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Effects of dietary lavender essential oil on growth performance, intestinal function, and antioxidant status of broiler chickens

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ABSTRACT

A total of 480 one-day-old male broiler chickens were used in a 42-d study to assess the effects of different inclusion levels of lavender essential oil (LEO) supplementation in comparison with an antibiotic growth promoter on growth performance and physiological characteristics of broiler chickens. On d 1, broiler chickens were randomly assigned into 4 experimental treatments (6 replicates of 20 broiler chickens each) including a basal diet without any additive (control, CON), the basal diet supplemented with 200 mg/kg virginiamycin (VIM), and the basal diet supplemented with either 300 (LEO_{300}) or 600 (LEO_{600}) mg/kg LEO. The results showed that experimental treatments had no significant effect on feed intake at any stage of the study. In addition, no effect of experimental treatments was observed on body weight gain (BWG) and feed conversion ratio (FCR) over the starter period (d 0–21). However, broiler chickens fed diets supplemented with LEO_{600} and VIM had greater BWG and exhibited a lower FCR during grower (d 21–42) and the entire production period (d 0–42; $P < 0.05$). Likewise, feeding LEO_{600} and VIM diets decreased *Escherichia coli* population in the ileum and cecal contents and increased villus height and villus height to crypt depth of jejunum compared with the CON diet ($P < 0.05$). Dietary supplementation of LEO increased activity of superoxide dismutase in serum and liver and glutathione peroxidase activity in serum, but reduced concentrations of malondialdehyde in the serum ($P < 0.05$). In conclusion, addition of 600 mg/kg LEO to broiler chicken diets has the potential to improve growth performance, gut microbiota balance, intestinal morphology, and antioxidant activity.

1. Introduction

In recent years, the increase of awareness of the adverse effects of antibiotic growth promoters (AGP) in animal feed has led to increased interest in producing animals without using AGP. However, by removing AGP from diets diseases problems may increase, negatively affecting growth performance (Liu et al., 2018). Hence, to prevent the undesirable effects of the removal of AGP from poultry diets, it is necessary to apply proper nutritional strategies. Medicinal plants or herbal extracts and essential oils are among the non-antibiotic feed additives commonly used for poultry production that could help in maintaining general health and growth performance (Toghyani et al., 2010; Shirani et al., 2019). The potential beneficial effects of essential oils and/or phytogenic products in poultry production have been extensively researched (Windisch et al., 2008). Mechanistically, phytogenic additives improve productivity in multiple and diverse pathways such as modifying mucosa architecture (Murugesan et al., 2015; Zeng et al., 2015a), enhancing antioxidant capacity and digestive enzymes activity (Hashemipour et al., 2013; Zeng et al., 2015a), stabilizing the enteric microbiota, as well as reducing lipid oxidation (Hashemipour et al., 2013; Shirani et al., 2019). However, results have not always been consistent and there are studies which failed to observe any positive responses of phytogenic inclusion in poultry diets on productive traits (Lee et al., 2003; Toghyani et al., 2010).

Lavender (*Lavandula angustifolia*) is a strongly aromatic shrub, belongs to the *Lamiaceae* family, which grows in various regions worldwide. Lavender essential oil (LEO) is extracted from lavender flowers, has therapeutic properties and is used as natural preservatives in the food industry (Jamroz et al., 2018). The main active compounds of LEO are linalool, linalyl acetate, lavandulyl acetate, alpha-terpineol, geranyl

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acetate, caryophyllene, and terpinen-4-ol (Fakhari et al., 2005; Evandri et al., 2005; Adaszynska and Szczerbinska, 2018). In vitro studies have investigated the antimicrobial and antifungal (Giovannini et al., 2016; de Rapper et al., 2016; Jamroz et al., 2018), antioxidant (Rashed et al., 2017; Jamroz et al., 2018), and anti-inflammatory properties of lavender (Sosa et al., 2005; Giovannini et al., 2016). A recent study has shown that addition of LEO to broiler chickens drinking water can improve growth performance and gut microbiota balance by reducing pathogenic bacteria colonization in favor of beneficial bacteria (Adaszynska-Skwirzynska and Szczerbinska, 2018). Furthermore, the administration of LEO to mice diet increased weight gain and protected the hepatocytes and renal cells against induced oxidative stress by Malathion through preventing lipid oxidation and increasing the activity of antioxidant enzymes (Selmi et al., 2015). However, not many studies have tested LEO as an AGP alternative in broiler chickens to determine the mechanism of action and pathways through which LEO may exerts its beneficial effects. Therefore, this experiment was conducted to evaluate the effects of LEO supplementation to basal diet on growth performance, intestinal microbiota and morphology, and antioxidant capacity of broilers, and accordingly estimate the potential of LEO as an alternative for AGP.

