

Productive parameters, cecal microflora, nutrient digestibility, antioxidant status, and thigh muscle fatty acid profile in broiler chickens fed with *Eucalyptus globulus* essential oil

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ABSTRACT This study was conducted to evaluate the effects of different dietary inclusion of eucalyptus essential oil (EEO) on growth performance, relative organ weight, cecal microflora, nutrient digestibility, serum biochemical parameters, and thigh muscle fatty acid profile in broiler chickens. A total of six hundred 1-day-old male broiler chickens were randomly allocated into 5 treatment groups with 8 replicate pens, and each pen contained 15 birds. The experiment lasted for 42 d. Dietary treatments included corn–soybean meal-based diet supplemented with 0, 250, 500, 750, and 1,000 mg/kg EEO. The results indicated that dietary treatments had no effect on growth performance parameters in the 1 to 10 d period. From day 11 to 24, dietary supplementation of EEO showed a linear decrease in feed conversion ratio (FCR, $P < 0.05$). From day 25 to 42 and the overall period (1–42 d), broilers fed with different levels of EEO showed a linear increase in body weight gain (BWG) and reduction in feed conversion ratio (linear, $P < 0.05$). The relative organ weight were unaffected by any of the dietary treatments. With increasing levels of EEO, the

cecal *Escherichia coli* (linear, $P = 0.085$) count showed a trend in reduction, and the cecal lactic acid bacteria population tended to increase (linear, $P = 0.063$). The apparent ileal digestibility of ether extract and organic matter were linearly and quadratically increased in response to increasing dietary EEO supplementation ($P < 0.05$). A trend of linear decrease in total cholesterol in the serum of birds fed with different levels of EEO was recorded ($P = 0.074$). Eucalyptus essential oil's inclusion increased serum superoxide dismutase linearly but reduced serum malondialdehyde linearly ($P < 0.05$). Dietary supplementation of EEO affected the fatty acid profile of thigh muscle so that increased the concentrations of total polyunsaturated fatty acids (linear, $P < 0.05$) and reduced total saturated fatty acid contents (linear, $P < 0.05$). Taken together, the inclusion of EEO increased BWG and decreased FCR during day 25 to 42 and day 1 to 42, and partially improved cecal microflora balance, nutrient digestibility, antioxidant activity, and thigh muscle fatty acid profile in broiler chickens.

Key words: antioxidant status, broiler chickens, eucalyptus, fatty acid profile, medicinal herb

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INTRODUCTION

Over the years, the use of prophylactic drugs and antibiotic growth promoters in animal feeding has become commonplace. However, the continuous use of these

compounds resulted in outcomes such as the emergence of resistant bacteria and antibiotic residues in meat and other livestock products which pose a risk to public health and the environment (Jazi et al., 2018a). This concern has led many countries to restrict the use of antibiotics in animal feed. Accordingly, it is necessary to identify cost-effective, safe, and eco-friendly alternatives to antibiotics.

Among feed additives, the use of medicinal herb plants or phytochemical products (such as essential oils) in livestock nutrition has recently received much greater attention, owing to their important biological activities

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including antioxidant, antimicrobial, and anti-inflammatory (Windisch et al., 2008; Brenes and Roura, 2010). These features have been attributed to bioactive compounds (i.e., hydrocarbons, phenols, esters, alcohols, acids, and steroids) present in phytogetic products, which have positive effects on animal production and health (Shirani et al., 2019). Other characteristics of phytogetic feed additives include an increase in the production of digestive secretions such as endogenous digestive enzymes, saliva, bile, and mucin (Brenes and Roura, 2010; Sharma et al., 2020). Therefore, phytogetic feed additives can influence animal productive and physiological responses, both quantitatively and qualitatively.

Previous studies have demonstrated the positive effects of phytogetic feed additives such as essential oils on growth performance and digestive functions of broiler chickens (Jamroz et al., 2003; Murugesan et al., 2015; Barbarestani et al., 2020). In addition to improving immune response, such additives also enhance antioxidant capacity and meat quality in broiler chickens (Hashemipour et al., 2013; Chowdhury et al., 2018a; Shirani et al., 2019). However, some studies on the use of phytogetic feed additives in poultry nutrition have indicated inconsistent results on productive animal performance, as reported by Jang et al. (2007) and Chamorro et al. (2013).

Eucalyptus (*Eucalyptus globulus*) is one of the most popular medicinal herbs in traditional medicine. It has been used to treat loss of appetite, infections, colds, influenza, bronchitis, sore throats, and pneumonia in humans (Dhakad et al., 2018). The main constituent found in eucalyptus essential oil (EEO) is 1,8-cineole (eucalyptol). Besides 1,8-cineole, EEO contains α -pinene, α -phellandrene, γ -terpinene, α -terpineol, cymene, limonene, and spathulenol (Song et al., 2009; Damjanovic-Vratnica et al., 2011; Luis et al., 2016; Dhakad et al., 2018). Previous reports have indicated the pharmacological and health benefits of EEO including antimicrobial, antiviral, antifungal, anti-inflammatory, and antioxidant activities (Boulekbache-Makhlouf et al., 2013; Luis et al., 2016; Mekonnen et al., 2016; Said et al., 2016; Dhakad et al., 2018). Also, Fathi et al. (2019), in a feeding trial, showed an improvement in intestinal microbial balance and immune response in rabbits with eucalyptus supplementation. Chen et al. (2018) reported that feeding laying hens with a diet supplemented with eucalyptus improved productive traits and meat quality and protected the liver against induced oxidative stress by ethanol through enhancing the antioxidant enzyme activity. However, there is limited information about the effects of eucalyptus supplementation, especially its essential oil in broiler chickens.

