowest values related to those fed the control diet.

Ileal microbial population

The effects of the experimental diets on the ileal microbial population are illustrated in Table 4. Dietary inclusion of 150 mg/kg SEO, significantly increased

Table 3 – Effect of experimental diets on growth performance of broiler chickens[1].

Treatment		Starter (d 0 -10)			Grower (d 11 - 24)			Finisher (d 25 - 42)			Overall (d 0 - 42)			
SEO level(mg/kg)		BWG (g/b/d)	FI (g/b/d)	FCR (g/g)	BWG (g/b/d)	FI (g/b/d)	FCR (g/g)	BWG (g/b/d)	FI (g/b/d)	FCR (g/g)	BWG (g/b/d)	FI (g/b/d)	FCR (g/g)	FBW (g)
SEO Level														
0		13.71	19.98	1.458	42.37^{b}	70.87	1.703^{a}	77.12^{b}	165.5	2.152^{a}	50.15^{b}	98.60	1.970^{a}	2170^{b}
150		13.19	18.64	1.414	44.98^{a}	70.68	1.577^{b}	84.35^{a}	162.9	1.930^{b}	54.12^{a}	97.77	1.809^{b}	2340^{a}
300		13.98	19.66	1.407	42.40^{ab}	71.93	1.685^{ab}	78.47^{b}	161.7	2.064^{ab}	50.84^{b}	96.96	1.908^{a}	2207^{b}
SEM		0.385	0.526	0.015	0.730	0.558	0.031	1.144	1.577	0.038	0.576	0.608	0.026	22.97
p-value		0.355	0.190	0.059	0.027	0.252	0.021	0.001	0.247	0.002	0.001	0.185	0.001	0.001
SEO Form														
NE		13.49	19.21	1.425	42.42	70.61	1.672	79.83	164.6	2.073	51.54	98.44	1.917	2211
E		13.76	19.64	1.428	44.08	71.70	1.638	80.13	162.1	2.028	51.87	97.11	1.875	2267
SEM		0.315	0.429	0.012	0.596	0.456	0.026	0.934	1.287	0.031	0.470	0.496	0.021	18.75
p-value		0.539	0.493	0.853	0.062	0.104	0.375	0.819	0.184	0.320	0.631	0.070	0.178	0.054
SEO level*Form														
0	NE	13.81	20.28	1.469	40.18	70.73	1.762	76.04	167.8	2.213	49.30^{c}	99.80	2.027	2120^{d}
0	E	13.60	19.68	1.448	44.55	71.00	1.645	78.19	163.1	2.091	51.00^{bc}	97.40	1.912	2220^{bcd}
150	NE	13.17	18.44	1.401	45.37	70.06	1.546	86.53	166.1	1.923	55.25^{a}	98.83	1.790	2374^{a}
150	E	13.21	18.83	1.427	44.58	71.29	1.608	82.17	159.7	1.947	52.99^{ab}	96.71	1.829	2306^{ab}
300	NE	13.48	18.92	1.405	41.71	71.04	1.708	76.19	159.9	2.082	50.08^{bc}	96.70	1.932	2138^{cd}
300	E	14.48	20.40	1.410	43.09	72.82	1.662	80.03	163.6	2.046	51.61^{bc}	97.22	1.884	2276^{abc}
SEM		0.545	0.744	0.022	1.033	0.790	0.045	1.619	2.230	0.054	0.815	0.860	0.036	32.48
p-value		0.512	0.391	0.569	0.061	0.635	0.160	0.061	0.071	0.413	0.037	0.194	0.134	0.011

[1] Data are mains of 5 replicates per treatment. SEM standard error of the means. SEO Level = Savory Essential Oil (0, 150 and 300mg/kg).NE = Non-Encapsulated, E = Encapsulated.
[a,b,c] Means within the same column with different superscripts differ significantly ($p<0.05$).

the concentration of Lactobacillus and decreased (p=0.002) the concentration of coliforms (p=0.010) in the ileal digesta in comparison to the control birds. SEO form and its interaction with the SEO level did not affect the microbial population of the ileum (p<0.05).

Table 4 – Effect of experimental diets on ileal microbial population at 42d of age.