2. Materials and methods

2.1. Broiler chickens, experimental design, and diets

All the procedures used and applied in this study were approved by Animal Care and Use Committee of University of Mohaghegh Ardabili, Ardabil, Iran. A total of 480 one-day-old male broiler chickens were purchased from a commercial hatchery (Arbor Acres; Dashte Kimia Co, Isfahan, Iran). Upon arrival, broiler chickens were weighed (43.5 ± 1.5 g) and assigned into 4 experimental treatments with 6 replicate cages, each with 20 birds. Broiler chickens were raised in a windowless and environmentally controlled house with wire-floored cages (180 × 120 × 70 cm), which allowed free access to water and experimental diets. The ambient temperature was maintained at 35 °C for the first 3 d and then gradually decreased by 3 °C per week until reaching 23 °C and then kept constant until the end of the experiment. The house lighting program followed the breeder manual. The dietary treatments included a corn-soybean meal basal diet formulated based on breeder recommendation (Aviagen, 2009), with the feeding phase adopted from NRC(1994; Table 1) without any additives serving as the control (CON), LEO_{300} diet (CON + 300 mg/kg lavender essential oil), LEO_{600} diet (CON + 600 mg/kg lavender essential oil) and VIM diet (CON + 200 mg/kg virginiamycin; Virginiamycin 10%, Tolide Darouhai Dami Iran Co., Iran). All the birds were vaccinated against Newcastle disease virus at 5 and 18 d of age, and infectious bursal disease virus at 14 d of age.

2.2. Lavender essential oil preparation

In order to extract the essential oil of lavender (*Lavandula angustifolia*), aerial parts of plant were harvested during the flowering stage and were air-dried at an environmental temperature in the shade, well-ventilated room for 3 d at 30 °C and relative humidity of 40%. Dried samples were hydro-distilled for 3 h using a Clevenger type apparatus, giving essential oil in 1.0% yield. The extracted essential oil was dried with anhydrous sodium sulfate and then stored at 4 °C in glass containers until gas chromatography/mass spectrometry (GC/MS) assays. The GC/MS analysis of the compositions of the extract was conducted with Agilent 6890N gas chromatograph and according to the method described by de Rapper et al. (2016). Briefly, the prepared samples of the essential oil (with a volume of 1 μL) was injected into gas chromatography with an autosampler (24.79 psi and an inlet temperature of 250 °C) and a fused silica capillary column with length 30 m and internal diameter 0.25 mm (Chrompack, Middelburg, Netherlands).

Table 1
Ingredient and calculated nutrient composition of the basal diets.

Ingredients (%)	Starter (d 0–21)	Grower (d 21–42)
Corn	51.28	61.45
Soybean meal	39.54	30.89
Corn gluten meal	2.00	-
Sunflower oil	2.88	3.95
Limestone	0.75	0.65
Dicalcium phosphate	2.05	1.67
Salt	0.3	0.3
Sodium bicarbonate	0.06	0.08
Vitamin premix[1]	0.25	0.25
Mineral premix[2]	0.25	0.25
DL-Met	0.31	0.27
L- Lys	0.22	0.16
L-Thr	0.11	0.08
Calculated composition		
ME (Kcal/kg)	3000	3120
CP (%)	22.5	18.7
Dig Lys (%)	1.23	0.98
Dig Met (%)	0.62	0.52
Dig Met + Cys (%)	0.92	0.77
Dig Thr (%)	0.83	0.66
Ca (%)	0.92	0.77
Available P (%)	0.46	0.38
Na (%)	0.15	0.15

[1] Supplied per kilogram of diet: 1.8 mg all-trans-retinyl acetate; 0.02 mg cholecalciferol; 8.3 mg alpha-tocopheryl acetate; 2.2 mg menadione; 2 mg pyridoxine HCl; 8 mg cyanocobalamin; 10 mg nicotine amid; 0.3 mg folic acid; 20 mg D-biotin; 160 mg choline chloride.