Hence, considering the pharmaceutical properties (antioxidant and antibacterial) of EEO reported in vitro and in vivo, the current study was designed to evaluate the effects of EEO supplementation on productive and physiological responses in broiler chickens. The physiological responses tested include cecal microflora, blood metabolites, apparent ileal digestibility (AID) of nutrients, antioxidant status, and thigh muscle fatty acid profile.

MATERIAL AND METHODS

All experimental procedures were approved by the Animal Care and Use Committee of the University of Mohaghegh Ardabili, Ardabil, Iran.

Birds and Housing

A total of 600 1-day-old male chicks (Ross 308, Aviagen Inc., Huntsville, AL) were purchased from a commercial hatchery. On arrival, the chicks were weighed and randomly assigned to 40 floor pens. Each pen measured 1.35 m \times 1.35 m and was equipped with a tube feeder and 2 cup drinkers with fresh wood shavings as bedding material. The ambient temperature was maintained at 33°C at the arrival of chicks for the initial 3 d and then reduced to 2.5°C per week until a temperature of 23°C was achieved. The lighting program and ventilation followed the recommendations of the Ross 308 breed management manual Aviagen (2019). Birds had ad libitum access to water and feed throughout the entire experiment.

Experimental Treatments and Design

The birds were randomly assigned into 5 dietary treatments, each replicated 8 times, with 15 chicks per replicate. The basal diets were formulated to meet or exceed Aviagen (2019) recommendations, as is shown in Table 1. Before formulating the diets, the main feed ingredients (i.e., corn, soybean meal, and corn gluten meal) were analyzed for CP (method 955.04), ether extract (EE, method 920.39), crude fiber (method 962.09), and ash (method 942.05) according to the standard procedures of AOAC International (2000). The dietary treatments consisted of a corn-soybean meal-based basal diet without any supplemental (as control, CON) and basal diet with different EEO concentrations, including 250, 500, 750, and 1,000 mg/kg. To prepare dietary treatments, small amounts of the basal diet were mixed with the respective amount of each essential oil in small liquid batches and later on with a larger amount of basal diet until the total amount of the respective diet yielded a homogeneous mixture. The broiler chicks received the experimental diets in 3 phases from day 1 to 10 (starter), day 11 to 24 (grower), and day 25 to 42 (finisher). From day 35 to 42, chromic oxide (5 g/kg) was added to all diets as an indigestible marker to allow for the determination of AID of nutrients.

Eucalyptus Essential Oil Preparation

To extract the essential oil of eucalyptus, fresh leaves of *Eucalyptus globulus*, *Myrtaceae* family, were collected from plants grown in the Kivi City, Ardabil province, Iran. The fresh plant leaves were air-dried at ambient temperature in a dark, well-ventilated room for 3 d at 28°C and relative humidity of 40%. Dried samples were hydrodistilled for 3 h using a Clevenger-type apparatus to produce the essential oil. The compositions of

Table 1. Ingredients and nutrient levels of the basal diets on as-is basis.

Ingredients, %	Starter phase (day 1–10)	Grower phase (day 11–24)	Finisher phase (day 25–42)
Corn	51.92	56.11	59.28
Soybean meal (CP, 44%)	38.50	36.35	32.68
Corn gluten meal	2.86	-	-
Sunflower oil	2.27	3.50	4.33
Limestone	0.80	0.71	0.65
Dicalcium phosphate	2.14	1.92	1.71
Sodium chloride	0.30	0.30	0.30
Sodium bicarbonate	0.08	0.09	0.10
Vitamin premix ¹	0.25	0.25	0.25
Mineral premix ²	0.25	0.25	0.25
DL-methionine	0.31	0.30	0.27
L-lysine HCL	0.24	0.15	0.12
L-threonine	0.08	0.07	0.06
Total	100	100	100
Calculated composition			
AME, kcal/kg	2,950	3,050	3,150
Crude protein, %	23.04	20.56	19.14
Dig Lys, %	1.24	1.10	1.00
Dig Met, %	0.62	0.57	0.52
Dig Met + Cys, %	0.92	0.84	0.78
Dig Thr, %	0.81	0.73	0.67
Ca, %	0.96	0.87	0.79
Available P, %	0.48	0.435	0.395
Ca: available P ratio	2.00	2.00	2.00
Na, %	0.16	0.16	0.16
Cl, %	0.23	0.23	0.23

¹Vitamin premix provided the following per kilogram of diet: vitamin A, 11,500 IU; cholecalciferol, 2,100 IU; vitamin E, 22 IU; vitamin K3, 1.50 mg; thiamine, 3 mg; riboflavin, 4.4 mg; pantothenic acid, 25 mg; niacin, 40 mg; choline chloride, 560 mg; biotin, 0.1 mg; folic acid, 0.8 mg; pyridoxine 10 mg; vitamin B12, 0.060 mg.

