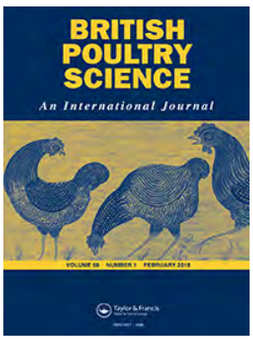

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Effects of essential oils from *Liquidambar orientalis* Mill. leaves on growth performance, carcass and some organ traits, some blood metabolites and intestinal microbiota in broilers

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ABSTRACT

1. This study was carried out to investigate the effects of liquidambar essential oils (LEO) isolated from Turkish sweet gum (*Liquidambar orientalis* Mill.) leaves on growth performance, carcass, edible inner organs (EIO), gastrointestinal traits (gut), some blood metabolites and jejunum microbiota in broilers.
2. A total of 375 one-d-old male broilers (Ross 308) were randomly allocated to 5 treatments with 5 pens with 15 birds. The birds were fed on diets without antibiotics (CONT), with antibiotic (50 mg per kg, AB), with LEOs at 0.0405 (0.04LEO), 0.0811 (0.08LEO) or 0.1622 (0.16LEO) g/kg feed up to 42 d of age. The levels of LEOs included to diets were determined according to *in vitro* antimicrobial activity.
3. From d 1 to 42, the 0.08LEO treatment had higher live weight gain (LWG) compared to others. The 0.08LEO treatment increased feed intake (FI) compared to the CONT, AB and 0.04LEO. However, the feed conversion ratio (FCR) of these birds was lower than those in the AB and 0.16LEO treatments. From 1 to 42 d of age for LWG, the effects were quadratic and cubic, while those for FI and FCR were cubic and quadratic, respectively. Birds that fed 0.08LEO and AB diets had higher and lower carcass weights (CW) than those that fed other diets. The effect of LEO levels was cubic on the CW. The 0.08LEO and 0.16LEO decreased abdominal fat (AF) weight compared to the AB. The blood cholesterol decreased by the 0.04LEO and 0.08LEO treatments compared to the CONT. For the blood cholesterol, the effects of LEO levels were cubic. The 0.08LEO treatments decreased *Escherichia coli* counts in jejunum compared to the CONT and 0.16LEO.
4. Feeding a diet with LEO at 0.0811 g/kg might increase the LWG, FI and weights of carcass and AF, whereas it might decrease blood cholesterol and *E. coli* counts without affecting blood high-density lipoprotein, low-density lipoprotein, triglyceride, glucose, aspartate transaminase and alanine transaminase concentrations.

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Cholesterol; essential oils; feed additive; microorganism; poultry; Turkish sweet gum

Introduction

The use of antibiotics to enhance growth and feed efficiency and reduce mortality has been banned in animal nutrition due to the emergence of microbes with cross-resistance and multiple resistance to antibiotics which are used to treat human and animal infections (Zeng et al. 2015) and due to consumer concern. Therefore, the search for non-antibiotic growth promoters with the antimicrobial and antioxidant activities including various dietary herbs and plant extracts, especially essential oils in poultry nutrition (Ocak et al. 2008; Ozturk et al. 2012), has been prompted. Plant essential oils have been studied intensively in recent years. It has been reported that essential oils have positive or beneficial effects on appetite and feed utilisation, carcass quality, health, shelf life of poultry products (meat, egg, etc.) and immunity-regulating cholesterol metabolism (Cabuk et al. 2006; Durape 2007; Brenes and Roura 2010; Amorati et al. 2013).