[2] Supplied per kilogram of diet: 32 mg Mn ($MnSO_4H_2O$); 16 mg Fe ($FeSO_4\_7H_2O$); 24 mg Zn (ZnO); 2 mg Cu ($CuSO_45H_2O$); 800 μg I (KI); 200 μg Co ($CoSO_4$); 60 μg Se ($NaSeO_3$).

Helium with a column head pressure of 552 kPa was used as carrier gas and sample injected was 1 μL into GC-MS with a flow rate of 1.0 mL/min. Column temperature program was initially 60 °C for 10 min, then the temperature was increased to 220 °C at a rate of 40 °C/min and finally, the column temperature increased to 240 °C at a rate of 2 °C/min. The identification of the essential oil components was based on a comparison of their retention indices and mass spectra with the standard data.

2.3. Measurement of performance parameters and sample collection

The body weight and feed intake (FI) per pen were recorded during the starter (d 0–21) and grower (d 21–42) period and calculated for the entire production period (d 0–42). Feed conversion ratio (FCR) was calculated for the same periods and corrected for mortality body weights. At the end of the trial (d 42), blood samples were randomly collected from 12 birds per treatment from the wing vein into sterilized tubes. The samples were allowed to coagulate for 30 min at room temperature and then centrifuged for 10 min at 2,000 g for serum separation; the serum samples were stored at –20 °C until analysis. Following blood collection, each of the sampled broiler chickens was immediately euthanized by cervical dislocation, and then samples from mid-duodenum and -jejunum were taken for histological analysis. To determine bacteria population, during slaughter fresh digesta samples from mid-ileum and -cecum was also collected. In addition, about 2 g liver was removed and immediately frozen in liquid nitrogen for antioxidant capacity assays.

2.4. Analysis of microbial profile and pH value

To study microbial characteristics, one gram of the collected sample from the ileum and cecum was diluted with 9 mL of sterile peptone water and mixed for 1 min on a vortex stirrer. Samples were serially blended from 10^{-1} to 10^{-6}, and were injected by 100 μL in 3 selective

agar media as follows; MRS agar for lactic acid bacteria (LAB), MacConkey agar for *Escherichia coli* (*E. coli*), violet red bile agar for coliform bacteria, and plate count agar for total anaerobic bacteria (TAB). Plates were then incubated aerobically at 37 ˚C for 24 h (MacConkey agar and plate count agar) or anaerobically at 37 ˚C for 24–48 h (violet red bile agar and MRS agar). The bacterial colonies were counted using a colony counter and finally, the results were reported as colony-forming units per gram of sample (log_{10} cfu/g). The pH value was measured using a digital pH meter (NWKbinar pH, K-21, Landsberg, Germany), according to the method of Jazi et al. (2017).

2.5. Assay of morphology of intestinal mucosa

The gut morphological examination was performed as previously described by Jazi et al. (2018a). Briefly, a 1 cm intestinal segment from the medial of the duodenum and jejunum were taken and placed in 10% buffered formaldehyde for 48 h. After dehydration, intestinal samples were embedded in paraffin wax. Then, tissue samples were sectioned (5 μm) using a microtome and stained with hematoxylin and eosin, and finally examined under a light microscope. Villus length (VL) was determined from the tip of the villus to the junction of villus and crypt, and villus width (VW) was defined as the midpoint between the top and bottom of villi. The crypt depth (CD) was measured from the base upwards to the region of transition between the crypt and villus, and the VL:CD ratio was also calculated. For statistical analysis, a minimum of 10 villi and associated crypts were randomly chosen and used.

2.6. Measurement of of blood metabolites

The serum concentrations of cholesterol, triglycerides, high-density lipoproteins cholesterol (HDL-C),total protein (TP), and glucose (GLU) were determined by spectrophotometric methods using a spectrophotometer following the instructions of the kit's manufacturer (Pars Azmoon Kits; Pars Azmoon, Tehran, Iran). Very low-density lipoprotein cholesterol (VLDL-C) values were calculated by dividing triglyceride values to unit 5 and low-density lipoprotein cholesterol (LDL-C) values subtracting total values of HDL-C and VLDL-C from total cholesterol (Jazi et al., 2018a).