	Treatment	Microbial Count (log CFU/g)	
SEO (mg/kg)	Level	Lactobacillus (Gram-Positive)	Coliforms (Gram-Negative)
0		7.027b	5.124a
150		8.040a	3.985b
300		7.426ab	4.606ab
SEM		0.182	0.239
p-value		0.002	0.010
SEO Form			
NE		7.574	4.795
E		7.421	4.348
SEM		0.149	0.195
p-value		0.475	0.119
SEO level*Form			
0	NE	6.878	5.515
0	E	7.177	4.732
150	NE	8.256	4.249
150	E	7.824	3.721
300	NE	7.589	4.620
300	E	7.263	4.591
SEM		0.258	0.338
p-value		0.327	0.534

a b c Means within the same column with different superscripts differ significantly (p<0.05).

SEM standard error of the means. SEO Level = Savory Essential Oil (0, 150 and 300 mg/kg). NE = Non-Encapsulated, E = Encapsulated.

Intestinal morphology

The effect of different treatments on the intestinal morphology indices at 42 d of age is summarized in Table 5. Unlike villus width, crypt depth, and villus height influenced by the dietary SEO level. The villus height was significantly lower in chicks fed the control diet than in the birds that consumed different levels of SEO (p<0.01). The crypt depth was also significantly higher in broilers fed 150 mg/kg SEO in comparison to those fed the control diet (p<0.05). SEO form, as well as the interaction between SEO level and SEO form, did not affect the morphological characteristics of the intestine (p<0.05).

DISCUSSION

Chemical composition

As noted in Table 2, the applied SEO in this experiment contained carvacrol (50.46%), γ-terpinene (16.91%), thymol (11.74%) and p-cymene (8.14%) as the main components. This information is consistent with reports from other researchers. In this regard, it is reported that volatile oil isolated from SEO has constituents of carvacrol, thymol, phenols, and flavonoids (Momtaz & Abdollahi, 2010). Several researchers have also mentioned, thymol (0.3–28%), γ-terpinene (15–40%), carvacrol (10–67%), and p-cymene (3–20%) are the main constituents of the savory volatile oils (Mihajilov-Krstev *et al.*, 2009; Hamidpour *et al.*, 2014; Tepe & Cilkiz, 2016). In another study, the major components of *Satureja hortensis* were detailed as thymol (40.5%), γ-terpinene (18.6%), carvacrol (14%), and p-cymene (9%) (Adiguzel *et al.*, 2007).

Table 5 – Effect of experimental diets on the jejunal morphology characteristics at 42 d of age.

Treatment		Morphology Parameter			
SEO Level (mg/kg)		Villus height (μm)	Villus width (μm)	Crypt depth (μm)	VCR
SEO Level					
0		1321^{b}	138.6	129.7^{b}	10.20
150		1373^{a}	140.9	135.4^{a}	10.15
300		1365^{a}	137.9	133.0^{ab}	10.27
SEM		6.137	1.483	1.514	0.113
p-value		0.001	0.343	0.044	0.743
SEO Form					
NE		1355	138.1	131.8	10.30
E		1350	140.2	133.6	10.12
SEM		5.011	1.211	1.236	0.092
p-value		0.487	0.225	0.313	0.182
SEO level*Form					
0	NE	1313	137.2	126.4	10.40
0	E	1329	140.0	133.0	10.01
150	NE	1379	140.2	136.4	10.12
150	E	1367	141.6	134.4	10.18
300	NE	374	136.8	132.6	10.37
300	E	1356	139.0	133.4	10.17
SEM		8.679	2.097	2.141	0.160
p-value		0.125	0.946	0.145	0.372

a b c Means within the same column with different superscripts differ significantly (p<0.05).

SEM standard error of the means.

SEO Level = Savory Essential Oil (0, 150 and 300mg/kg).NE = Non-Encapsulated, E = Encapsulated

VCR = Villus height Crypt depth Ratio

It turns out that the concentrations of SEO components such as linalool, carvacrol, g-terpinene, p-cymene, and b-caryophyllene exhibit broad variations depending on environmental conditions, geographic origin and the stage of herb development (Milos *et al.*, 2001).