The tree genus *Liquidambar*, from the *Hamamelidaceae* family, is widespread, ranging from North America to East Asia (Ozturk et al. 2008). Commonly known as the oriental sweet gum (English), the tree is also frequently called the *Gunluk Agaci* (Turkish), meaning frankincense or myrrh tree owing to its fragrance, or the *Sigla agaci* (Turkish) due to the balsam seeping from the tree trunk when injured (Ozturk et al. 2008). Furthermore, Turkish sweet gum (*L. orientalis* Mill.) is one of the endemic forest tree species, reared in districts of Marmaris, Koycegiz, Dalaman and Fethiye in the Mugla province of Turkey (Ozturk et al. 2008). The major compounds of essential oils isolated from sweet gum leaves are terpinen-4-ol (35%), α-terpinol (1.9%), sabinene (13%) and γ-terpinene (15%) (Hafizoglu et al. 1996; Duru et al. 2002) or styrene (70.4%), α-pinene (19%), limonene (1.2%) and β-pinene (4,3%) (Fernandez et al. 2005) with antimicrobial and antioxidant activity. Medicinal plants, such as herbs, extracts or essential oils, are used to improve poultry performance and have gained much attention for their potential as alternatives to antibiotics. Although it has been determined that the LEO from Turkish sweet gum leaves have an *in vitro* antimicrobial activity (Duru et al. 2002), a systematic approach to investigate the efficacy and safety of LEO as feed additives for broilers is still missing. Therefore, we hypothesised that LEO with antimicrobial activity would exert measurable variation or beneficial effects in or on broilers in terms of the growth-promoting, cholesterol-lowering and antimicrobial effects, and blood metabolites which are related directly to animal

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health. Accordingly, the aim of the study reported herein was to investigate the effects of LEO on growth performance, some non-carcass parts, intestinal microbiota and blood metabolites in broiler.

Materials and methods

A total of 375 one-d-old Ross 308 male broiler chickens, with an average initial weight of 40 ± 0.02 g, obtained from a commercial hatchery (Ross Breeders Anadolu, Turkey) were used in this study. All birds were fed on a starter diet for 1–21 d old, grower diet for 22–35 d old, and finisher diet for 36–42 d old (Table 1). The base diet was in mash form. The vegetable oil compound was added when preparing the 5 experimental diets. LEO were mixed with vegetable oil and then added to the base diet. All diets were provided *ad libitum* in mash form. Broiler chicks were divided into 5 experimental groups with 5 replicate cages, each including 15 birds. Dietary treatments were control (CONT, basal diet without antibiotic and LEO), antibiotic (AB, basal diet supplemented with 50 g chlortetracycline/kg of feed), and basal diets supplemented with 0.0405 (0.04LEO), 0.0811 (0.08LEO) or 0.1622 (0.16LEO) g LEO/kg feed. The antimicrobial activity of LEOs was assessed using the agar diffusion method as recommended by Aureli et al. (1992) and Ozcan et al. (2004). For this purpose, reference microorganisms (*Staphylococcus aureus* ATCC 25923^T, *Escherichia coli* ATCC 25922^T, *Lactobacillus acidophilus* ATCC 11975^T, *Enterococcus faecalis* ATCC 29212^T and *Clostridium perfringens* ATCC 10 388) obtained from Ondokuz Mayis University, Faculty of Arts and Sciences, Department of Biological Sciences and Faculty of Veterinary were used. Therefore, LEO levels to be added to the base diet were determined according to the findings of minimum inhibitory concentration (MIC) values (MIC values; 0.6 mg/ml for *E. coli*, 0.4 mg/ml for *S. aureus*, 0.9 mg/ml *E. faecalis*, >12 mg/ml for *L. Acidophilus*, >5 mg/ml for *C. perfringens*).

All groups were subjected to similar feeding and management practices (vaccination, lighting, feeding and watering) as mentioned in Management Guide for Ross 308 broilers (AVIAGEN 2014). Birds were fed by using cylindrical hanging feeders and watered by hanging drinkers. Feeder and drinker spaces were 2 cm per bird. Lights were on continuously for the first 3 d after hatching, after which a 23L:1D lighting schedule by 2 fluorescent bulbs was maintained for the duration of the experiment. Ambient temperature was gradually decreased from 33°C at 7 d to 21°C till 21 d old and then kept constant.

Turkish sweet gum leaves were freshly collected at the beginning of July in the Koycegiz (36°57′33″ N; 28°40′30″ E) in Mugla, Turkey. Then these leaves were air-dried at ambient temperature in a dark, well-ventilated room by air conditioner for 3 d (mean temperature = 30°C, and mean relative humidity of 40%). Dried leaves were hydrodistilled for 4 h using a Clevenger apparatus, giving essential oil in 1.1% yield. The essential oil was dried over anhydrous sodium sulphate and then stored at 4°C (Hadian et al. 2011). Qualitative and quantitative analyses of the LEO were performed using gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). The GC analysis of the LEO was carried out on a Shimadzu GC-17 AAF, V3, 230V series gas chromatography (Japan), equipped with a split injector, attached to DB-1 column (30 m × 0.25 mm, 0.25-µm-film thickness) and fitted to flame ionization detector (FID). Carrier gas flow rate (He) was 1.4 ml/min, split ratio 1:50, injector temperature was 250°C, detector temperature 270°C. The initial oven temperature for both analyses were held at 60°C for 5 min, then increased up to 240°C with 4°C/min increments and held at this temperature for 10 min. The same analytical conditions were employed for the GC/MS analysis, where Varian Saturn 2100T (USA) system equipped with DB-1 column (30 m × 0.25 mm, 0.25-µm-film thickness) was used. Transfer line temperature was heated at 290°C. Mass