2.7. Assay of antioxidant enzymes in serum and liver

The liver tissue samples collected on day 42, were first homogenized on ice-cold 0.86% physiological saline and then centrifuged at 4,000 × g for 15 min at 4 ˚C. The supernatant of the liver homogenization solution and already prepared serum samples were used to determine the superoxide dismutase activity (SOD), glutathione peroxidase activity (GSH-Px), and malondialdehyde concentration (MDA) using corresponding diagnostic kits (ZellBio GmbH kit, Germany), according to the manufacturer's instructions. The activity of SOD was measured by the xanthine oxidase method, which monitors the inhibition of the reduction of nitro blue tetrazolium by the sample. The activity of GSH-Px was detected with 5,5′-dithiobis-p-nitrobenzoic acid, and the change of absorbance at 412 nm was monitored using a spectrophotometer. The MDA content was assayed with 2-TBA, and the change of absorbance at 532 nm was monitored by a spectrophotometer.

2.8. Statistical analysis

All the data obtained were checked for normality and then subjected to analysis of variance procedure appropriate for a completely randomized design using the GLM procedures of SAS statistical software (SAS, 2010). Differences among groups were examined by Tukey's multiple range test. Significance (*P*-value) was evaluated at 0.05. Data were presented as means and their pooled standard errors.

Table 2
The components of lavender essential oil (analyzed values).

Compound	Percentage (%)	Retention index
α-Pinene	0.40	935
Camphene	0.85	942
β-Pinene	0.70	975
Myrcene	1.10	982
α-Phellanderene	0.14	1001
3-Carene	0.37	1010
p-Cymene	1.06	1020
1,8-Cineole	1.45	1031
Cis-β-ocimene	0.61	1037
Trans-β-ocimene	0.48	1045
Linalool oxide	0.52	1071
Linalool	38.12	1098
Camphor	1.02	1131
Borneol	0.93	1165
Terpinen-4-ol	1.20	1177
α-Terpineol	4.20	1195
Linalyl acetate	25.79	1254
Bornyl acetate	0.10	1279
Lavandulol acetate	10.63	1284
Carvacrol	2.10	1300
Geranyl acetate	1.87	1365
β-Caryophyllene	3.75	1421
β-Farnesene	0.20	1448
Germcrene-D	0.10	1485
Oxygenated monoterpenes	88.07	–
Sesquiterpene hydrocarbones	5.67	–
Monoterpene hydrocarbones	3.95	–

3. Results

3.1. Chemical composition of lavender essential oil

Chemical compositions identified in the LEO are listed in Table 2. Twenty-four constituents were identified in the essential oil representing 97.7% of the total oil constituents. The most abundant chemical categories for LEO are oxygenated monoterpenes (88.07%), followed by sesquiterpene hydrocarbons (5.67%) and monoterpene hydrocarbons (3.95%). The predominant compounds of the LEO are linalool (38.12%), linalyl acetate (25.79%), lavandulyl acetate (10.63%), α-terpineol (4.20%), and β-caryophyllene (3.75%).

3.2. Growth performance

The results of the growth performance of broiler chickens in response to the dietary treatments are summarized in Table 3. During the 0-21 d period, body weight gain (BWG) and FCR were not influenced by dietary treatments. However, in the 21-42 d period, broiler chickens fed diets supplemented by LEO_{600} and VIM had higher BWG and lower FCR compared to the LEO_{300} and CON diets ($P < 0.05$). Likewise, similar results were observed for the 0–42 d period ($P < 0.05$). Feed consumption was not affected by the dietary treatments at any stage of the study.

3.3. Microbial count and pH

Table 4 depicts the effects of dietary treatments on ileal and cecal microbiota profile and pH recorded in broiler chickens on d 42. Feeding broiler chickens with diets containing 600 mg/kg LEO and VIM decreased *E. coli* counts in both ileum and cecal compared to the CON treatment ($P < 0.05$). Likewise, the population of coliforms in ileum section decreased in birds fed the VIM and LEO_{600} diets ($P < 0.05$). Furthermore, LAB counts were higher in cecal of broiler chickens fed diets supplemented with 600 mg/kg LEO than the other treatments ($P < 0.05$). However, no significant difference was observed for the pH value and TAB count among treatment groups in the ileum and cecal.

