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Efficacy of Different Blends of Essential Oils on Growth Performance, Blood Metabolites, Gut Microflora, and Meat Quality of Broilers

Maziar Mohiti-Asli[1], Milad Khedmatgozar[1], Hassan Darmani-Kuhi[1], Mohsen Farzaneh[2]

[1]Department of Animal Sciences, Faculty of Agriculture Sciences, University of Guilan, Rasht, Iran
[2]Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran

Abstract:

BACKGROUND: The application of phytogenic additives in the form of essential oils have attracted considerable attention in poultry industry.

OBJECTIVES: An experiment was conducted to evaluate effects of the blends of essential oils (EO) isolated from some medicinal plants including savory, thyme, fennel and rosemary on performance, blood metabolites, intestinal microflora, and meat quality of broilers.

METHODS: Two hundred 1-day-old broilers were randomly allocated to 5 treatments with 4 replicates of 10 chicks. The dietary treatments included 1) NC (negative control; without EO), 2) PC (positive control; 300 mg commercial EO of oregano/kg diet), 3) SR (300 mg EOs of savory and rosemary/kg diet), 4) SRT (300 mg EOs of savory, rosemary and thyme/kg diet), 5) SRTF (300 mg EOs of savory, rosemary, thyme and fennel/kg diet).

RESULTS: Neither PC nor combinations of EOs affected growth performance of broilers. Serum cholesterol reduced (P<0.05) by feeding the blend of EOs compared with NC. The lowest ileal *Lactobacillus* counts were observed in chicks fed diets supplemented with EO of oregano or SR (P<0.05). Dietary supplementation of oregano EO decreased (P<0.05) lipid peroxidation of thigh meat after 30 and 60 days of frozen storage compared with NC group.

CONCLUSIONS: Broilers fed diets supplemented with EOs of oregano or SR had the lowest ileal *Lactobacillus* spp. counts probably due to their large amount of carvacrol contents which suppress growth and proliferation of Gram-positive bacteria. Contrary to blended EOs, oregano EO did not reduce serum cholesterol concentration, suggesting hypocholesterolemic effect of other compounds except carvacrol in EOs. The highest stability to meat oxidation was achieved in oregano EOs-fed broilers. This antioxidative effect was lower in treatments with less carvacrol contents.

Keywords:

Broiler, Essential oils, Gut microflora, Meat quality, Performance

Correspondence

Maziar Mohiti-Asli, Department of Animal Sciences, Faculty of Agriculture Sciences, University of Guilan, Rasht, Iran

Tel: +98(13) 33690274, Fax: +98(13) 33690281, Email: mmohiti@guilan.ac.ir

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Introduction

The growing resistance of microorganisms to conventional chemicals and antibiotics has become a major concern in global public health that has prompted research toward the identification of potential antimicrobial agents with broad activity (Diaz-Sanchez et al., 2015). Essential oils (EO) are concentrated hydrophobic liquids containing the volatile aroma compounds produced as secondary metabolites in plants. Most EOs are made up of 20 to 60 compounds from a variety of chemical classes, mainly constituted by alcohols, aldehydes, esters, ethers, ketones, phenols, and terpenes. The EOs have been known for important biological activities, such as antimicrobial, antioxidant and inflammatory activity (Steiner, 2010). The antibacterial activity of EOs would be closely attributed to the incidence of phenolic components, while other constituents are believed to have little effect, interactions among these compounds should not be ignored (Burt, 2004). Regular combinations of EOs or their purified main components targets several biochemical processes in the bacteria leading to a synergistic, additive, or occasionally even antagonistic effects. The mechanisms of action of the EO blend are dependent on their chemical composition. Phenolic compounds are hydrophobic molecules with antimicrobial activity. The major phenolic compounds of thyme oil are carvacrol and thymol which exert similar antimicrobial effect from different mechanisms. The hydrophobicity of the phenolics allows them to easily penetrate to the lipid bilayer of the cytoplasmic membrane, leading to the leakage of ions and other critical molecules, resulting in death of the cell (Nazzaro et al., 2013). The efficacy of EO on gut microflora in poultry is not consistent as it was supplemented to the diets at the levels of 20-200 mg EO/kg diet, which is below the range found in several in vitro studies (0.03 to 2.0%) to have antimicrobial activity (Hammer et al., 1999). Few studies could demonstrate reduced intestinal microbiota, including *Escherichia coli* and Clostridium perfringens, in broilers (Jamroz et al., 2003; Clavijo and Flórez, 2017). It was emphasized that selection of proper plants, active components, and efficacious dietary doses are important for suitability of these substances to influence poultry performance. However, essential oils have been extensively studied and have been used in aviculture to improve feed safety, further research is required to confirm if they can improve the productive parameters and poultry health (Diaz-Sanchez et al., 2015).