²Trace mineral premix provided the following in milligrams per kilogram of diet: iron, 50 mg; zinc, 55 mg; manganese, 75 mg; iodine, 1.8 mg; copper, 8 mg; selenium, 0.3 mg; cobalt, 0.2 mg.

plant essential oil were analyzed by gas chromatography/mass spectrometry according to the method described by [Said et al. \(2016\)](#) at the Research Center of University of Mohaghegh Ardabili. The contents of main constituents of EEO were as follows: 1.8-cineole (67.85%), α -pinene (12.69%), α -terpineol (5.37%), α -phellandrene (4.15%), cymene (2.94%), and limonene (1.60%).

Performance Data

Body weight and feed intake (**FI**) of broiler chickens were recorded weekly, and then body weight gain (**BWG**), FI, and feed conversion ratio (**FCR**) were calculated at the end of the starter, grower, and finisher periods and overall experiment. Mortality was recorded daily in each group, and FCR was corrected for mortality.

Sample Collection

At the end of the experiment (day 42), 3 birds from each replicate were randomly selected and sampled. To evaluate serum indices, 5 mL blood samples were obtained from the wing vein, and then serum from each sample was collected by centrifugation at $2,000 \times g$ for 10 min at 4°C. Next, serum samples were frozen at -20°C in Eppendorf tubes for further analysis. After blood sampling, the aforementioned 3 birds were individually weighed and then killed by cervical dislocation to measure the weight of the carcass, breast, thighs,

abdominal fat, heart, liver, spleen, pancreas, and bursa of Fabricius. The relative weight of the organs was expressed as a percentage of body weight. After that, digesta samples of the ileum and ceca were collected in sterile tubes to determine the apparent digestibility of nutrients and bacterial enumeration. In addition, the thigh muscle of each bird was removed, vacuum packed, and stored at -20°C for the determination of fatty acid composition.

Serum Metabolites

The values of triglyceride, total cholesterol, high-density lipoproteins, total protein, and glucose in the serum were analyzed by an automatic biochemical analyzer (Clima, Ral. Co, Barcelona, Spain) and using specific commercial kits (Pars Azmoon Company, Tehran, Iran), according to the manufacturer's instructions. Also, the activities of superoxide dismutase (**SOD**) and glutathione peroxidase (**GSH-Px**), and malondialdehyde (**MDA**) content in serum samples were determined using commercial assay kits (Cayman Chemical Company., Ann Arbor, MI) according to the manufacturer's standard procedures.

Cecal Microbial Population

The enumeration of bacterial target groups in the cecal was performed via bacteria culture, as described in our previous studies ([Jazi et al., 2018a,b](#)). For this purpose, 1 g of cecal digesta contents was diluted

serially in 0.9% sterile saline solution. Afterward, 100 μL of each dilution was plated onto MRS agar, tryptose sulfite cycloserine agar, eosin methylene blue agar, and MacConkey agar to enumerate the lactic acid bacteria (**LAB**), *Clostridium* spp., *Escherichia coli*, and coliforms, respectively. Tryptose sulfite cycloserine agar and MRS agar plates were incubated anaerobically for 24 to 48 h at 37°C. The MacConkey agar and eosin methylene blue agar plates were incubated aerobically for 24 h at 37°C. After the incubation period, the bacterial colonies were counted in a colony counter, and the results were expressed as \log_{10} colony-forming units per gram of sample.

Apparent Ileal Nutrient Digestibility

To determine the AID of nutrients, the digesta were collected from the lower half of the ileum, as described by Ravindran et al. (2005). The ileum was considered as the portion of the small intestine from Meckel's diverticulum to a point about ~ 40 mm proximal to the ileocecal junction. The ileal digesta of all birds within each pen was pooled, immediately frozen (-20°C), and stored until further analysis (dry matter [DM], CP, EE, and organic matter [OM]). At the time of analysis, ileal digesta collected were dried at 65°C for at least 24 h prior to being ground in a coffee grinder. After that, the amount of DM (method 934.01), CP (method 955.04), EE (method 920.39), and OM (method 942.05) were determined in the diet and digesta samples according to methods of AOAC (2000). The concentration of chromium in the diet and digesta samples were determined by spectrophotometry (450 nm; Spark 10 M; Tecan Group Ltd., Mannedorf, Switzerland), following wet-ash digestion with perchloric and nitric acid (Fenton and Fenton, 1979). The AID of nutrients was calculated using the following equation:

$$\text{AID} = 1 - \left[\frac{(\text{Nutrient}_{\text{digesta}} \times \text{Marker}_{\text{diet}})}{(\text{Nutrient}_{\text{diet}} \times \text{Marker}_{\text{digesta}})} \right]$$

Where $\text{Nutrient}_{\text{digesta}}$ and $\text{Marker}_{\text{digesta}}$ are the concentrations of nutrient and indigestible-marker in the ileal digesta; $\text{Nutrient}_{\text{diet}}$ and $\text{Marker}_{\text{diet}}$ are the concentrations of nutrients and indigestible-marker in the diet.