Table 3
Influence of dietary treatments on the growth performance of broiler chickens.[1]

Item[3]	Dietary treatment[2]				Pooled SEM	*P*-value
	CON	VIM	LEO_{300}	LEO_{600}		
Starter (d 0–21)						
BWG, g	725	753	739	760	13.70	0.320
FI, g	1089	1106	1118	1120	14.91	0.395
FCR	1.50	1.47	1.51	1.48	0.02	0.566
Grower (d 21–42)						
BWG, g	1628[b]	1753[a]	1674[b]	1763[a]	15.84	0.001
FI, g	3075	3028	3105	3014	18.85	0.128
FCR	1.89[a]	1.73[b]	1.85[a]	1.71[b]	0.01	0.001
Overall (d 0–42)						
BWG, g	2352[b]	2506[a]	2413[b]	2524[a]	26.73	0.004
FI, g	4163	4133	4224	4134	30.62	0.379
FCR	1.77[a]	1.65[b]	1.75[a]	1.64[b]	0.01	0.001

[a-b] Means with different superscripts in each row are significantly different ($P < 0.05$).
[1] Value are the means of 6 replicate pens of 20 chicks.
[2] CON, control (basal diet); VIM, basal diet + 20 mg/kg of virginiamycin; LEO_{300}, basal diet + 300 mg/kg of lavender essential oil; LEO_{600}, basal diet + 600 mg/kg of lavender essential oil.
[3] BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio.

Table 4
Influence of dietary treatments on the microflora composition and pH of ileum and cecum.[1]

Item[3]	Dietary treatment[2]				Pooled SEM	*P*-value
	CON	VIM	LEO_{300}	LEO_{600}		
Ileum						
TAB, log_{10} cfu/g	6.72	6.47	6.85	6.64	0.12	0.321
LAB, log_{10} cfu/g	6.68	6.57	6.80	7.01	0.15	0.250
E. coli, log_{10} cfu/g	5.45[a]	4.83[b]	5.26[a]	4.98[b]	0.11	0.005
Coliforms, log_{10} cfu/g	4.20[a]	3.52[b]	3.97[a]	3.61[b]	0.13	0.002
pH	6.30	6.35	6.30	6.23	0.05	0.450
Cecum						
TAB, log_{10} cfu/g	6.90	6.76	7.05	6.85	0.11	0.296
LAB, log_{10} cfu/g	7.54[b]	7.48[b]	7.71[b]	8.15[a]	0.13	0.001
E. coli, log_{10} cfu/g	4.89[a]	4.18[c]	4.68[ab]	4.40[b]	0.08	0.015
Coliforms, log_{10} cfu/g	4.11	3.84	4.04	3.87	0.12	0.364
pH	6.08	6.12	5.98	5.92	0.10	0.262

[a-c] Means with different superscripts in each row are significantly different ($P < 0.05$).
[1] Data represent means of 2 broiler chickens per pen.
[2] CON, control (basal diet); VIM, basal diet + 20 mg/kg of virginiamycin; LEO_{300}, basal diet + 300 mg/kg of lavender essential oil; LEO_{600}, basal diet + 600 mg/kg of lavender essential oil.
[3] TAB, total anaerobic bacteria; LAB, lactic acid bacteria.

3.4. Intestinal morphology

Table 5 shows the histomorphometric changes in the duodenum and jejunum of broiler chickens fed with dietary supplements. Dietary supplementation of VIM and LEO_{600} significantly increased the VL and VL:CD in the jejunum as compared to the CON group ($P < 0.05$). Furthermore, supplementing diets with VIM and both levels of LEO lowered the jejunum CD ($P < 0.05$). Dietary supplements had no effect on VW in the duodenum and jejunum and VL, CD, and VL:CD in the duodenum.

3.5. Serum biochemical parameters

The blood serum metabolites of broiler chicks in response to the dietary treatments on d 42 are shown in Table 6. Feeding broiler chickens with diets containing 600 mg/kg LEO decreased serum concentrations of cholesterol and LDL-C compared to other treatments

Table 5
Influence of dietary treatments on morphological parameters of duodenum and jejunum.[1]

Item[3]	Dietary treatment[2]				Pooled SEM	*P*-value
	CON	VIM	LEO_{300}	LEO_{600}		
Duodenum						
VL, μm	982	1018	1002	1015	11.94	0.142
VW, μm	140	143	142	144	2.39	0.726
CD, μm	130	131	128	130	2.32	0.831
VL:CD	7.56	7.75	7.82	7.80	0.09	0.214
Jejunum						
VL, μm	707[b]	780[a]	731[ab]	769[a]	16.57	0.010
VW, μm	125	122	126	125	1.74	0.458
CD, μm	105[a]	96[b]	102[a]	98[ab]	2.14	0.034
VL:CD	6.72[b]	8.23[a]	7.15[b]	7.86[a]	0.18	0.001

[a-b] Means with different superscripts in each row are significantly different ($P < 0.05$).
[1] Data represent means of 2 broiler chickens per pen.
[2] CON, control (basal diet); VIM, basal diet + 20 mg/kg of virginiamycin; LEO_{300}, basal diet + 300 mg/kg of lavender essential oil; LEO_{600}, basal diet + 600 mg/kg of lavender essential oil.
[3] VL, villus length; VW, villus width; CD, crypt depth.