Table 1. Ingredient composition and chemical analysis of basal diets used in the experiment (as fed on basis).

Ingredients	Starter diet (1–21 days)	Grower diet (22–35 days)	Finisher diet (36–42 days)
Maize	494.00	510.50	558.00
Soybean meal (47%)	310.00	288.00	228.00
Full-fat soybean	120.00	120.00	130.00
Vegetable oil	37.30	44.00	49.00
Limestone	10.00	10.00	9.00
Dicalcium phosphate	19.00	19.00	17.00
Sodium chloride	3.00	3.00	3.00
Vitamin, Mineral premix*	2.50	2.50	2.50
L-lysine (78%)	0.60	0.30	0.50
DL-methionine (99%)	2.60	1.70	2.00
Sodium bicarbonate	1.00	1.00	1.00
Total	1000.00	1000.00	1000.00
Analysed composition (% dry matter, DM)			
Crude protein	23.68	22.16	20.64
Ether extract	7.15	8.05	8.32
Crude fibre	2.95	3.06	2.89
Crude ash	5.71	6.05	6.11
Calculated composition			
Metabolizable energy, MJ/kg[b]	12.8	13.2	13.5
Lysine	1.43	1.24	1.09
Methionine	0.51	0.45	0.41
Methionine-cysteine	1.07	0.95	0.86
Calcium	1.05	0.90	0.85
Available phosphorus	0.50	0.45	0.42

[a]Contained per kg of premix: retinyl acetate, 3.60 mg; cholecalciferol, 0.06 mg; DL-α-tocopheryl acetate, 40 mg; menadione, 4 mg; thiamine, 3 mg; riboflavin, 6 mg; niacine, 25 mg; calcium-D-pantothenat, 10 mg; pyridoxine, 5 mg; cyanocobalamin, 0.03 mg; D-biotin, 0.05 mg; folic acid, 1 mg; Mn, 80 mg; Zn, 60 mg; Fe, 60 mg; Cu, 5 mg; Co, 0.2 mg; I, 1 mg; Se, 0.15 mg; cholinechloride, 200 mg.
[b]Metabolizable energy was calculated based on chemical composition.

spectrum was acquired in EI mode (70 eV), in m/z range 28–650. Identification of compounds of the essential oil was based on GC retention indices and computer matching with the Wiley, NIST-2005 and TRLIB Library as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature (Adams 1989; Duru et al. 2002) and, whenever possible, by co-injection with authentic compounds.

During the experimental period, health status was evaluated daily, whereas live weight (LW) and feed intake (FI) were measured at 21 and 42 days of age. Feed conversion ratio (FCR) was calculated as the ratio of FI to LW gain (LWG). At the end of the experiment, two birds from each replication with LW within 1 standard deviation of the mean treatment weight (10 birds per treatment or totally 50 birds) were slaughtered to determine weights of edible inner organs (EIO) (gizzard + heart + liver), abdominal fat (AF) and gastrointestinal tract (gut), and length of gut. Relative weights and lengths (when appropriate) of carcass and non-carcass parts were calculated as percentages of the LW (g or cm/100 g LW).

For analyses of blood metabolites such as glucose, triglyceride, total cholesterol, high-density (HDL) and low (LDL)-density cholesterol, glucose, alanine transaminase (ALT) and aspartate transaminase (AST) at the end of the experiment, two birds per replicate (10 birds with similar LW for each treatment) were selected and fasted overnight. Blood samples were collected (about 4 cm^3) from the brachial wing vein to vacutainer heparinesed steryl tubes (BD Bioscience, Franklin Lakes, NJ) at the age of 43 d. Then the samples were separated by centrifugation (2325 g for 10 min) to get blood for glucose, triglyceride, total cholesterol, HDL, LDL, AST and ALT analysis by using suggested kits (Biolab, Maizy, France) in an automatic analyser (Airone-200, RA, Italy).