Fatty Acid Profile

The thigh muscle specimens were evaluated for fatty acid analysis. Fatty acid methyl esters from tissue total lipid extracts were prepared by acid-catalyzed transesterification of total lipids, similar to the method of Christie (1982) except that the reaction was performed at 80°C for 3 h. The fatty acid methyl esters were prepared by reaction with 4% HCl in methanol for 20 min at 60°C. Then, analyses of fatty acid methyl esters was determined with a Hewlett-Packard 5890 gas

chromatograph equipped with an autosampler, a flame ionization detector, and fused silica capillary column (30 m \times 0.25 mm i.d.; Chrompack, Middelburg, The Netherlands). Each sample (1 μL) was injected with helium as a carrier gas onto the column programmed for ramped oven temperatures (initial temperature was 110°C, held for 1 min, then ramped at 150°C/min to 190°C and held for 55 min, then ramped at 5°C/min to 230°C and held for 5 min). Inlet and detector temperatures were both 220°C. Fatty acid methyl esters identification was carried out by comparison of their retention times with the authentic external standards and were expressed as a percentage of fatty acid methyl esters or as g/100 g of sample (Apperson and Cherian, 2017).

Statistical Analysis

The data were analyzed using the GLM procedures of SAS software (SAS Institute, 2010). Tukey's studentized range test was applied to compare the difference among groups. Orthogonal polynomial contrasts were used to determine the linear and quadratic effects of different levels of the EEO on all measurements. All statements of significance were considered as $P < 0.001$ or $P < 0.05$, with a trend between $P > 0.05$ and $P < 0.10$.

RESULTS

Growth Performance and Relative Organ Weight

The data presented in Table 2 show the results of broiler chickens' growth performance in response to the dietary treatments. At the end of the starter period, no treatment effect was observed on BWG, FI, and FCR.

Similar results were recorded between the dietary treatments for BWG and FI at the end of the growth period. However, in the same period, the inclusion of EEO to broiler diets linearly decreased ($P < 0.05$) FCR compared with the CON diet. During the finisher phase, as well as the whole experimental period, the supplementation of EEO linearly increased ($P < 0.05$) BWG but reduced ($P < 0.05$) FCR without any effect on FI. The mortality rate was not influenced by dietary treatments during the study.

In terms of relative organ weight, dietary supplementation with EEO had no significant effect on relative weights of carcass (71.54 vs. 70.90%), breast (25.86 vs. 25.23%), thigh (20.30 vs. 20.47%), abdominal fat (1.50 vs. 1.47%), liver (2.86 vs. 2.91%), spleen (0.13 vs. 0.10%), and bursa of Fabricius (0.18 vs. 0.15%), pancreas (0.24 vs. 0.22%), and heart (0.51 vs. 0.57%) as compared with the CON group.

Table 2. The effect of supplementation of eucalyptus essential oil (EEO) on growth performance in broiler chickens.¹

Items	EEO (mg/kg of diet)					SEM	P-value	
	0	250	500	750	1,000		Linear	Quadratic
Starter phase (day 1–10)								
BWG ² , kg/bird	0.240	0.236	0.245	0.253	0.250	0.021	0.204	0.872
FI ³ , kg/bird	0.260	0.260	0.263	0.282	0.264	0.013	0.285	0.920
FCR ⁴	1.086	1.102	1.072	1.112	1.053	0.029	0.553	0.739
Grower phase (day 11–24)								
BWG, kg/bird	0.787	0.796	0.808	0.817	0.823	0.038	0.128	0.801
FI, kg/bird	1.246	1.250	1.254	1.264	1.240	0.017	0.973	0.633
FCR	1.584 ^a	1.569 ^a	1.551 ^{a,b}	1.546 ^{a,b}	1.506 ^b	0.018	0.005	0.773
Finisher phase (day 25–42)								
BWG, kg/bird	1.380 ^c	1.383 ^c	1.405 ^{b,c}	1.433 ^b	1.474 ^a	0.013	0.001	0.213
FI, kg/bird	2.870	2.897	2.904	2.916	2.920	0.048	0.126	0.696
FCR	2.079 ^{a,b}	2.094 ^a	2.067 ^{a,b}	2.034 ^b	1.981 ^c	0.010	0.002	0.171
Overall phase (day 1–42)								
BWG, kg/bird	2.407 ^c	2.415 ^c	2.458 ^{b,c}	2.504 ^{a,b}	2.548 ^a	0.026	0.005	0.561
FI, kg/bird	4.376	4.406	4.221	4.462	4.424	0.032	0.226	0.602
FCR	1.818 ^{a,b}	1.824 ^a	1.798 ^{a,b}	1.781 ^b	1.737 ^c	0.012	0.001	0.284
Mortality, %	2.50	1.66	4.16	1.66	2.50	0.240	0.675	0.979

^{a-c}Means in the same row with different superscripts differ ($P < 0.05$).