Table 6
Influence of dietary treatments on the serum biochemical values.[1]

Item[3]	Dietary treatment[2]				Pooled SEM	*P*-value
	CON	VIM	LEO_{300}	LEO_{600}		
Cholesterol, mg/dL	147.8[a]	156.4[a]	141.3[a]	122.0[b]	6.06	0.008
Triglycerides, mg/dL	90.5	93.8	89.3	83.1	4.97	0.641
HDL-C, mg/dL	73.3	76.4	78.5	79.1	3.13	0.564
VLDL-C, mg/dL	18.1	18.8	17.8	16.6	1.19	0.641
LDL-C, mg/dL	56.4[a]	61.2[a]	49.9[a]	26.3[b]	4.76	<.001
GLU, mg/dL	213.2	220.1	210.1	207.2	5.35	0.365
TP, g/dL	3.80	3.75	3.98	3.93	0.16	0.731

[a-b] Means with different superscripts in each row are significantly different ($P < 0.05$).
[1] Data represent means of 2 broiler chickens per pen.
[2] CON, control (basal diet); VIM, basal diet + 20 mg/kg of virginiamycin; LEO_{300}, basal diet + 300 mg/kg of lavender essential oil; LEO_{600}, basal diet + 600 mg/kg of lavender essential oil.
[3] HDL-C, high-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; GLU, glucose; TP, total protein.

Table 7
Influence of dietary treatments on the antioxidant capacity of serum and liver.[1]

Item[3]	Dietary treatment[2]				Pooled SEM	*P*-value
	CON	VIM	LEO_{300}	LEO_{600}		
Serum						
SOD, U/mL prot	126[b]	129[b]	145[a]	153[a]	5.24	0.001
GSH-Px, U/mL prot	181[c]	185[bc]	201[ab]	212[a]	6.12	0.003
MDA, nmol/mL prot	7.60[a]	7.78[a]	7.03[b]	6.83[b]	0.14	<.001
Liver						
SOD, U/mg prot	295[b]	303[b]	329[a]	337[a]	5.96	<.001
GSH-Px, U/mg prot	3.26	3.19	3.34	3.32	0.08	0.603
MDA, nmol/mg prot	4.05	4.12	3.98	3.95	0.17	0.133

[a-c] Means with different superscripts in each row are significantly different ($P < 0.05$).
[1] Data represent means of 2 broiler chickens per pen.
[2] CON, control (basal diet); VIM, basal diet + 20 mg/kg of virginiamycin; LEO_{300}, basal diet + 300 mg/kg of lavender essential oil; LEO_{600}, basal diet + 600 mg/kg of lavender essential oil.
[3] SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde.

($P < 0.05$). The triglycerides, VLDL-C, HDL-C, GLU, and TP level in serum was not affected by dietary treatments.

3.6. Antioxidant capacity in serum and liver

As indicated in Table 7, compared to the VIM and CON treatments, supplementing diets with LEO (300 and 600 mg/kg of diet) increased the serum SOD and GSH-Px activities and decreased serum MDA content ($P < 0.05$). Additionally, broiler chickens fed diets supplemented with both levels of LEO showed higher SOD activity in the liver than the other treatments ($P < 0.05$). However, dietary treatments had no effect on GSH-Px activity and MDA content in the liver.

4. Discussion

In the current investigation, linalool, linalyl acetate, and lavandulyl acetate were the main compounds of LEO. Similarly, Fakhari et al. (2005) reported that the main compounds of the lavender cultivated in Iran include linalool (35.3%), linalyl acetate (13.4%), and lavandulyl acetate (10.9%). The LEO harvested in India, linalyl acetate (47.6%), linalool (28.1%) and lavandulyl acetate (4.3%) were reported as principal compounds (Verma et al., 2010). Also, in another study, linalool (22.1%), lavandulyl acetate (15.3%) and linalyl acetate (14.7%) were reported as main compounds found in lavender cultivated in Turkey (Kirimer et al., 2017). In general, according to literature, LEO consists mainly of oxygenated monoterpenes such as linalool, which its antibacterial and antioxidant properties have been the focus of a large number of studies (Giovannini et al., 2016; Nicsik et al., 2017; Rashed et al., 2017; Jamroz et al., 2018).