Antibacterial activity

The results of this survey demonstrated that SEO has more antimicrobial activity on gram-negative bacteria

(*E. coli*) in comparison to the gram-positive bacteria (*S. aureus*). These activities have been ascribed to the presence of active volatile components in the SEO.

Nowadays, it has become clear that thymol and carvacrol play a vital role in antimicrobial activities against tested strains. Many findings have shown that the important constituents of the Satureja spp, contain thymol and carvacrol, which are mostly accompanying γ-Terpinene, paracymene, and linalool. This class of phenolic compounds has antimicrobial activates (Mirjana & Nada 2004; Sefidkon & Jamzad, 2005). Although, it's usually expected that EOs should be more efficient against gram-positive bacteria because of the fundamental interactions between the cell membrane and hydrophobic ingredients of the EOs (Soković *et al.*, 2010). However, in an experiment by Dorman & Deans (2000), phenolic compounds include thymol, and carvacrol reacted otherwise against gram-positive and gram-negative bacteria, which was in line with the results of the present study. In this regard, Burt (2004) declared that thymol and carvacrol can lead to the collapse of the external membrane of gram-negative bacteria, releasing lipopolysaccharides and amplifying the permeability of the cytoplasmic membrane to ATP. Concerning the mechanism of action, it's believed that phenolic compounds interfere with the cell membrane role and eventually result in the leak of ions, and thus, they operate their bactericidal function. The chemical structure of herbal essential oil and its volatile compounds have a great impact on the antimicrobial activities of EOs. Bacterial count, type of culture medium, extraction manners, growth phase, pH, incubation time and also temperature are the main factors affecting the antimicrobial activities of the herbal EOs, so the findings obtained from different experiments are sometimes accompanying with differences (Burt, 2004).

On the other hand, the broilers receiving ESEO showed higher microbial activity against the *E. coli* population compared to birds that received NSEO. These data revealed that the supplementation of ESEO prevented more the growth of the pathogen in the broiler intestine. In line with our findings, Michiels *et al.,* (2008) exhibited that carvacrol was wholly imbibed in the abdomen and the proximal small intestine of piglets after oral consumption, obviously stipulating the need for insulating carvacrol and efficient transport to the poultry intestine. Although, it seems synergism between EOs and different processing methods will also require to be more probed before they can be exerted commercially (Calo *et al.,* 2015).

Antioxidant activity

The main constituents of the *Satureja* species such as carvacrol and thymol are extensively reported to possess high levels of antioxidants (Ćavar *et al.*, 2008; Oke *et al.*, 2009; Khoury *et al.*, 2016). Moreover, the synergistic effects functions between the various molecules of the EOs and their monoterpenoid components have been published (Bakkali *et al.,* 2008). Trifan *et al.,* (2015) indicated that phenolic chemotype (carvacrol) existing in SEO was exhibited a considerable antioxidant activity with potential applicability in the preservation of sensitive matrices from free radical-mediated oxidative stress, comprising ionizing radiation-induced oxidative damage. Fazel *et al.*, (2007) also declared the antioxidant activity of the essential oils of savory based on IC50 was 5.8 mg/ml, whereas IC50, 68 µg/ml in our survey showed good antioxidant activity. Our findings also demonstrated that ESEO had more antioxidant properties than the NSEO. It seems the antioxidant properties of essential oils linked to their capability to act as an anti-inflammatory factor. A large amount of reactive oxygen species (ROS) are produced by monocytes, neutrophils, eosinophils, and macrophages through the process of bacterial phagocytosis. ROS oxidative damage on biological macromolecules such as proteins, lipids, and DNA is considered as the initial phase of various diseases, aging, and cancer (Dickinson *et al.,* 2011). It is proven that essential oils can scavenge ROS and thus decrease the oxidative damage of a tissue that has been linked to the reduction of inflammation (Miguel *et al.,* 2010). The difference between these antioxidant capacities is probably due to the insolubility of ESEO in methanol.

Growth performance

As noted in Tables 3, except for the starter phase, in other periods, broilers which received 150 mg/kg SEO had significantly higher BWG and lower FCR compared to the birds fed the control diet. The FBW was also higher in chicks which received 150 mg/kg SEO in comparison to the others. Whereas, all of these parameters for chicks treated by 300 mg/kg SEO were similar to those fed the control diet.