¹Growth performance data are the means of 8 replicate pens with 15 broiler chickens per each.

²Body weight gain.

³Feed intake.

⁴Feed conversion ratio.

Cecal Microflora

The effects of dietary supplementation with EEO on cecal microbial population are summarized in Table 3. The number of LAB in cecal digesta showed a linear increase trend with increasing dietary EEO supplementation ($P = 0.063$). On the other hand, with the increasing level of EEO, the cecal *E. coli* population (linear; $P = 0.085$) showed a trend in reduction. However, no treatment effects were observed on *Clostridium* spp. and coliforms in cecal of birds fed the diets containing EEO.

Apparent Ileal Digestibility of Nutrients

As shown in Table 4, the AID of OM and EE increased linearly and quadratically in broiler chickens fed diets supplemented with 0 to 1,000 mg/kg EEO ($P < 0.05$). However, no significant effects were observed on apparent DM and CP digestibility.

Serum Biochemical Parameters

Concentrations of glucose, total protein, triglycerides, and high-density lipoproteins were not affected by

dietary treatments in this experiment (Table 5). However, a trend of linear ($P = 0.074$) reduction in cholesterol was observed as the dietary EEO supplementation increased. Furthermore, no significant difference was recorded among the treatment groups for GSH-Px activity, whereas the activity of SOD was increased linearly as the dietary levels of EEO increased (Table 5; $P < 0.05$). On the other hand, the content of serum MDA linearly decreased in birds fed diets supplemented with 0 to 1,000 mg/kg EEO ($P < 0.05$).

Fatty Acid in Thigh Muscle

The effects of experimental treatments on fatty acid compositions in the thigh muscle of broiler chickens are presented in Table 6. The administration of EEO reduced concentrations of C14:0, C16:0, C18:0, and total saturated fatty acid (SFA) linearly, whereas increased contents of C18:2, C18:3, C20:4, and total polyunsaturated fatty acid (PUFA) linearly ($P < 0.05$). However, no significant differences were found between the treatment groups for C20:0, C14:1, C16:1, C18:1, C20:1, and total monounsaturated fatty acid (MUFA) contents in the thigh muscle.

Table 3. The effect of supplementation of eucalyptus essential oil (EEO) on cecal microbial population (\log_{10} cfu/g) in broiler chickens.¹

Items	EEO (mg/kg of diet)					SEM	P-value	
	0	250	500	750	1,000		Linear	Quadratic
Lactic acid bacteria	6.72	6.65	6.85	6.90	7.07	0.103	0.073	0.895
<i>Escherichia coli</i>	4.21	4.30	3.92	4.11	3.89	0.141	0.085	0.455
<i>Clostridium</i> spp.	2.46	2.35	2.20	2.23	2.10	0.195	0.178	0.683
Coliforms	7.28	7.32	7.14	7.07	6.96	0.131	0.216	0.888

¹Data represent the mean of 8 replicate pens (3 birds/pen) per treatment.

Table 4. The effect of supplementation of eucalyptus essential oil (EEO) on apparent ileal digestibility of nutrients (%) in broiler chickens.¹

Items	EEO (mg/kg of diet)					SEM	P-value	
	0	250	500	750	1,000		Linear	Quadratic
Dry matter	65.4	65.8	66.5	65.9	66.1	1.340	0.491	0.797
Organic matter	68.2 ^b	68.1 ^b	68.4 ^b	73.4 ^a	74.6 ^a	1.256	0.005	0.023
Crude protein	72.1	72.0	71.6	71.5	72.4	1.062	0.247	0.564
Ether extract	78.3 ^b	80.0 ^{a,b}	81.8 ^{a,b}	85.3 ^a	85.5 ^a	0.951	0.004	0.015

^{a-b}Means in the same row with different superscripts differ ($P < 0.05$).

¹Data represent the mean of 8 replicate pens (3 birds/pen) per treatment.

DISCUSSION

Pharmacological researches have shown that EEO has multiple biochemical and physiological functions in the body, owing to its active components, a variety of antioxidant phytochemicals, and bioactive ingredients (Arise et al., 2009; Dhakad et al., 2018). However, there is a lack of data on the efficacy of EEO in broiler production. Therefore, the present study was attempted to assess the role of EEO as a natural growth promoter for broiler chickens. The results of the current study revealed that the inclusion of EEO in broiler diets linearly increased BWG and decreased FCR over the growth and overall periods. Recently, Mashayekhi et al. (2018) reported that the addition of 0.5% eucalyptus powder in broiler diets improved BWG and reduced FCR over the growth and overall periods. Chen et al. (2018) observed that the addition of 0.8 g/kg polyphenols in eucalyptus leaves to laying hen diets improved productive traits. Additionally, Giannenas et al. (2003) reported significant improvements in BWG and FCR of broiler chickens fed diet containing oregano essential oil compared with the control group. Other studies also indicated that supplementation of broiler diet with *Pulicaria gnaphalodes* powder (the main compound of the *Pulicaria gnaphalodes* plant is 1.8-cineole, which is similar to eucalyptus) might potentially improve growth performance, antioxidant status, and fatty acid profile (Shirani et al., 2019). According to the literature, the positive influence of EEO on growth performance of