Here, we evaluated the effects of LEO supplementation on growth performance, gut function, and antioxidant capacity, as well as its possibility to serve as an alternative to AGP in broiler chickens. According to the current data, dietary supplementation of LEO at a dosage of 600 mg/kg increased BWG and improved FCR of birds, indicating the efficient utilization of feed. These findings are in agreement with the study of Adaszynska-Skwirzynska and Szczerbinska (2018), who reported that BWG and FCR were significantly improved by the addition of LEO in broiler chickens' drinking water. Interestingly, in the present experiment growth performance of broiler chickens fed the diets containing 600 mg/kg LEO was similar to those broilers fed the diets supplemented with VIM. In a trial reviewed by Murugesan et al. (2015), a significant improvement in performance parameters was reported when the broiler chickens were fed a diet containing a combination of essential oils, compared with birds fed a control diet, but were similar to the AGP birds. According to these authors, essential oils could increase the growth performance of broiler chickens by exerting beneficial actions within the digestive tract and thereby increasing nutrients availability (Murugesan et al., 2015). However, several studies did not find a positive effect on production traits in broiler chickens (Lee et al., 2003; Toghyani et al., 2010). This inconsistency may be related to differences in the composition and inclusion level of the phytogenic additives used, animal age, hygiene, the approaches of administration, diet composition and environmental factors (Zeng et al., 2015b).

In recent years, the manipulation of the gastrointestinal microbiota balance in favor of beneficial and more desirable bacteria through the inclusion of feed additives such as probiotics, prebiotics, and phytogenic products has attracted the attention of many researchers (Jazi et al., 2018a,b; Shirani et al., 2019). Various in vitro studies have shown that the major components of LEO such as linalool and linalyl acetate possess potent antimicrobial activity against gram-negative bacteria such as *E. coli* and *Salmonella* typhimurium, and *Campylobacter* spp. (Sokovic et al., 2010; Duarte et al., 2016; de Rapper et al., 2016). In the present study, dietary supplementation of LEO at a dosage of 600 mg/kg increased the cecal LAB counts and reduced coliform and *E. coli* in the ileum and cecal contents. Similar to this finding, Adaszynska and Szczerbinsk (2018) showed that the LEO supplementation stabilizes the gut microbiota and thereby reduces the colonization of coliforms and *E. coli* in the ileum. Several in vivo studies indicated that essential oils increased the *Lactobacillus* ssp. and decreased coliforms and *E. coli* in broiler chickens (Cetin et al., 2016; Liu et al., 2017; Giannenas et al., 2018). The antimicrobial activity of essential oils is related to their hydrophobicity, which disrupts cell membranes permeability and cell homeostasis with the consequence of loss of cellular materials and leakage of other components, or even cell death (Zhai et al., 2018). On the other hand, it has been reported that LAB plays an important role in stabilizing the gut ecosystem of animals by competing with pathogenic microorganisms and promoting the population of beneficial bacteria in the gut (Jazi et al., 2017 and 2018b).

The integrity of the gut barrier plays an essential role in maintaining the normal functions of the intestine. Changes in the intestinal mucosal structure such as an increase in the VL and VL:CD can indicate an improvement in intestinal health and an increase in surface area for nutrient absorption. In addition, previous studies suggest that the increase in VL results in enhanced digestive enzymes activities and hence greater nutrient digestibility (Jazi et al., 2018a, b). On the other hand, the crypts are considered as origin areas for the production of villus because they contain stem cells. Deeper crypt indicates fast epithelial cell turnover and the demand for energy and protein sources for new tissue. Therefore, additional cellular turnover increases the nutrients need for maintenance, which is associated with reduced animal efficiency (Xu et al., 2003). This may partly explain the superior FCR in groups receiving a higher level of LEO in the present study. Supplementation of LEO_{600} and VIM in the diets had positive effects on the VL, CD, and VL:CD in the jejunum, suggesting the reduced endogenous secretion and enhanced ability of digestion and absorption of available nutrients. The current results are in agreement with those reported by Murugesan et al. (2015) who showed that diet supplementation with phytogenic feed additives improved intestinal mucosa morphology. The close proximity of the mucosal surface and the intestinal contents can motive oxidative stress caused by digestive process (Windisch et al., 2008). Phytogenic products can positively affect the antioxidant enzyme activity such as SOD which in return reduces the production of reactive oxygen species, known as inflammatory factors on tissues and cells, causing intestinal atrophy and intestinal epithelial barrier disturbance (Moretti et al., 2018). Therefore, the antioxidant function of LEO in the gut might lead to improving the intestinal morphology and health. In addition, the enhanced count of LAB in the intestine would produce more useful metabolites, which may be relative to the improvement of the proliferation of epithelial cells and intestinal morphology in the LEO_{600} group (Jazi et al., 2017). The improved intestinal mucosa morphology in response to dietary LEO_{600} and VIM observed in the current study can further support the superior performance of broiler chickens in these groups.