Jejunum is one of the most important organs that reflect the microbial distribution and is used as an alternative to antibiotics in intestinal microflora to determine the effects of feed additives. In this study, jejunum content (from the end of the duodenum to Meckel's diverticulum) from 10 birds from each treatment were, therefore, aseptically collected and transferred to peptone buffer in test tubes and sterile 'whirl-pack' plastic bags for bacteriological culture that was carried out on the same day. Then these samples were further subjected to a 10fold dilution series. Appropriate dilutions were plated, using the pour plate technique, in selective culture media to count specific numbers of 7 groups of jejunum bacteria. For the enumeration of *Clostridium, Enterococcus, Lactobacilli spp., Staphylococcus* and *E. coli*, TSC (Tryptose Sulfite Cyclocerine Agar, Merck 1.11972), Slanetz and Bartley (Oxoid CM0377), MRS (Lactobacillus Agar acc. to De Man, Rogosa and Sharpe Agar Merck 1.10660), Baird Parker (Oxoid CM275), CVA (BD-BBL 297 246), and the incorporation in Eosin Methylene Blue Agars (Oxoid CM0069) were used, respective to test microorganisms, in an appropriate incubation condition for each bacteria. All bacteria counts were expressed as log_{10}CFU/g.

Statistical analysis

All statistical analyses were performed by means of SPSS 15.0 for Windows software (SPSS Inc., NY, USA). For performance data, pen means served as the experimental unit for statistical analysis. For data of relative weights and length of gut and blood metabolites, individual birds were considered as the experimental unit (Ozturk et al. 2012). Levene's test and the Shapiro–Wilk test were firstly used for equality of variance and for normality assumption, respectively, of the traits (LWG, FI etc.) for the 5 treatments (CONT, AB, 0.04LEO, 0.08LEO and 0.16LEO) ($P > 0.05$). Then, one-way analysis of variance and Tukey HSD multiple comparison tests were used to determine the differences among the groups in terms of the traits. Also, results from feeding treatment diets 1 through 4 (CONT, 0.04LEO, 0.08LEO and 0.16LEO) were analysed as an orthogonal polynomial. Linear, quadratic and cubic effects were determined by orthogonal polynomial contrasts. Results are presented as means and a standard error of mean (SEM). *P*-values of less than 0.05 were considered statistically significant.

Results

Although 33 compounds were identified (Table 2), the compounds in LEO were dominated by the terpinen-4-ol (31.86%), γ-terpinen (14.38%), α-terpinen (8.69%), sabinen (8.61%) and germacrene (5.80%). Although the LWG, FI and FCR were not affected by the treatments until 21 d of age, the birds in 0.08LEO treatment had the best performance between 22 and 42 d of age in terms of FCR and LWG (Table 3). Effects for LWG and FI were cubic ($P < 0.01$), while for FCR they were quadratic and cubic ($P < 0.01$). The birds in 0.08LEO treatment had a higher LWG (approximately 6.7–11.5%) during the entire experiment compared to other treatments ($P < 0.05$). From d 1 to 42 of the experiment, FI of broilers fed 0.08LEO diet was

Table 2. The compounds of *Liquidambar orientalis* Mill. essential oil.