broiler chickens could be attributed to EEO bioactive compounds ability in stimulating the digestive and pancreatic enzymes secretion (Hashemipour et al., 2013), improving gut morphology (Giannenas et al., 2018), and enhancing immune function (Chowdhury et al., 2018b). In addition, the appetite-stimulating properties and antimicrobial effects of herbs, spices, and various essential oils are likely to be other factors explaining increased growth performance (Windisch et al., 2008). On the other hand, according to the findings of the present study, the enhancement of growth performance observed in the current study could be because of increasing the antioxidant activities, improving the digestibility of nutrients, and maintaining the beneficial microbial population.

The intestinal microflora plays an important role in protecting the integrity of the intestinal mucosa (Jazi et al., 2020). Impairing this integrity leads to a progressive increase of mucosal permeability, which facilitates pathogens infection. One of the most important biological properties of phytochemical products is their antibacterial activity against less favorable and pathogenic bacteria residing in the gut (Brenes and Roura, 2010). In vivo researches in poultry indicated that phytochemical products such as cinnamon essential oil and herbal extracts rich in phenolic compounds could act against colonization of intestinal pathogens such as *E. coli* and *Clostridium perfringens* because of the antibacterial action of their phenolic ingredients (Chowdhury et al., 2018b; Giannenas et al., 2018). Furthermore, there is

Table 5. The effect of supplementation of eucalyptus essential oil (EEO) on blood biochemical profile and serum antioxidant enzyme in broiler chickens.¹

Items	EEO (mg/kg of diet)					SEM	P-value	
	0	250	500	750	1,000		Linear	Quadratic
Biochemical profile								
Glucose, mg/dL	267	260	264	262	258	3.465	0.614	0.539
Total Protein, g/dL	3.59	3.63	3.80	3.91	3.90	0.203	0.187	0.241
Cholesterol, mg/dL	138.6	138.4	132.5	129.5	127.2	5.154	0.074	0.689
Triglycerides, mg/dL	87.4	88.1	86.9	82.5	83.9	4.574	0.369	0.793
HDL, mg/dL	74.1	69.3	72.2	74.3	76.6	3.126	0.898	0.829
Antioxidant enzyme								
SOD, U/mg prot	144 ^b	149 ^b	155 ^{a,b}	160 ^{a,b}	170 ^a	1.890	0.015	0.345
GSH-Px, U/mg prot	168	171	176	175	176	2.121	0.124	0.569
MDA, nmol/mg prot	6.15 ^a	5.88 ^{a,b}	5.52 ^b	5.40 ^b	4.95 ^c	0.216	0.005	0.101

^{a-c}Means in the same row with different superscripts differ ($P < 0.05$).

Abbreviations: GSH-Px, glutathione peroxidase; HDL, high density lipoprotein; MDA, malondialdehyde; SOD, superoxide dismutase.

¹Data represent the mean of 8 replicate pens (3 birds/pen) per treatment.

Table 6. The effect of supplementation of eucalyptus essential oil (EEO) on fatty acid profile (% of total fatty acid) of thigh muscle in broiler chickens.¹

Items	EEO (mg/kg of diet)					SEM	P-value	
	0	250	500	750	1,000		Linear	Quadratic
SFA	37.80 ^a	37.82 ^a	36.80 ^{a,b}	35.68 ^{a,b}	34.16 ^b	0.784	0.002	0.235
C14:0	0.81 ^a	0.80 ^a	0.77 ^a	0.69 ^b	0.65 ^b	0.022	0.032	0.453
C16:0	30.86 ^a	30.81 ^a	30.06 ^a	29.56 ^{a,b}	28.29 ^b	0.433	0.005	0.184
C18:0	5.92 ^a	6.01 ^a	5.75 ^a	5.23 ^b	5.03 ^b	0.086	0.001	0.673
C20:0	0.21	0.20	0.22	0.20	0.19	0.011	0.654	0.784
MUFA	42.34	42.85	42.37	42.65	43.21	1.090	0.514	0.869
C14:1	0.25	0.24	0.25	0.23	0.22	0.012	0.342	0.654
C16:1	6.10	6.07	5.98	5.95	5.96	0.866	0.496	0.828
C18:1	35.23	35.76	35.32	35.67	36.22	0.951	0.221	0.743
C20:1	0.76	0.78	0.82	0.80	0.81	0.020	0.680	0.965
PUFA	29.67 ^b	29.91 ^b	31.06 ^{a,b}	32.19 ^{a,b}	33.31 ^a	0.573	0.003	0.486
C18:2	24.85 ^b	25.01 ^b	25.77 ^{a,b}	26.54 ^a	26.93 ^a	1.121	0.005	0.791
C18:3	1.65 ^b	1.70 ^b	1.83 ^b	2.10 ^{a,b}	2.48 ^a	0.498	0.023	0.255
C20:4	3.17 ^b	3.20 ^b	3.46 ^{a,b}	3.55 ^{a,b}	3.80 ^a	0.767	0.001	0.206

^{a-b}Means in the same row with different superscripts differ ($P < 0.05$).