Although sufficient information on the encapsulated SEO and its possible use as an alternative to antibiotics for poultry is not available, few studies on savory utilize in poultry nutrition have been carried out in recent years. The obtained findings of this experiment in regard to using 150 mg/kg SEO, were in agreement with the results of Zamani Moghaddam *et al.*, (2007), and Goodarzi *et al.,* (2014) who observed significant improvements in FCR, BWG and FBW of broilers, due to the utilization of SEO. Rajaian *et al.,* (2013) also stated that *Satureja hortensis* powder has a positive effect on FBW and no significant impact of FI and FCR. In another experiment whose results were the opposite of Rajaian *et al.,* (2013), it was concluded that, unlike BWG that was not impressed by savory extract, FCR was significantly improved when birds fed 400 mg/kg savory extract, compared to control birds (Movahhedkhah *et al.,* 2019). This part of our results also disagreed with the experiments carried out by Ghalamkari *et al.*, (2011), and Yeganeparast *et al.,* (2016), who claimed that SEO did not have a positive effect on the growth performance of broilers. However, these reports are consistent with our data regarding the use of 300 mg/kg SEO, which did not make any significant difference with the control group. Similarly, Zamani Moghaddam *et al.,* (2007) concluded that although the use of 3 g/kg savory had an advantageous effect on growth performance in broilers, higher dosage had a detrimental impact on performance. In another experiment similar to ours, Azarbad *et al.,* (2019) reported that adding various levels of *Satureja* essential oil (400 and 500 mg/kg) in conventional and microencapsulated forms to the diet of broilers did not affect feed consumption and FCR of broilers. These authors also showed that experimental treatments containing conventional and microencapsulated types of *Satureja* essential oil caused a significant decrease in the final body weight of the birds relative to the control treatment. These researchers attributed these findings to the presence of polyphenolic compounds, such as carvacrol, which is known as a bitter-tasting substance. It has also been claimed this active compound can modulate appetite and reduce FI by affecting the central nervous system (Brenes & Roura, 2010; Azarbad *et al.,* 2019).

These differences in the mentioned results could be attributed to the combined effect of variations in the sample type and dose, duration and processing of the herb, EOs, different dietary compositions, environment, management, and age differences (Zeng *et al.,* 2015). Despite all these contradictions, it is generally believed that EOs have numerous lucrative functions in poultry diet, involving increased FI (Jamroz *et al.,* 2005; Jang *et al.,* 2007), digestibility improvement (Lee *et al.,* 2003), secretion of digestive enzymes (Jamroz *et al.,* 2005; Lee *et al.,* 2003), and modification of gastrointestinal microflora (Liolios *et al.,* 2009). Also, other reasons, such as growth-stimulating of broilers via enhancement of glucose influx into the tissues (Goodarzi *et al.,* 2013), and elevation of serum testosterone concentration (Khosravinia, 2014), have been mentioned in the interpretation of improved bird performance. These factors appear to be able to conspicuously vindicate the performance improvement of the chicks which received 150 mg/kg SEO. The utilization of 300 mg/kg SEO appear to have had no significant effect on performance indices, according to Zamani Moghaddam *et al.,* (2007). As mentioned earlier, carvacrol found in SEO can modulate appetite and decrease the growth performance of birds by affecting the central nervous system. Also, with the increase in SEO content in the diet, the relative decline in broilers performance due to the bitter taste of carvacrol will not be surprising (Brenes & Roura, 2010; Azarbad *et al.,* 2019).

