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Effect of Dietary Oregano and Rosemary Essential Oil Supplementation on Growth Performance and Cecal Microbiota of Broilers

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ABSTRACT: In this study, the effect of dietary supplementation of oregano and rosemary essential oils (EO) on growth performance and cecal microbiota of broilers were investigated. A total of 450 1-d-old male Ross-308 broilers were divided into 5-experimental groups (10 replicates of 9 chickens): a Control (C), fed a basal diet; four treatments, which received a basal diet supplemented with oregano and rosemary EOs individually (O, 300 mg/kg oregano EO; R, 300 mg/kg rosemary EO) and combined (OR1, 150 mg/kg oregano EO + 150 mg/kg rosemary EO; OR2, 200 mg/kg oregano EO + 200 mg/kg rosemary EO). Body weight (BW), feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), and cecal microbiota (coliforms, clostridia and lactobacilli) were determined weekly, and at 42 d, respectively. BW in R ($p<0.05$) and OR2 ($p<0.001$), and BWG and FCR in OR2 ($p<0.05$) were significantly higher than C at 42 d, despite no difference in FI in any group during experimental period. Counts of cecal coliforms ($p<0.001$) and clostridia ($p<0.01$) decreased, and lactobacilli ($p<0.001$) increased substantially between C and treatment groups. Results indicated that combined oregano and rosemary EO (200 mg/kg ea) supplementation significantly increased BW and BWG, improved FCR in 1-42 d, lowered coliform and clostridial, and increased lactobacilli counts suggesting a beneficial shift in cecal microbiota.

Keywords: broiler, essential oil, growth performance, cecal microbiota

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INTRODUCTION

In recent years phytobiotics, which are generally recognized as safe (GRAS), are used in the food and feed industry. Particularly in broilers, they are used as growth promoters by modulating the intestinal microbial flora (Franciosini et al., 2016; Sugiharto, 2016). Different parts of the plant (such as flowers, leaves, seeds, etc.) or the essential oils (EOs) obtained from these parts can be utilized as phytobiotics (O'Bryan et al., 2015). In addition to their distinct effects as stimulating secretion of digestive enzymes, and gastric/intestinal mobility, EOs of aromatic plants are known to have antimicrobial, antiviral, antiparasitic, antifungal, immunomodulating, antioxidant and anti-inflammatory activities (Giannenas et al., 2016; Wati et al., 2015; Zeng et al., 2015).

Oregano (*Origanum vulgare* L.), an aromatic plant widely grown in the Mediterranean region and Asia, has EOs containing highly volatile aroma compounds such as thymol and carvacrol so is used as a feed additive in animal nutrition (Silva et al., 2012). These major compounds have anti-inflammatory and antioxidative activities, and exert their antimicrobial effects by disrupting inorganic ion balance and disturbing pH homeostasis in bacterial membranes (Roofchaee et al., 2011; Zou et al., 2016). Rosemary (*Rosmarinus officinalis* L.) has several naturally active compounds with antioxidant activity, mainly the phenolic diterpenes, such as carnosol, rosmanol, and their acid forms or flavonoids (Cetin et al., 2017). Also, major components of rosemary EOs are monoterpenes such as α-pinene, 1,8-cineole, myrecene and borneol, which possess strong antimicrobial activities (Khazaei et al., 2017; Yesilbag et al., 2011). As is known, the individual or mixed use of EOs in poultry feeds is not new. There are previous reports on the individual and combined use of various levels of oregano (25-1200 mg/kg) and rosemary (100-500 mg/kg) EOs in poultry feed for determining their effect on growth performance and intestinal flora (Abd El-Latif et al., 2013; Al-Kassie et al., 2008; Basmacioglu et al., 2004; Cetin et al., 2016; Franciosini et al., 2016). However, little comparative information is available on the effects of combined usage levels of oregano and rosemary EOs (especially no data on 200 mg/kg each) in broiler diets. These effects can originate from the synergy of different active substances from oregano and rosemary EOs.

Thus, the objective of the present study is to identify the comparative effects of combined usage of oregano and rosemary essential oils in broiler diets at two different levels (150 mg/kg and 200 mg/kg each) on broiler performance and cecal microbiata.