Serum biochemical indicators can display the nutrient's metabolism and body physiological state (Toghyani et al., 2012). Our data showed that dietary supplementation of LEO at 600 mg/kg level improved the serum lipid profile by reducing cholesterol and LDL-C concentration in serum compared to other dietary treatments. The active substances in LEO such as linalool have been reported to possess cholesterol-lowering effects (Eissa et al., 2017). Investigating the hypercholesterolemia properties of linalool, Cho et al. (2011) have shown that oral administrating of linalool to mice lowers 3-hydroxy-3-methyl glutaryl-CoA reductase protein expression (as a marker for hepatic cholesterol synthesis; HMG-CoA) leading to decreased total cholesterol and LDL-C concentrations. Similarly, other research studies with broilers and laying hens concerning the hypercholesterolemia properties of herbal essential oils have suggested similar mechanisms such as decreased activity of HMG-CoA reductase and cholesterol-7 hydroxylase fatty acid synthase, and inhibiting the activity of the pentose phosphate pathway by 6-amino nicotinamide (Chowdhury et al., 2018; Torki et al., 2018). On the other hand, an increase in the number of LAB in the intestine could explain the

lower concentration of cholesterol in broiler chicks fed the diet supplemented with 600 mg LEO/kg. One of the proposed mechanisms of lipid alteration due to increased LAB in the gut includes inhibition of HMG-CoA reductase activity in the liver (Jazi et al., 2018a).

The SOD and GSH-Px scavenge free oxygen radicals and are the most potent enzymes against reactive oxygen species (ROS) products. These enzymes can clear the superoxide and lipid hydroperoxide, reducing the damage of organic hydrogen peroxide to the body (Shirani et al., 2019). Malondialdehyde is one of the most known secondary products of lipid peroxidation, and it can be used as a marker of cell membrane injury. Several in vitro studies have shown that LEO and its main bioactive compound, linalool, has potent antioxidant activities. For example, Gulcin et al. (2004) reported LEO had a powerful antioxidant activity comparable to standard antioxidants such as a-tocopherol, and butylated hydroxytoluene. These authors attributed the antioxidant activity of lavender to its strong hydrogen donating and metal chelating ability, and concluded that the phenolic compounds are responsible for these antioxidant activities. Jabir et al. (2018) indicated that linalool exhibits antioxidant activity comparable to ascorbic acid, by donating hydrogen atoms and removing electrons from DPPH and thus can be considered as a natural antioxidant source. In the present study, dietary supplementation of LEO at both levels increased the activities of SOD and GSH-Px and decreased the content of MDA in the serum. Other researchers also showed that dietary LEO administration improved SOD and GSH-Px activities and reduced MDA contents in the kidney and liver of rats (Hancianu et al., 2013; Selmi et al., 2015). Accordingly, our results suggest that LEO has the potential to protect broiler chickens against damage from ROS and free radicals.

5. Conclusion

In summary, the findings obtained in this study suggest that supplementing broiler chicken diets with 600 mg LEO/kg effectively improves growth performance mainly through ameliorating gut microbiota balance, intestinal structure, and antioxidant capacity. In addition, dietary supplementation of LEO_{600} lowered blood cholesterol of broiler chickens. Therefore, the inclusion of 600 mg LEO/kg might provide a good alternative to promote broiler chicken production comparable to an AGP source.

CRediT authorship contribution statement

S. Yarmohammadi Barbarestani: Methodology, Software, Formal analysis, Data curation, Investigation. **V. Jazi:** Conceptualization, Writing - original draft. **H. Mohebodini:** Conceptualization, Methodology, Investigation, Supervision, Project administration, Funding acquisition. **A. Ashayerizadeh:** Resources, Project administration. **A. Shabani:** Resources, Investigation. **M. Toghyani:** Conceptualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.livsci.2020.103958.

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