Compounds	RI[a]	%[b]	Identification methods
α-Pinene	912	5.18	Co-GC, MS, RI
Camphene	934	0.12	Co-GC, MS, RI
Sabinene	971	8.61	Co-GC, MS, RI
β-Pinene	975	1.92	Co-GC, MS, RI
Myrcene	998	1.65	Co-GC, MS, RI
α-Phellandrene	1006	0.81	Co-GC, MS, RI
α-Terpinene	1018	8.69	Co-GC, MS, RI
p-Cymene	1025	1.74	Co-GC, MS, RI
Limonene	1042	0.91	Co-GC, MS, RI
β-Phellandrene	1045	2.05	Co-GC, MS, RI
γ-Terpinen	1059	14.38	Co-GC, MS, RI
Terpinolen	1089	3.17	Co-GC, MS, RI
Menthol	1141	0.82	Co-GC, MS, RI
Terpinen-4-ol	1150	31.86	Co-GC, MS, RI
α-Terpineol	1164	3.94	Co-GC, MS, RI
Myrtenol	1171	0.10	Co-GC, MS, RI
trans-Carveol	1178	0.45	Co-GC, MS, RI
cis-Myrtanol	1185	tr	Co-GC, MS, RI
trans-Myrtanol	1189	0.12	Co-GC, MS, RI
Viridiflorene	1223	1.93	MS, RI
trans-Carvyl acetate	1227	0.83	MS, RI
α-Longipinene	1232	0.71	MS, RI
β-Caryophyllene	1247	0.92	Co-GC, MS, RI
β-Gurjunene	1252	0.54	MS, RI
Aromadendren	1255	0.20	MS, RI
Germacrene D	1267	5.80	Co-GC, MS, RI
Epi-bcyclosesquiphellandrene	1279	0.36	MS, RI
δ-Cadinene	1287	0.42	MS, RI
Spathulenol	1298	0.65	Co-GC, MS, RI
β-Caryophyllene oxide	1301	0.42	MS, RI
β-Cadinene	1305	0.18	MS, RI
tau-Kadinol	1316	0.30	MS, RI
tau-Muurolol	1325	0.22	MS, RI

Co-GC: Co-injection with authentic compounds, RI: Retention Index literature comparison, tr: trace (< 0.1%).
[a]Kovats index on DB-1-fused silica column.
[b]Percentage concentration.

Table 3. Live weight gain, feed intake and feed conversion ratio of broilers fed diet with essential oil from *Liquidambar orientalis* Mill. leaves.

	Diets[†]							Effect		
	CONT	AB	0.04LEO	0.08LEO	0.16LEO	SEM	*P*	L	Q	C
Live weight gain (g/bird)										
1–21 d	897	924	901	919	911	5.7	0.564	NS	NS	NS
22–42 d	1485^{b}	1373^{c}	1519^{b}	1676^{a}	1501^{b}	24.8	0.001	NS	NS	*
1–42 d	2382^{b}	2297^{b}	2420^{b}	2595^{a}	2413^{b}	26.0	0.001	NS	*	*
Feed intake (g/bird)										
1 to 21 d	1420	1461	1441	1468	1454	7.5	0.300	NS	NS	NS
22 to 42 d	3012^{bc}	2869^{c}	3019^{bc}	3243^{a}	3076^{b}	32.7	0.001	NS	NS	*
1 to 42 d	4433^{b}	4330^{b}	4460^{b}	4711^{a}	4530^{ab}	35.3	0.003	NS	NS	*
Feed conversion ratio (g g^{-1})										
1 to 21 d	1.59	1.58	1.60	1.60	1.59	0.006	0.919	NS	NS	NS
22 to 42 d	2.03^{bc}	2.09^{a}	1.98^{cd}	1.93^{d}	2.05^{ab}	0.014	0.001	NS	**	*
1 to 42 d	1.86^{ab}	1.89^{a}	1.84^{ab}	1.82^{b}	1.88^{a}	0.007	0.002	NS	**	NS

[a,b]Mean values within the same row not sharing a common superscript differ significantly (*$P < 0.05$). NS = Not significant, $P > 0.05$; ** $P < 0.01$. SEM = standard error of the mean.

[†]The dietary treatments were as follows: control (CONT, basal diet without antibiotic and LEO), antibiotic (AB, basal diet supplemented with 50 g of chlortetracycline per kg), and basal diets supplemented with 0.0405 (0.04LEO), 0.0811 (0.08LEO) or 0.1622 (0.16LEO) g *Liquidambar orientalis* essential oil (LEO) per kg feed. L, linear; Q, quadratic; C, cubic.

higher than those fed CONT, AB and 0.04LEO diets ($P < 0.05$). However, the FCR of these birds was lower than of those in the AB and 0.16LEO treatments ($P < 0.05$). At the entire experiment (from 1 to 42 d of age) for LWG, the effects were quadratic and cubic ($P < 0.05$), while for FI and FCR they were cubic ($P < 0.05$) and quadratic, respectively ($P < 0.01$).