MATERIALS AND METHODS

Birds and housing environment

The study was carried out in Bursa Uludag University Veterinary Faculty Animal Health and Research Center (AHRC) between October - November 2014. A total of 450 one day old male Ross-308 broiler chicks were used, following the protocols of the Ethics Committee of the Bursa Uludag University, Turkey (Ethics Committee No: 2014-11/02). Individually weighed chicks were divided into 5 groups of 90 birds each. Chicks were randomly distributed into 50 floor pens with 9 chicks per pen in the experiment (10 replicate pens for each experimental group). Each pen (1 m x 1 m) was furnished with rice husk in ambient temperature at 32-36 ºC during the first week, then gradually lowered to 25-27 ºC in the next two weeks, while chicks were exposed to natural environmental conditions until 42 d. Birds were exposed to 23 h light - 1 h darkness to day 7, and 20 h light - 4 h darkness from day 8 to day 42. All birds were vaccinated for Marek, Infectious Bursal Disease, Infectious Bronchitis (IB), and Newcastle in the hatchery and against Newcastle and IB at the 12 d.

Experimental design and diets

Five experimental groups of a Control (C) fed a maize-soybean meal basal diet, four treatment groups specifically designed to investigate both broiler performance parameters and microbial population analysis of cecal content received a basal diet supplemented with oregano and rosemary EO individually (O, 300 mg/kg oregano EO; R, 300 mg/kg rosemary EO) or combined (OR1, 150 mg/kg oregano EO + 150 mg/kg rosemary EO; OR2, 200 mg/kg oregano EO + 200 mg/kg rosemary EO). EOs were introduced into the soybean oil, which was added to the feed. Calculated chemical compositions of basal diets for starter (1-10 d), grower (11-24 d) and finisher (25-42 d) broiler growth periods (Table 1) were formulated as isoenergetic and isonitrogenous as recommended for Ross 308 (Ross 308, 2014). Diets were prepared for each period in mash form and stored in airtight containers. Feed and drinking water were provided *ad libitum*. Chemical analyses of diets were performed according to the AOAC methods (AOAC, 2000). Metabolizable Energy (ME) level estimations were calculated by the formula (ME kcal/kg = (37.07 x crude protein) + (82 x crude fat) + (39.89 x starch) + (31.1 x sugar) indicated in Turkish

Standard on Animal Feeds - Determination of Metabolizable Energy (Chemical Method) (TSE, 1991). EOs added to basal diet were supplied from Farmavet® International Feed and Water Additives Specialist, Manisa, Turkey, and were stored in a cool and dry place with no sunlight. Components of oregano EO (major component carvacrol), and rosemary EO (major components α-pinene and 1,8-cineole), determined by gas chromatography, was provided by the supplier (Table 2). Analyses were carried out on a MS-Thermo Polaris Q GC Thermo Trace GC (Thermo Fisher Inc., MA, USA). Chromatograms were determined using MS (Mass Spectrometer). Data were calculated using internal standards (Pala-Paul et al., 2004).

Table 1. Chemical analysis of the experimental diets with varying essential oil concentrations

Ingredient (g/kg-as fed)	**Basal diets**		
	Starter (1-10 d)	**Grower** (11-24 d)	**Finisher** (25-42 d)
Maize	530.4	596.6	642.5
Soybean meal	300.0	171.0	127.2
Full-fat soybean meal	64.0	78.0	77.8
Maize gluten meal	47.1	100.0	100.0
Soybean oil[1]	20.0	20.0	20.0
Limestone	14.7	12.5	12.1
Dicalcium phosphate	9.8	8.2	7.3
Sodium chloride	2.9	3.0	3.0
Sodium bicarbonate	1.0	1.0	1.0
Cholin chloride 70%	1.5	2.0	2.2
L-Lysine	4.2	4.5	4.1
DL-Methionine	2.4	1.2	0.8
Vitamin and mineral premix*	2.0	2.0	2.0
Analysed chemical composition (g/kg-as fed)			
ME (MJ/kg)†	12.9	12.8	13.2
Dry matter	882.0	918.0	922.0
Crude protein	221.0	201.0	205.0
Crude fat	62.3	65.0	59.0
Crude ash	58.3	45.0	45.0
Starch	349.0	358.0	399.1
Sugar	51.4	44.4	40.3

[1]Oregano and rosemary essential oil were included in soybean oil.
*Supplied per kg diet: Vitamin A (retinol) 3 g, Vitamin D3 (cholecalciferol) 38 mg, Vitamin E (dl-alpha tocopherol) 18.2 g, Vitamin K3 (menadione) 3 mg, Vitamin B1 (thiamine) 60 mg, Vitamin B2 (riboflavin) 12.5 mg, Vitamin B6 (pyridoxal) 5 mg, Vitamin B12 (cobalamine) 0.015 mg, Vitamin B5 (panthotenic acid) 15 mg, Vitamin B3 (niacin) 60 mg, Vitamin B9 (folic acid) 2 mg, Mn 80 mg, Fe 60 mg, Zn 60 mg, Cu 5 mg, I 1 mg, Co 0.5 mg, Se 0.15 mg. †Metabolisable energy values for the experimental diets were calculated in kcal/kg according to TSE, 1999, and converted to MJ/kg.