Ileal microbial population

Consistent with the in vitro findings, our results, confirmed that all the bacteria were susceptible with different degrees to SEO. Our data were at variance with the outcomes detailed by Yeganeparast *et al.,* (2016), revealing the ineffectuality of 150 mg/kg SEO on the intestinal microflora population. Although there are not many reports on the effect of this essential oil on the intestinal microflora, other ESOs have registered impacts in this area. Many researchers believed that phytogenic additives cause positive changes in gut microbiota by restricting the growth of harmful bacteria, reduction of their metabolites, growth improvement of the beneficial bacteria, enhancement of the feed breaks down, and increasing nutrient availability to the host animal (Jamroz *et al.*, 2003; McReynolds *et al.*, 2009; Tiihonen *et al.*, 2010; Mountzouris *et al.*, 2011; Oso *et al.*, 2019). However, other studies have not exhibited significant effects of herb-byproducts on the gut microbiota of birds (Cross *et al.,* 2007; Cao *et al.,* 2010; Kirkpinar *et al.,* 2011).

SEO and its active constituents (especially carvacrol and thymol) have been shown to exhibit a wide spectrum of activity against microorganisms (Mirjana & Nada, 2004). The antimicrobial mode of action is mainly attributed to ESO's potential to entrance into the bacterial cell membrane, collapse these constructions, and cause intracellular materials leakage. These additives were also claimed to stimulate intestinal secretion of mucus in broilers, which was supposed to reduce the adhesion of pathogens (Jamroz *et al.,* 2006). These interpretations support the theory that phytogenic additives may positively modify gut functions, howbeit the number of *in-vivo* studies with birds is still quite finite.

On the other hand, unlike the *in vitro* data, *in vivo* results demonstrated that SEO form had no *in vivo* effects on the viability of *Lactobacillus* spp. and *E. coli* in the ileum of the bird.

Intestinal morphology

For years, there has been a consensus that, mucosa status and their microscopic anatomy can be proper indices of the intestinal tract reaction to active substances in diets. As depicted in Table 5, villus height was significantly higher in birds consumed different levels of SEO in comparison to the birds fed the control diet. The crypt depth was also significantly higher in broilers fed 150 mg/kg SEO in comparison to those fed the control diet.

In a similar experiment, Azarbad *et al.,* (2019) illuminated that adding different levels of *Satureja* essential oil (400 and 500 mg/kg) in conventional and capsulated forms (0.5 and 1%) to the diet of broiler chickens had no effect on the width of villi, depth of crypt and length of villi to depth of crypt ratio was reported. These authors also remarked the level of 0.5 percent capsulated *Satureja* essential oil caused a significant decrease in length to width of the villi ratio. Although the reason for that is not yet fully understood, these authors attributed the lack of efficacy to factors such as inadequate or incorrect use of active plant ingredients, and specific conditions and different responses in the animals tested.

Insomuch, increased villus height is associated with improved gut health, the diets containing 150 and 300 mg/kg SEO, offer a relative privilege over the control diet in modifying the gut health status of the broilers in the present study. As was the case with performance, almost the same trend was observed, and there was a reasoning match in this regard. It's supposed that an elevated villus height is collimated by an enhanced absorptive and digestive activity of the intestine as a consequence of increased absorptive surface area, activation of brush border enzymes, nutrient transfer systems and a subsequently improved performance (Caspary 1992; Iji *et al.,* 2001; Montagne *et al.,* 2003; Maneewan & Yamauchi, 2004; Oso *et al.,* 2019).

Although encapsulation has been proposed as an impressive approach for use in targeted delivery to the gastrointestinal tract, our data failed to evidence any positive effect of this process on growth performance, intestinal morphology, and microbial population in broilers. If we want to summarize, this ineffectiveness related to factors such as inadequacy of the active plant material, incorrect utilizes of the samples, inappropriate concentration of the applied materials, specific conditions, and different responses of the treated animals and other things like that. More research is needed to prove the efficacy of this essential oil and its encapsulated form in poultry nutrition.

CONCLUSIONS

It can be concluded that dietary supplementation of SEO, especially at 150 mg/kg level, was effective in raising the populations of beneficial bacteria in the gastrointestinal tract as well as improving intestinal morphology and growth performance of broilers. These facts may have a prominent influence on the physiology and biochemistry of the intestine. However, our data failed to demonstrate any positive effect of ESEO in comparison to NSEO form on growth performance, intestinal morphology, and microbial population in broilers. Subsequent animal surveys will supply better insight into the potential of these bioactive substances to operate as prebiotics in the host's intestinal tract.

COMPLIANCE WITH ETHICAL STANDARDS

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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