Means for the carcass weight (CW), dressing percentage and traits of EIO and gut are shown in Table 4. The CW of birds fed 0.08LEO diet was higher, while those of AB diets were lower than those fed other diets ($P < 0.05$). The effect of LEO levels was linear, quadratic and cubic on the CW ($P < 0.01$). The relative AF weight of birds from the 0.08LEO and 0.16LEO treatments was lower than of those of the AB treatment, but not CONT ($P > 0.05$). There were no differences among the experimental groups in terms of the dressing percentage, the relative weight and length of gut, and the relative weight of EIO ($P > 0.05$).

The studied blood metabolites, except for cholesterol level (Table 5), and microbiota of jejunum, except for *E. coli* counts (Table 6), were not affected by the treatments. The cholesterol level decreased by the 0.04LEO and 0.08LEO treatments compared to the CONT treatment ($P < 0.01$). The 0.08LEO treatment decreased *E. coli* counts compared to the CONT and 0.16LEO treatments ($P < 0.01$). The effects of the cholesterol level ($P < 0.01$) and the *E. coli* counts were quadratic ($P < 0.01$).

Table 4. Carcass weight (CW, g), dressing percentage (DP, %), edible inner organ (EIO, g/100 g LW), abdominal fat (AF, g/100 g LW), relative length of gut (RLG, cm/100 g LW) and relative weight of gut (RWG, g/100 g LW) of broilers fed on diets with essential oil from *Liquidambar orientalis* Mill. leaves.

	Diets[†]							Effect		
Metabolites	CONT	AB	0.04LEO	0.08LEO	0.16LEO	SEM	*P*	L	Q	C
CW	1742^{b}	1659^{c}	1769^{b}	1906^{a}	1784^{b}	17.2	0.001	**	**	**
DP	72.8	73.0	73.4	73.6	73.4	0.23	0.837	NS	NS	NS
EIO	3.84	3.82	4.26	3.99	3.96	0.085	0.515	NS	NS	NS
AF	1.45^{ab}	2.25^{a}	1.47^{ab}	1.17^{b}	1.31^{b}	0.117	0.018	NS	NS	*
RLG[‡]	12.54	12.79	12.38	11.95	12.12	0.141	0.360	NS	NS	NS
RWG[‡]	11.50	11.89	12.19	11.09	11.27	0.244	0.364	NS	NS	NS

[a,b]Mean values within the same row not sharing a common superscript differ significantly *($P < 0.05$). NS = Not significant, $P > 0.05$; ** $P < 0.01$. SEM = Standard error of the mean.

[†]The dietary treatments were as follows: control (CONT, basal diet without antibiotic and LEO), antibiotic (AB, basal diet supplemented with 50 g of chlortetracycline per kg), and basal diets supplemented with 0.0405 (0.04LEO), 0.0811 (0.08LEO) or 0.1622 (0.16LEO) g *Liquidambar orientalis* essential oil (LEO) per kg feed. L, linear; Q, quadratic; C, cubic.

[‡]The values are means of the 5 replicates (pens)

Table 5. Effect of treatments on blood metabolites (mg/dl) of broilers at 42 d of age.

	Diets[†]							Effect		
Metabolites[‡]	CONT	AB	0.04LEO	0.08LEO	0.16LEO	SEM	*P*	L	Q	C
Cholesterol	134.31^{a}	127.76^{ab}	115.28^{b}	114.08^{b}	123.92^{ab}	2.148	0.019	NS	**	NS
HDL	71.93	72.03	73.08	70.83	78.70	2.321	0.867	NS	NS	NS
LDL	44.00	39.82	39.16	37.40	37.84	1.685	0.858	NS	NS	NS
Triglyceride	66.72	67.30	66.40	65.10	64.65	0.643	0.644	NS	NS	NS
Glucose	249.94	254.92	243.37	250.06	244.07	3.736	0.856	NS	NS	NS
AST (U/L)	311.20	296.40	327.00	319.00	316.20	4.567	0.268	NS	NS	NS
ALT (U/L)	7.45	6.66	7.53	6.43	7.21	0.438	0.929	NS	NS	NS

[a,b] Mean values within the same row not sharing a common superscript differ significantly (*$P < 0.05$). NS = Not significant, $P > 0.05$; ** $P < 0.01$. SEM = standard error of the mean.