Table 2. Components of oregano and rosemary essential oils

Components			
Oregano oil (%)		**Rosemary oil (%)**	
Carvacrol	61.31	α-pinene	26.00
Linalool	19.58	1,8 Cineole	26.20
γ-terpinene	3.09	Borneol	21.00
ρ-cymene	2.24	Camphene	11.40
β-bisabolene	2.12	β-pinene	6.53
Borneol	2.12	Limonene	2.00
Thymol	2.02	Camphor	2.00
β-caryophyllene	1.43	β-myrcene	1.08
Terpinen-4-ol	1.10	γ-terpinene	0.75
α-terpinene	1.00	α-terpinene	0.40
β-myrcene	0.75	Undefined	2.64
β-pinene	0.38		
Sabinene	0.36		
Undefined	2.50		

Broiler performance parameters

For body weight (BW), each bird was weighed at the first day and weekly. The feeds given and taken back were weighed weekly. Body weight gain (BWG) and feed intake (FI) data were calculated weekly based on mean of replicates per pen. Feed conversion ratio (FCR) was calculated by dividing FI into body weight gain (BWG) (g/g) each week. Number of dead birds was recorded daily, as adjusting the total number of birds determines the total FI per bird.

Collection and preparation of cecal content

Fifty cecal content samples (one sample from a slaughtered broiler from each replicate pen) were collected for microbial population analyses from 42 d old broilers slaughtered in the pilot slaughter facility of Food Unit in AHRC. Following the removal of gastrointestinal tract, ileum, cecum and colon were aseptically divided into sections by ligating with light twine before separating the cecum from the small intestine, and were transferred to the laboratory in cold chain (Mountzoirus et al., 2011). For each animal, after opening the cecum, all content was placed into sterile stomacher bags, hand-massaged form outside, and one g was weighed into a sterile test tube containing 9 ml (0.1% v/v) of peptone water. Serial dilutions from 10^{-1} to 10^{-7} were performed (ISO, 2017).

Intestinal microbial population analysis of cecal microbiota

Coliform counts were determined after parallel-plating as double layered pour plates on Violet Red Bile (VRB, CM0107, OXOID, London, UK) agar from respective dilutions incubated at 37°C for 24-48 h, and was done accordingly (Yasar et al., 2011). For lactobacilli counts, samples from respective dilutions were parallel-plated as double layered pour plates using De Man Rogosa and Sharpe (MRS, CM 0361, OXOID, London, UK) agar, and incubated at 35°C for 3 d under 5% CO_2 (AnaeroJar AG0025, OXOID, London, UK) with Anaerogen (AN0025, OXOID, London, UK) packs (Franciosini et al., 2016). Enumeration of clostridia from samples was performed using Reinforced Clostridial (RCM, CM0151, OXOID, London, UK) agar. Parallel-plated as double layered pour plates were incubated anaerobically at 37°C for 20±2 h in AnaeroJar (AG0025, OXOID) with Anaerogen (AN0025, OXOID) packs and colonies were counted and recorded according to Mountzouris et al. (2011). All bacterial counts were converted to $\log_{10}$ cfu/g for statistical analysis.

Statistical analyses

One-Way Analysis of Variance (ANOVA) was used with General Linear Models (GLM) procedure of the IBM SPSS statistics software v. 22.0, 2013 (SPSS, 2013). Significant differences within mean values of groups were subjected to Tukey's test as post-test. Chi-Square test was used for calculating and statistics of mortality rates. Differences were considered significant when $p<0.05$ (Dawson and Trapp, 2001).

RESULTS

When effects of oregano and rosemary EO supplementation on broiler growth performance during rearing period were considered (Table 3); BW in C was significantly lower than R ($p<0.05$) and OR2 ($p<0.001$) by 42 d. Within treatment groups, birds in R had significantly higher BW than those in OR1 and OR2 treatments on 21 d ($p<0.05$). While there was no statistically significant difference in BWG and FCR values within groups from 1 to 35 d, OR2 group had better BWG and FCR than C ($p<0.05$) in 42 d. No meaningful difference between FI data of groups during the rearing period was observed ($p>0.05$).