Recently, different commercial combinations of EOs have been studied in poultry diets; however, their biological efficacy presents a scattered picture (Hafeez et al., 2016). Therefore, there is a need to move beyond a perfunctory performance-based, product-driven research to a more mechanistic approach to fully understand where and how plant-derived compounds are working in our domestic livestock and poultry species (Steiner, 2010). The main objectives of the current study were to determine the components of freshly prepared EOs from some indigenous herbs grown in Iran and evaluate their antimicrobial and antioxidant effects in an in vivo trial on broiler chickens.

Materials and Methods

Preparation of EOs: Aerial parts of fresh

Thymus daenensis celak, Satureja khuzistanica, Foeniculum vulgare and Rosemarinus officinalis were prepared from Medical Plant and Drugs Research Institute (Shahid Beheshti University, Tehran, Iran), dried in dark at room temperature and then powdered with a blender after verifying the species and elimination of trash bodies. The EOs were isolated by means of hydrodistillation, using Clevenger-type apparatus. The extraction procedure was performed for 3 h. Distilled oils were dried by anhydrous sodium sulfate, poured in opaque vials and stored at 4 °C until required.

GC-FID and GC–MS analysis: GC analysis was performed with a Thermoquest (San Jose, CA) gas chromatograph with a flame ionization detector (FID). Analysis carried out using fused silica capillary DB-1 column (60 m × 0.25 mm i.d.; film thickness 0.25 μm). Operating conditions were as follows: injector and detector temperatures were 250 °C and 300 °C, respectively. Nitrogen was used as carrier gas at a flow rate of 1 ml/min; oven temperature programmed, 60 °C-250 °C at the rate of 0.067 °C/s, and finally held isothermally for 60 s.

GC-MS analysis was also performed by using Thermoquest-Finnigan (San Jose, CA) gas chromatograph equipped with column described above and coupled with a TRACE mass quadrupole detector. Helium was used as carrier gas with ionization voltage of 70eV. Ion source and interface temperatures were 200 °C and 250 °C, respectively. Mass range was from m/z 43-456. Gas chromatographic conditions were as given for GC.

Identification of compounds: The retention indices were calculated for all volatile constituents using a homologous series of n-alkanes C6 - C24. Identification of individual compounds was carried out by comparison of their mass spectra with those of similar compounds from a database (Wiely/NBS library) or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those reported in the literature (Özcan et al., 2006). For quantification purpose, relative area percentages obtained by FID were used without using correction factors.

Birds, diets, and management: The management of birds and all the experimental procedures performed in this study were approved by the Animal Care and Use Committee of University of Guilan. A total of 200 one-day-old straight-run broiler chicks (Ross 308; Navid Morgh Guilan Co., Guilan, Iran) were randomly allotted by BW into one of 5 treatments, each having 4 replicate floor pens with 10 chicks per pen. Chicks in each replicate were kept in a 1×1 m floor pen, deep littered with clean softwood shavings and equipped with a round feeder and a round drinker. The experiment was performed as a completely randomized design and the dietary treatments included 1) NC (negative control; basal diet without EO), 2) PC (positive control; 300 mg commercial EO of oregano/kg basal diet), 3) SR (300 mg EOs of savory and rosemary/kg basal diet), 4) SRT (300 mg EOs of savory, rosemary and thyme/kg basal diet), 5) SRTF (300 mg EOs of savory, rosemary, thyme and fennel/kg basal diet). Each of the EO blends consisted of the oils in equal ratios which were mixed with calcium carbonate as a feed grade carrier to compose 5% (w/w) dilution. The commercial EO of oregano used as PC was in the form of a powder called Orego-Stim® (Meriden Animal Health Ltd., Luton, UK) that contains

5% EO of Origanum vulgare subsp. Hirtum plants and 95% natural feed grade inert carrier. Diets were formulated according to Ross 308 broiler manual (Aviagen, 2014) and prepared in mash form at pilot feed mill at University of Guilan. Table 1 presents ingredients and nutrient composition of the basal diet. Chicks were reared from 1 d old on the experimental diets and were allowed ad libitum access to both feed and water throughout experiment. Temperature and relative humidity were maintained within the optimum range. There was continuous light regimen for the first 2 d, and then 23 h lighting was applied thereafter. All the chicks were vaccinated based on a routine program.

Sampling and measurements: Chicks were weighed every week and feed consumption in each pen was recorded at weekly intervals. Mortality was recorded daily and weighed at occurrence. From these data, average daily body weight gain (BWG), average daily feed intake (ADFI) and feed conversion ratio (FCR) were calculated by week and for the entire experimental period.

Two broilers were selected from each pen with body weight closest to the mean pen weight and were killed by exsanguinations at 42 days of age. The body cavity was immediately opened and the ileum section was dissected and ileal digesta was aseptically flushed out with deionized water from the distal half of the ileum to sterile microtubes and kept frozen at -20 ºC until enumeration of microbial populations. The entire thigh meat was dissected from left half-carcass, deboned, individually wrapped in aluminium foil then placed inside Ziploc freezer bag and stored at -20 °C for 30 and 60 days.