Abbreviations: MUFA, mono unsaturated fatty acids; SFA, saturated fatty acids; PUFA, polyunsaturated fatty acid.

¹Data represent the mean of 8 replicate pens (3 birds/pen) per treatment.

evidence that phytonics in some cases enhances the proliferation of beneficial bacteria in the gut. For example, [Murugesan et al. \(2015\)](#) reported that adding a commercial phytonic feed additive (combination of 30 essential oils) to broiler diets increased LAB counts in cecal digesta. Other reports also showed that essential oils (thymol, eugenol, carvacrol, and cinnamaldehyde) fortify the gut microflora by reducing harmful bacteria numbers and increasing beneficial bacteria populations ([Jamroz et al., 2003](#); [McReynolds et al., 2009](#)). The antibacterial activities of EEO against pathogens such as *E. coli*, *Salmonella typhi*, *Moraxella catarrhalis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* has significantly been demonstrated in vitro ([Ghalem and Mohamed, 2008](#); [Mekonnen et al., 2016](#); [Dhakad et al., 2018](#)). These effects could be attributed because of the presence of ingredients such as 1,8-cineole, limonene, and α -pinene ([Dhakad et al., 2018](#)). Our study indicated that EEO dietary supplementation tended to reduce *E. coli* in the ceca digesta contents. Also, we found that EEO's dietary supplement led to a trend of the increased LAB population in cecal. The mode of actions of phytonic products to exert their antibacterial activity varies among such sources of products. Nonetheless, the general proposed mechanism of action is through affecting bacterial cell permeability ([Windisch et al., 2008](#)), dissipating the pH gradient in the bacterial cell ([Jim et al., 2020](#)), providing the substrate for growth and proliferation of lactic acid-producing bacteria in intestinal such as *Lactobacillus* which utilizes phenolic compounds of essential oils as carbon sources ([Pacheco-Ordaz et al., 2018](#)). In addition, phytonic products were reported to stimulate intestinal production of mucus in broiler chickens, an effect that was assumed to impair adhesion of pathogenic bacteria such as coliforms and *E. coli* and thus to contribute to stabilizing the microbial eubiosis in the gut of the animals ([Windisch et al., 2008](#)). Accordingly, the improvement in the microbial population

observed in the ceca of birds fed EEO diets could be contributed to the increased digestibility of OM and EE and improved growth performance.

A well-functioning and healthy gut is the cornerstone of the optimum performance of animals. When the gut function and health are impaired, digestion and absorption of nutrients are affected, and thus, the health and performance might be compromised. Determination of nutrients digestibility is a quantitative assessment of the nutritional and physiological phenomena associated with digestion capacity and gut functionality ([Soumei et al., 2019](#)). In response to increasing EEO supplementation, the AID of OM and EE were linearly and quadratically increased at the end of the study. Potential modes of action of phytonic products are an increase in digestive secretions such as mucin, saliva, bile acid, and enzyme activity enhancement. In line with our results, dietary supplementation with phytonic extracts containing carvacrol, cinnamaldehyde, and capsaicin improved nutrient digestibility in broiler chickens ([Jamroz et al., 2003](#)). [Hashemipour et al. \(2013\)](#) reported that dietary supplementation phytonic products containing an equal mixture of carvacrol and thymol at 4 dosages (0, 60, 100, and 200 mg/kg) in broiler diets linearly increased activities of trypsin, lipase, and protease in the pancreas and intestinal. [Lee et al. \(2003\)](#) also demonstrated that ileal digestibility coefficients for starch and protein in broiler chickens fed diets containing essential oils (thymol and carvacrol) were higher owing to greater the activity of amylase, compared with the control group. Furthermore, the inclusion of phytonic products in broiler diets has shown positive effects on intestinal morphology, gut microbial balance, antioxidant status, and also an intestinal barrier and nonspecific immunity ([Hashemipour et al., 2013](#); [Chowdhury et al., 2018b](#); [Giannenas et al., 2018](#); [Mohebodini et al.,](#)

2019), which could lead to better nutrient digestion and absorption.