The total mortality rates were 4.4, 3.3, 5.6, 0 and 3.3% for C, O, R, OR1 and OR2, respectively at 42 d (data not shown in Table 3). No significant difference was found between mortality rates of groups throughout the trial ($p>0.05$).

Effect of dietary supplementation with EO on cecal microbiota for coliforms, clostridia and lactobacilli counts at 42 d (Table 4) showed approximately 1 log reduction in coliforms and clostridia counts, and 1 log increase in lactobacilli counts in C and treatment groups. Significant differences in coliforms and lactobacilli counts ($p<0.001$), and clostridia counts ($p<0.01$) between C and all treatments, and no difference within treatment groups ($p>0.05$) were observed.

DISCUSSION

There are many studies supplementing diets with EO at 25-1200 mg/kg, when summarized, indicated inconsistent efficacy of oregano and rosemary EOs on growth performance in poultry. In detail, positive effects on BW and BWG when used as 25 ppm oregano (Giannenas et al., 2016), 100 mg/kg oregano and rosemary (Mathlouthi et al., 2012), 150 ppm rosemary (Yesilbag et al., 2011), 500 mg/kg rosemary (Manafi et al., 2014) EOs were reported, despite others findings no ef-

Table 3. Effect of oregano and rosemary essential oil supplementation on growth performance in broiler

Parameters	Treatments (Mean ± SD)									
	C	n	O	n	R	n	OR1	n	OR2	n
Day 1										
BW (g)	44.25±3.65	90	44.81±3.64	90	45.32±3.63	90	44.41±3.32	90	44.83±3.83	90
Day 1-7										
BW (g)	147.95±19.11	88	148.89±20.95	87	151.00±14.88	88	145.82±21.83	90	148.76±22.55	89
BWG (g)	103.72±8.52	10	104.19±12.39	10	105.67±6.73	10	102.53±6.84	10	105.82±9.67	10
FI (g)	186.44±11.51	10	167.98±19.01	10	176.73±18.07	10	180.38±13.96	10	176.32±12.82	10
FCR (g/g)	1.81±0.21	10	1.62±0.18	10	1.67±0.15	10	1.76±0.11	10	1.67±0.15	10
Day 1-14										
BW (g)	411.62±46.10	88	418.19±58.87	87	428.10±51.65	88	416.87±46.00	90	424.10±53.97	89
BWG (g)	367.71±16.84	10	374.11±32.12	10	382.61±22.07	10	372.46±17.42	10	381.20±21.95	10
FI (g)	603.06±46.91	10	621.63±34.96	10	608.99±32.27	10	601.42±29.35	10	629.83±32.54	10
FCR (g/g)	1.64±0.16	10	1.67±0.12	10	1.60±0.11	10	1.62±0.09	10	1.66±0.12	10
Day 1-21										
BW (g)*	797.72^{ab}±89.87	87	796.27^{ab}±98.59	87	823.25^{b}±92.13	88	780.92^{a}±93.83	90	783.97^{a}±98.55	89
BWG (g)	755.92±43.46	10	752.94±44.80	10	776.95±45.95	10	736.52±54.55	10	742.99±41.20	10
FI (g)	1232.01±99.60	10	1260.04±56.26	10	1245.06±72.65	10	1205.25±76.77	10	1254.44±89.18	10
FCR (g/g)	1.63±0.11	10	1.68±0.07	10	1.60±0.08	10	1.64±0.05	10	1.69±0.12	10
Day 1-28										
BW (g)	1369.16±179.91	87	1371.32±189.93	87	1400.99±155.87	87	1365.67±163.62	90	1400.05±185.36	89
BWG (g)	1327.68±75.94	10	1328.45±104.13	10	1355.10±65.64	10	1331.20±106.41	10	1354.67±67.27	10
FI (g)	2086.37±145.85	10	2108.70±121.82	10	2112.22±103.69	10	2048.23±145.65	10	2152.89±82.92	10
FCR (g/g)	1.57±0.10	10	1.59±0.10	10	1.56±0.06	10	1.54±0.10	10	1.59±0.07	10
Day 1-35										
BW (g)	2060.18±269.96	87	2090.68±275.74	87	2125.03±261.89	87	2099.07±264.98	90	2110.43±279.22	89
BWG (g)	2020.55±132.22	10	2047.25±124.61	10	2079.12±125.37	10	2054.67±147.78	10	2063.88±85.49	10
FI (g)	3250.04±233.29	10	3272.03±143.52	10	3306.31±159.60	10	3257.99±195.99	10	3338.20±146.56	10
FCR (g/g)	1.61±0.07	10	1.60±0.05	10	1.59±0.06	10	1.59±0.05	10	1.62±0.07	10
Day 1-42										
BW (g)**	2606.98^{a}±292.78	86	2685.74^{ab}±310.12	87	2757.80^{b}±299.44	86	2701.57^{ab}±326.99	90	2801.25^{b}±316.58	87
BWG (g)*	2568.28^{a}±141.02	10	2641.09^{ab}±143.36	10	2711.69^{ab}±127.16	10	2657.16^{ab}±167.87	10	2755.22^{b}±85.69	10
FI (g)	4498.59±258.15	10	4580.20±166.84	10	4603.26±224.09	10	4540.28±277.15	10	4632.22±153.46	10
FCR (g/g)*	1.75^{b}±0.07	10	1.74^{ab}±0.07	10	1.70^{ab}±0.04	10	1.71^{ab}±0.02	10	1.68^{a}±0.04	10