[†]The dietary treatments were as follows: control (CONT, basal diet without antibiotic and LEO), antibiotic (AB, basal diet supplemented with 50 g of chlortetracycline per kg), and basal diets supplemented with 0.0405 (0.04LEO), 0.0811 (0.08LEO) or 0.1622 (0.16LEO) g *Liquidambar orientalis* essential oil (LEO) per kg diet. L, linear; Q, quadratic; C, cubic.

[‡]The values are means of the 5 replicates (pens).

Table 6. Effect of treatments on jejunum microbial population (log CFU/g of digesta) of broilers at 42 d of age.

	Diets[†]							Effect		
Parameters[‡]	CONT	AB	0.04LEO	0.08LEO	0.16LEO	SEM	*P*	L	Q	C
L. acidophilus	7.24	7.22	7.22	7.18	7.15	0.034	0.943	NS	NS	NS
E. faecalis	6.91	6.80	6.59	6.59	6.70	0.050	0.158	NS	NS	NS
C. perfringens	6.06	6.06	5.82	5.81	5.76	0.051	0.158	NS	NS	NS
S. aureus	6.73	6.67	6.58	6.73	6.74	0.044	0.777	NS	NS	NS
E. coli	8.00[a]	7.93[ab]	7.59[ab]	7.22[b]	7.99[a]	0.072	0.016	NS	**	NS

[a,b]Mean values within the same column not sharing a common superscript differ significantly (*$P < 0.05$). NS = Not significant, $P > 0.05$; **$P < 0.01$. SEM = standard error of the mean.

[†]The dietary treatments were as follows: control (CONT, basal diet without antibiotic and LEO), antibiotic (AB, basal diet supplemented with 50 g chlortetracycline of per kg diet), and basal diets supplemented with 0.0405 (0.04LEO), 0.0811 (0.08LEO) or 0.1622 (0.16LEO) g *Liquidambar orientalis* essential oil (LEO) per kg diet. L, linear; Q, quadratic; C, cubic.

[‡]The values are means of the 5 replicates (pens).

Discussion

The major compounds of LEO used in the present study were not similar to previous results reported by Hafizoglu et al. (1996), Duru et al. (2002) and Fernandez et al. (2005). These differences can be explained by the location of *L. orientalis* or the hydrodistillation method (Duru et al. 2002). Indeed in the present study, the MIC values of essential oils from *L. orientalis* leaves collected in the Koycegiz, Marmaris, Fethiye in Mugla, Turkey, were significantly different (data not shown). Thus, this result supports the idea that the chemical composition of medicinal plants is known to be variable.

The results of the present study show that feeding a diet with LEO at 0.0811 g/kg might increase LWG, FI and CW and decrease blood cholesterol and *E. coli* counts without affecting carcass yield, EIO and gut traits, and the other metabolites that were studied. These results suggest that there was a beneficial effect of LEO at 0.0811 g/kg on broilers in terms of growth promoting and cholesterol- and *E. coli*-lowering effects. The improved growth performance (LWG, FI and FCR) by LEO supplementation could be attributed to the encouragement of secretions of endogenous digestive enzymes by the presence of essential oils digesta to enhance nutrient digestion and gut passage rate in chickens (Lee et al. 2004; Hashemipour et al. 2013; Hashemipour et al. 2016). Although the secretion of digestive juices was not investigated in the present study, it has been reported that plant essential oils enhances the secretion of digestive juices, which has appetising and antimicrobial effects (Costa et al. 2013; Hashemipour et al. 2016; Masouri et al. 2017). Also, our results with respect to MIC values and jejunum bacteria counts (Table 6) refer to the fact that the essential oils of *L. orientalis* in the digestive system improved feed efficiency via their antimicrobial impact on pathogenic microbiota (Sagdic et al. 2005; Oskay and Sari 2007).

Although studies testing the palatability of diets with supplementation of phytogenes have been limited, most studies have observed only their effect on FI and LWG through performance tests (Oetting et al. 2006). The LEO has a characteristically bitter taste and strong odour (Hafizoglu et al. 1996; Duru et al. 2002; Fernandez et al. 2005; Sagdic et al. 2005). Therefore, in the current study, the increased FI by the 0.08LEO diet compared to CONT, AB and 0.04LEO may be attributed to the sharp smell of LEO. As reported by Masouri et al. (2017), the underlying mechanisms by which phytogenics affect bird performance are not yet clear. Amad et al. (2011) concluded that the variation in bird responses to phytogenics may be related to differences in the composition of the various bioactive substances, diet type, animal age, hygiene and environmental factors.