The serum biochemical parameters can reflect the physiological and metabolic status of animals. These parameters can be altered by diet and environmental agents. [Arise et al. \(2009\)](#) reported that the administration of aqueous extract of eucalyptus in the feed of rats reduced the cholesterol and low-density lipoprotein cholesterol of serum. In the current study, also serum cholesterol concentration had tended to reduce linearly in birds fed different concentrations of EEO. Similarly, [Chen et al. \(2018\)](#) reported that the inclusion of polyphenols in eucalyptus leaves at 0.8 g/kg could reduce cholesterol content in egg yolk. In an in vitro study, [Kladniew et al. \(2014\)](#) observed that 1.8-cineole (as the main compound of the eucalyptus) could inhibit steps in cholesterol synthesis by blocking 3-hydroxy-3-methylglutaryl-CoA reductase (the key indicator of the cholesterol biosynthesis pathway) levels. In vivo experiments also have shown that the possible mechanism for lowering the cholesterol synthesis by phytochemical products (ajwain, clove, and cinnamon essential oils) may be because of the inhibitory effects of its active compounds on the reductase enzyme 3-hydroxy 3-methylglutaryl-CoA or increased catabolism of lipoproteins ([Chowdhury et al., 2018b](#)). In addition, part of the decrease in blood cholesterol may be related to well-known LAB activities following the improvement of intestinal microflora balance ([Ashayerizadeh et al., 2018](#); [Jazi et al., 2018b](#)).

Oxidative stress is a common process in life, which means the imbalance between the production of free radicals and the body's antioxidant system's ability to remove these reactive species ([Birben et al., 2012](#)). The SOD and GSH-Px enzymes are the most important antioxidant defense enzymes protecting cells by preventing the formation of free radicals and increasing the antioxidant defense ([Mohebodini et al., 2019](#)). Furthermore, lipid peroxidation is a major consequence of oxidative stress, leading to increased MDA contents. Therefore, MDA concentration is usually used as a biomarker to assess the extent of lipid oxidation ([Shirani et al., 2019](#)). Our findings indicated the dietary supplementation of EEO enhanced the serum antioxidant capacity of broiler chickens by increasing the activity of SOD and reducing MDA content. This means that the EEO may provide a more efficient free radical-scavenging activity in broiler chickens. There are almost no data about the antioxidant effects of EEO in broiler chickens, but there is evidence of antioxidant effects from in vitro studies. A study by [Luis et al. \(2016\)](#) examined the antioxidant activity of EEO by test systems of antioxidant activity such as DPPH. In that study, the researchers stated that EEO could be considered as potential substitutes of synthetic ones, considering their radical scavenging properties and lipid peroxidation inhibition capacity ([Luis et al., 2016](#)). These authors ascribed the observed antioxidant properties to 1.8 cineole. [Horvathova et al. \(2014\)](#) also observed that 1.8 cineole exhibited different degrees of reducing power, radical

scavenging, chelating, in addition to DNA-protective capacity. The exact mechanism by which EEO and essential oils improve antioxidant capacity is not fully understood. However, the possible mechanism of aromatic plants or their essential oils for this observation may be attributed to their ability to donate hydrogens or electrons and also delocalizing the unpaired electron within the aromatic structure ([Jin et al., 2020](#)).

In terms of consumers' health, the evaluation of the compositions of meat fatty acid can be considered as an important parameter in the meat quality. Despite the extensive investigation about the influence of phytochemical products on the production performance of broiler chicks, scarce data are available on the effect of these products on meat compositions. In the current study, the total contents of PUFA in muscle increased in response to the increase of dietary EEO supplementation. The enhanced PUFA amount in the muscle could be because of the amplification of the antioxidant system such as SOD in birds receiving EEO diets, although this requires further investigation. Birds fed different levels of EEO had lower SFA content as well. This decrease should be attributed to a notable reduction in the concentrations of C14:0, C16:0, and C18:0. Some studies have shown that, among the SFA, myristic acid (C14:0) and palmitic acid (C16:0) can enhance low-density lipoprotein cholesterol content and therefore increase serum cholesterol concentration ([Rioux et al., 2005](#)). Hence, dietary SFA have high importance because of their hypercholesterolemic effects, which are associated with coronary artery disease ([Ashayerizadeh et al., 2018](#)). Also, clinical data strongly support a relationship between coronary heart disease and dietary intake cholesterol and SFA ([Zhou et al., 2009](#)). In the current study, the thigh muscle of broilers fed the diet supplemented with 1000 mg/kg EEO had a lower SFA content than that of broilers in the control group. Therefore, the present results show that humans' consumption of these kinds of meats may help reduce the risk of coronary heart disease. In line with our results, [Hashemipour et al. \(2013\)](#) reported that supplementation of phytochemical products (thymol and carvacrol) reduced total SFA contents and increased PUFA and n-6 fatty acid in the serum and thigh muscle and increased MUFA in thigh muscle of broiler chickens. Therefore, considering the hypercholesterolemic effects of SFA, the current findings concluded that EEO's dietary supplement might be an effective strategy to reduce the thigh meat SFA concentrations in favor of MUFA and PUFA.

In summary, the findings obtained in this study indicated that supplementation with increasing levels of EEO improved growth performance, enhanced the AID of OM and EE, modified the cecal microbial population, and improved the antioxidant status of broiler chickens. In addition, dietary EEO supplementation can positively affect fatty acid compositions in the thigh muscle. Among the 4 dosages of EEO, the addition of 1,000 mg/kg EEO had the best performing in broiler chickens. However, further research is warranted to validate these

results in the enteric disease challenge model or commercial farm conditions.

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DISCLOSURES

The authors did not provide a conflict of interest statement.

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