SD: Standard deviation, BW: Body weight, FI: Feed intake, FCR: Feed conversion ratio, C: Control group, O: 300 mg/kg oregano oil, R: 300 mg/kg rosemary oil, OR1:150 mg/kg oregano oil and 150 mg/kg rosemary oil, OR2: 200 mg/kg oregano oil and 200 mg/kg rosemary oil. $^{a,b:}$ Means within the same row with different superscripts are significantly different ($^{*}p<0.05$) ($^{**}p<0.001$)

Table 4. Effect of oregano and rosemary essential oil supplementation on cecal microbiota in broiler diets

Cecal microbiota (log_{10} cfu/g)	Treatments (Mean ± SD)									
	C	n	O	n	R	n	OR1	n	OR2	n
Coliforms**	6.19^{a} ± 0.09	10	5.19^{b} ± 0.15	10	5.28^{b} ± 0.22	10	5.43^{b} ± 0.14	10	5.49^{b} ± 0.12	10
Lactobacilli**	7.16^{b} ± 0.17	10	8.33^{a} ± 0.12	10	7.97^{a} ± 0.14	10	8.18^{a} ± 0.15	10	8.01^{a} ± 0.17	10
Clostridia***	7.02^{a} ± 0.12	10	6.23^{b} ± 0.24	10	6.05^{b} ± 0.09	10	6.18^{b} ± 0.20	10	6.02^{b} ± 0.18	10

SD: Standard deviation, C: Control group, O: 300 mg/kg oregano oil, R: 300 mg/kg rosemary oil, OR1: 150 mg/kg oregano oil and 150 mg/kg rosemary oil, OR2: 200 mg/kg oregano oil and 200 mg/kg rosemary oil. ^{a,b}: Means within the same row with different superscripts are significantly different (**p<0.001) (***p<0.01)

fect (Alp et al., 2012; Basmacioglu et al., 2004; Botsoglou et al., 2002; Bugdayci and Ergun, 2011; Cetin et al., 2016). Roofchaee et al. (2011) noted improvement in FCR without affecting BWG and FI with 600 mg/kg and 1200 mg/kg levels of oregano EO supplementation, compared to a lower level (300 mg/kg), which did not affect FCR. Additionally, there are several reports for enhanced FCRs at levels of 25 ppm oregano EO (Giannenas et al., 2016), 100 mg/kg oregano

and rosemary EOs (Mathlouthi et al., 2012), 200 ppm rosemary EO (Bugdayci and Ergun, 2011), 300 mg/kg oregano EO (Alp et al., 2012), despite non-significant data (Basmacioglu et al., 2004; Botsoglou et al., 2002; Cetin et al., 2016; Kirkpinar et al., 2011; Manafi et al., 2014). Interestingly, a considerable decrease in BW with insignificant and negative effect on FCR was reported by supplementing 300 mg/kg oregano EO (Kirkpinar et al., 2011) and 200 mg/kg rosemary EO (Abd El-Latif et al., 2013) in diets, respectively. In earlier studies using these EOs in combination, Mathlouthi et al. (2012) found improvement in BW, BWG and FCR when feed was supplemented with 100 mg/kg (50 mg/kg oregano + 50 mg/kg rosemary) at 42 d, while Basmacioglu et al. (2004) observed no effect in live weight and FCR by 150 mg/kg (75 mg/kg oregano + 75 mg/kg rosemary) and 300 mg/kg (150 mg/kg oregano + 150 mg/kg rosemary) supplementation to diet.