An increase in the AF of birds fed with AB compared to higher levels (0.0811 and 0.1622 g/kg) of LEO may most likely be related to differences in microbiota composition, influencing fat accumulation (Pourhossein et al. 2012) and the increasing effect of fat mobilisation by active ingredients (especially terpinen-4-ol as in the LEO) in essential oils (Barreto et al. 2008). It has been reported that the supplementation of aromatic herbs or essential oils in diets affects lipid metabolism in body tissues and organs, which causes less fat accumulation in the organism (Ertas et al. 2005; Guler et al. 2005; Guler et al. 2006). Thus, it can be said that this essential oil may interfere with the accessibility of fat for the formation of fat tissue in the birds due to a reduction in the AF of birds fed with LEO supplemented diets, especially 0.04LEO and 0.08LEO, as reported by Ashayerizadeh et al. (2011) for several biological feed additives.

Like for the present findings, the effects of medicinal herbs were not significant on some carcass traits such as hot or cold CW, carcass yield, breast yield, edible organ weight etc. (Lee et al. 2003; Hernandez et al. 2004; Jang et al. 2007; Ocak et al. 2008; Toghyani et al. 2010; Amad et al. 2011). Lee et al. (2003) concluded that the use of highly digestible feed ingredients in the diet and hygienic conditions in research studies could mask the beneficial effects of phytogenic additives on growth performance and carcass traits. Inconclusive outcomes among previous and the present study may result from the fact that the studies differed not only in the poultry species and strains used but also in the husbandry conditions (fully environmental-controlled house, semi-environmental-controlled house or natural ventilation, cage or floor pen, vaccination etc.), slaughtering conditions (cervical dislocation, euthatal, slaughter by sharp knife or automatic system), genetics (Cobb, Ross, Arbor-acres etc.), growing period and age of the birds (0–28, 0–35 and 0–42 or 7–28, 21–28 day), as well as the difference in the parameters studied (sera, plasma or whole blood) and essential oils used (Ocak et al. 2008; Lee et al. 2003; Hernandez et al. 2004). The result with respect to mortality shows that broilers in the present study were kept in a clean environment, possibly leading to diminished efficacy, if any, of the antibiotic and LEO.

The fact that LEO-supplemented diets decrease blood cholesterol without affecting HDL, LDL, triglyceride, glucose, AST and ALT shows that LEO supplementation did not affect

the health status of the animals to any large extent. Some studies have indicated that herbal extracts (such as turmeric, oregano, anise, cinnamon, garlic and citrus peel) could reduce the level of cholesterol in poultry (Supuka et al. 2015) by acting on acylCoA-cholesterol acyltransferase, which esterifies cholesterol to its esters in tissues (Ciftci et al. 2010). Therefore, the action mechanism of LEO on total cholesterol may be the inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase, a regulating enzyme in cholesterol synthesis (Crowell 1999). Of course, the absence or presence of the cholesterolaemic effect of essential oils depends upon animal breed, gender, age and feed biochemistry (Lee et al. 2003).

In the present study, the 0.08LEO treatment has an antimicrobial effect against only *E.coli*, as reported in *in vitro* (Smith-Palmer et al. 1998; Du et al. 2015) and *in vivo* studies (Sarica et al. 2005) for some essential oils such as cinnamon, clove and thyme. Unfortunately, it is not understood why LEO has antimicrobial effect against *E. coli* alone. As reported in here, there are studies indicating that dietary essential oils have no effect on the other intestinal microbiota (Cross et al. 2007; Muhl and Liebert 2007). These results may be related to the fact that the population and characteristics of inhabitant microbiota in the gut of host animals vary based on the animal species and age as well as segment of gut, composition of diet and environment (Lee et al. 2003; Giannenas et al. 2013).

The results of the present study indicated that LEO supplementation to broiler diets, especially in the 0.08LEO treatment, improved growth performance and carcass traits and reduced blood cholesterol levels and *E. coli* counts. Since the efficacy of LEO has been assessed for the first time in the present study, there is still a need to clarify the effect and mode of action of its active compounds on performance and meat quality in poultry.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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