Similar to our study, supplementation with these EOs individually did not affect FI in studies by Cetin et al. (2016), Kirkpinar et al. (2011) and Yesilbag et al. (2011), besides significant reductions in FI reported by Alp et al. (2012), Bugdayci and Ergun (2011), Mathlouthi et al. (2012), and significant increases in FI by Abd El-Latif et al. (2013) and Manafi et al. (2014). Also, inclusion of these EOs in combination as 100 mg/kg (50 mg/kg oregano + 50 mg/kg rosemary) to the diet caused an increase in FI (Mathlouthi et al., 2012), whereas other two combinations at the levels of 150 mg/kg (75 mg/kg oregano + 75 mg/kg rosemary) and 300 mg/kg (150 mg/kg oregano + 150 mg/kg rosemary) by Basmacioglu et al. (2004) indicated no effect at 0-42 d. In this study, dietary supplementation with combination of 200 mg/kg EOs (oregano 200 mg/kg + rosemary 200 mg/kg) improved BW, BWG and FCR without affecting FI. Also, individual rosemary EO supplementation improved BW. However, dietary supplementation with oregano EO, and also combination of two EOs in lower levels (150 mg/kg each) did not affect BW, BWG, FCR and FI at 1-42 d (Table 3). As it is known, the diet levels and the chemical structures of EOs, managing conditions, and dietary formulations used in trials can lead to different performance results. However, it is interesting that differences in performance parameters from negative to positive occur when EO is added to broiler feeds at close levels. Data in this study indicated that using oregano and rosemary EO can positively effect BWG and FCR of the broilers due to diet level in last week but not in early weeks. The insignificant differences in performance parameters in the previous weeks may be due to the fact that young animals had higher growth rates, and the amount of EO consumed per animal was lower compared to the the final week.

In this study, cecal coliforms in C were 6.19 $\log_{10}$ cfu/g, while counts decreased almost 1 log in all treatments, and was significantly. However, there was no statistical difference in coliform counts within treatment groups (Table 4). Similar to our study, considerable reductions in coliforms, regardless of the amount used, with the individual supplementation of 15 mg/kg and 30 mg/kg oregano, 5% oregano, 0.5% and 1% rosemary EOs to the feeds were reported previously by Al-Kassie et al. (2008), by Cetin et al. (2016), and by Giannenas et al. (2016), respectively. Also, in a study by Cetin et al. (2016), combined addition of oregano, fennel, and rosemary EOs in three different concentrations (100, 200 and 400 mg/kg) to feed caused significant declines in coliform counts. Another finding in these studies coinciding with ours is no statistical difference in coliform counts within treatment groups when EOs were used individually or combined. In contrast, effect of an EO mix of different (80, 125, 250 mg/kg) levels including oregano on coliform reductions was found statistically insignificant by Mountzouris et al. (2011). One more additional contradictory outcome by Franciosini et al. (2016) reported statistical differences between increasing coliform counts in individual (2 g/kg), and decreasing counts in combined (1 g/kg each) treatments of oregano and rosemary aqueous extract treatment groups in 57 d. These differences could be mainly related to rearing period of the experiment, sampling day of the cecal content, EO form used, use of EOs with other phytobiotics, inclusion level of EOs to diet.

Lactobacilli counts in cecal content of C group were 7.16 $\log_{10}$ cfu/g, while these counts inclined almost 1 log in all treatments. This increase in lactobacilli counts with individual or combined addition of EOs to the feed was found statistically significant. However, there was no considerable difference in counts within treatment groups (Table 4). There are reports indicating statistically significant increases in lactobacilli counts in broiler cecal microbiota by the use of different levels of oregano and rosemary EOs in feeds (Cetin et al., 2016; Franciosini et al., 2016; Giannenas et al., 2016; Mountzouris et al., 2011). In-

dividual and combined use of 5% oregano and laurel EOs by Giannenas et al. (2016) was found to have no influence on lactobacilli counts, which is similar to our 'dose and combined use' finding. In contrast, Franciosini et al. (2016) found statistically significant decreases in lactobacillus counts in their studies using 2 g/kg thyme aqueous extract. Additionally, in studies by Al-Kassie et al. (2008), Aksu and Bozkurt (2009), and Cho et al. (2014), lactobacilli counts remained stable in both control and rosemary supplemented groups. The primary reasons for such a difference may be the use of different forms of EO, the levels of inclusion in the diets, and the use with other phytobiotics. In all experimental groups, especially OR2 and R groups, which have the highest inhibitory effect, were found to have almost 1 log reduction in clostridia numbers when compared to group C. Decline in clostridia counts with individual or combined addition of EOs to feed was found statistically significant. However, there was no considerable difference in clostridia counts within treatment groups (Table 4). Parallel to our findings, where influence of 300 mg/kg oregano EO supplementation in broiler diets to intestinal flora was investigated, oregano (5.57 $\log_{10}$ cfu/g) was found to cause a statistically significant (1 log) reduction in the initial *Clostridium* counts compared to 6.00 $\log_{10}$ cfu/g counts in control (Kirkpinar et al., 2011). Contrarily, in another study, where oregano, anise and citrus mix was used in 80, 125, 250 mg/kg doses in broiler feeds, there was no significant difference between treatment groups and 7.9 $\log_{10}$ cfu/g *Clostridium* in cecal microbiota of the control group (Mountzouris et al., 2011).

Current study results suggested that combined oregano and rosemary EO (200 mg/kg each) supplementation significantly increased BW, BWG and improved FCR in 42 d, as well as beneficially influenced cecal microbiota of broilers by lowering coliform and clostridia, and increasing lactobacilli counts. It may not be correct to attribute this improvement in broiler performance parameters to the positive effect of EO on intestinal microbiota only, but it is possible to attribute synergetic positive effects of EOs for their anti-inflammatory, anti-oxidant properties, and digestive enzyme activation.

Thus, future research will lead the way of the efficient use of oregano and rosemary EO as feed additives in broiler nutrition, and also it is important to ensure the sustainability of both aromatic plants, which are the source of EOs and natural resources such as feedstuffs.

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REFERENCES

Abd El-Latif AS, Saleh NS, Allam TS, Ghazy EW (2013) The effects of rosemary (Rosemarinus officinalis) and garlic (Allium sativum) essential oils on performance, haematological, biochemical and immunological parameters of broiler chickens. Brit J Poult Sci 2: 16-24.

Aksu T, Bozkurt AS (2009) Effect of dietary essential oils and/or humic acids on broiler performance, microbial population of intestinal content and antibody titres in the summer season. J Fac Vet Med Kafkas Univ 15: 185-190.

Al-Kassie GAM, Mohammed MF, Hamood MF, Jameel YJ (2008) The effect of anise and rosemary on the microbial balance in gastro intestinal tract for broiler chicks. Int J Poult Sci 7: 610-612.

Alp M, Midilli M, Kocabagli N, Yilmaz H, Turan N, Gargili A, Acar N (2012) The effect of dietary oregano essential oil on live performance, carcass yield, serum immunoglobulin g level, and oocyst count in broilers. J Appl Poult Res 21: 630-636.

AOAC (2000) Official Methods for Analysis. 18th ed. Arlington USA: Association of Official Analytical Chemists.

Basmacioglu H, Tokusoglu O, Ergul M (2004) The effect of oregano and rosemary essential oils or alpha-tocopheryl acetate on performance and lipid oxidation of meat enriched with n-3 PUFA's in broilers. S Afr J Anim Sci 34: 197-210.

Botsoglou NA, Florou-Paneri P, Christaki E (2002) Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. Br Poult Sci 43: 223-230.

Bugdayci KE, Ergun A (2011) The effects of supplemental essential oil and/or probiotic on performance, immune system and some blood parameters in broilers. Vet J Ankara Univ 58: 279-284.

Cassir N, Benamar S, La Scola B (2016) Clostridium butyricum: form beneficial to a new emerging pathogen. Clin Microbiol Infect 22: 37-45.

Cetin E, Yibar A, Yesilbag D, Cetin I, Cengiz SS (2016) The effect of volatile mixtures on the performance and ilio-cecal microbiota of broiler chickens. Br Poult Sci 57: 780-787.

Cetin I, Yesilbag D, Cengiz SS, Belenli D (2017) Effects of supplementation with rosemary (Rosmarinus officinalis L.) volatile oil on growth performance, meat MDA level and selected plasma antioxidant parameters in quail diets. Kafkas Univ Vet Fak Derg 23 (2): 283-288.

Cho JH, Kim HJ, Kim IH (2014) Effects of phytogenic feed additive on growth performance digestibility, blood metabolites, intestinal microbiota, meat color and relative organ weight after oral challenge with Clostridium perfringens in broilers. Livest Sci 160: 82-88.

Dawson B, Trapp RG (2001) Basic and Clinical Biostatistics. 3rd ed. New York: Lange Medical Books/ McGraw-Hill Medical Publishing Division.

Franciosini MP, Casagrande-Proietti P, Forte C (2016) Effects of oregano (Origanum vulgare L.) and rosemary (Rosmarinus officinalis L.) aqueous extracts on broiler performance, immune function and intestinal microbial population. J Appl Anim Res 44: 474-479.

Giannenas I, Tzora A, Sarakatsianos I, Karamoutsios A, Skoufos S, Papaioannou N, Anastasiou A, Skoufos I (2016) The effectiveness of the use of oregano and laurel essential oils in chicken feeding. Ann Anim

Sci 16: 779-796.
ISO (2017) International Organization for Standardization. Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 1: General rules for the preparation of the initial suspension and decimal dilutions. Switzerland: Geneva. ISO 6887-1:2017.
Khazaei R, Esmailzadeh L, Seidavi A, Simões J (2017) Comparison between rosemary and commercial antioxidant blend on performance, caecal coliform flora and immunity in broiler chickens fed with diets containing different levels of poultry fat. J Appl Anim Res 45: 263-267.
Kirkpinar F, Unlu HB, Ozdemir G (2011) Effects of oregano and garlic essential oils on performance, carcase, organ and blood characteristics and intestinal microbiota of broilers. Livest Sci 137: 219-225.
Manafi M, Hedayati M, Yari M (2014) Effectiveness of rosemary (Rosmarinus officinalis L.) essence on performance and immune parameters of broilers during aflatoxicosis. Adv Life Sci 4: 166-173.
Mathlouthi N, Bouzaienne T, Oueslati I (2012) Use of rosemary, oregano, and commercial blend of essential oils in broiler chickens: In vitro antimicrobial activities and effects on growth performance. J Anim Sci 90: 813-823.
Mountzouris KC, Paraskevas V, Tsirtsikos P (2011) Assessment of a phytogenic feed additive effect on broiler growth performance, nutrient digestibility and cecal microbiota composition. Anim Feed Sci Technol 168: 223-231.
O'Bryan CA, Pendleton SJ, Crandall PG, Ricke SC (2015) Potential of plant essential oils and their components in animal agriculture-in vitro studies on antibacterial mode of action. Front Vet Sci 2: 1-8.
Ross 308 (2014) Nutrition Specifications. Aviagen, Brochure No: 0814-AVNR-035 tmea.staging.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-308-Broiler Nutrition-Specs-2014r17-EN.pdf
Pala-Paul J, Garcia-Jimenez R, Perez-Alonso MJ, Velasco-Negueruela A, Sanz J (2004) Essential oil composition of the leaves and stems of Meum athamanticum Jacq., from Spain. J Chromatogr 1036: 245-247.
Roofchaee A, Irani M, Ebrahimzadeh MA, Akbari MR (2011) Effect of dietary oregano (Origanum vulgare L.) essential oil on growth performance, cecal microbiota and serum antioxidant activity of broiler chickens. Afr J Biotechnol 10: 6177-6183.
Silva N, Alves S, Goncalves A, Amaral JS, Poeta P (2012) Antimicrobial activity of essential oils from Mediterranean aromatic plants against several foodborne and spoilage bacteria. Food Sci Technol Int 19: 503-510.
SPSS (2013) Statistical Package for the Social Sciences. Version 22.0. Armonk NY: IBM Corporation.
Sugiharto S (2016) Role of nutraceuticals in gut health and growth performance of poultry. J Saudi Soc Agri Sci 15: 99-111.
TSI (1991) Turkish Standards Institution. Animal Feeds - Determination of Metabolizable Energy (Chemical Method). Ankara: TS 9610.
Wati T, Ghosh TK, Syed B, Haldar S (2015) Comparative efficacy of a phytogenic feed additive and an antibiotic growth promoter on production performance, caecal microbial population and humoral immune response of broiler chickens inoculated with enteric pathogens. Anim Nutr 1: 213-219.
Yasar S, Namik D, Fatih G, Gokcimer A, Selcuk K (2011) Effects of inclusion of aeriel dried parts of some herbs in broiler diets. J Anim Plant Sci 21: 465-476.
Yesilbag D, Eren M, Agel H, Kovanlikaya A, Balci F (2011) Effects of dietary rosemary, rosemary volatile oil and vitamin e on broiler performance, meat quality and serum SOD activity. Br Poult Sci 52: 472-482.
Zeng Z, Zhang S, Wang H, Piao X (2015) Essential oil and aromatic plants as feed additives in non-ruminant nutrition: A review. J Anim Sci Technol 6: 1-10.
Zou Y, Xiang Q, Wang J, Peng J, Wei H (2016) Oregano essential oil improves intestinal morphology and expression of tight junction proteins associated with modulation of selected intestinal bacteria and immune status in a pig model. Biomed Res Int 1-11.