Serum metabolites: Blood samples were collected by vena puncture of left wing vein of 2 birds, one male and one female, from each replicate at 42 days of age for subsequent determination of serum metabolites. Blood samples were immediately centrifuged for 15 min at 3000 rpm to separate serum and the sera were kept in Eppendorf tubes at -20 °C until analyzed. The concentrations of total protein, glucose, triglyceride and total cholesterol in serum samples were measured by a spectrophotometer (Biochrom Libra S22, UK) according to the enzymatic colorimetric methods using commercial reagent kits (Pars Azmoon Inc., Tehran, Iran).

Intestinal microbial populations: Ileum digesta samples from each bird were evaluated for *E. coli* and *Lactobacillus* spp. counts using culture techniques described by Dibaji et al. (2014). In brief, before enumeration, frozen digesta samples were incubated at 4 °C for 10 h. Thereafter, 0.2 g of digesta was taken from each sample and was diluted in 2 ml sterile physiological saline (pH 7.0), and 10-fold (10% wt/vol) serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) were made from diluted samples. Subsequently, 100 µl of each three last dilutions (10^{-4}, 10^{-5} and 10^{-6}) was inoculated into Petri dish containing the selective agar media for further bacterial enumeration. *Lactobacillus* spp. Was incubated using de Man, Rogosa, and Sharpe (MRS) agar in an anaerobic jar at 37 ºC for 48 h, and generic *E. coli* colonies were incubated using MacConkey agar at 37 ºC for 24h. Counting of bacteria in the Petri dishes was performed by a colony counter. The bacterial counts were reported as $\log_{10}$ colony-forming units (CFU) per gram of ileal contents.

Determination of moisture and pH in meat

The method of Corzo et al. (2009) was used to measure meat moisture. The ground

meat samples were dried for 12-16 h in a vacuum-oven at 103 °C and the moisture of meat was calculated. The pH of meat samples was determined according to the method of Biswas et al. (2007). Approximately, 5 g of tissue samples were homogenized with 50 ml of distilled deionized water using a homogenizer (Yellowline, IKA Works, Wilmington, NC, USA) at 10,000 rpm for 1 min, then transferred to a 50 mL beaker. The pH of homogenate was determined using a digital pH meter (Inolab WTW, Germany) at room temperature.

Lipid peroxidation of thigh meat: Lipid oxidation was determined by thiobarbituric acid reactive substances (TBARS) assay according to spectrophotometric method as described by Botsoglou et al. (1994). In brief, samples were thoroughly homogenized (Yellowline homogenizer, IKA Works, Wilmington, NC, USA) in presence of 8 mL of 5% aqueous trichloroacetic acid and 5 ml of 0.8% butylated hydroxytoluene in hexane, and the mixture was centrifuged. The top layer was discarded and a 2.5 mL aliquot from the bottom layer was mixed with 1.5 mL of aqueous 2-thiobarbituric acid (8 g/L) to be further incubated at 70 ºC for 30 min. Following incubation, the mixture was cooled to room temperature and submitted to spectrophotometry (Biochrom Libra S22, UK) in 532-600 nm. The following equation (Heath and Packer, 1968) was used to read the concentration of TBARS of meat (ng/g).

TBARS = 1000[(Abs 523- Abs 600nm) /155]

Statistical analysis: Normal distribution of residuals and variance homogeneity of the data was tested by UNIVARIATE procedure and the Levene's test, respectively. Statistical analyses of data were conducted according to a completely randomized design using the GLM procedure of SAS (SAS Institute, 2015) with individual pens as experimental unit. The number of CFUs was expressed as logarithmic (log_{10}) transformation per gram of intestinal digesta. All microbiological concentrations from each bird were subjected to log-transformation prior to statistical analysis. Differences among the individual means were compared by Tukey test ($P< 0.05$).

Results

Chemical composition of the EOs: The chemical composition of the EOs identified by GC and GC–MS analysis are presented in Table 2. More than twenty compounds were identified in the oil of Origanum vulgare, with the main constituents being carvacrol (81.89%), γ-terpinene (5.1%), p-cymene (3.76%), and thymol (2.42%). The major bioactive constituents of freshly prepared EOs were: Satureja khuzistanica oil contained carvacrol (80.05%), thymol (11.86%), and p-cymene (1.85%), Rosemarinus officinalis oil contained α-pinene (26.02%), 1,8-cineol (19.26%), camphene (5.11%), berbenone (14.85%), and camphor (6.73%), Thymus daenensis celak oil contained thymol (72.3%), carvacrol (7.1%), and p-cymene (5.4%), and Foeniculum vulgare oil contained trans-anethole (75.8%), fenchone (7.2%), α-phellandrene (4.6%), and γ-terpinene (4.8%).

Growth performance: All broilers appeared healthy and average livability value was 97.4±0.8 for the entire experiment and there was no difference among treatments ($P> 0.05$). The effects of dietary treatments on BWG, ADFI, and FCR during 0 to 21 d, 22 to 42 d, and 1 to 42 d of age are presented in Table 3. Dietary supplementation of any

Table 1. Feed ingredients and nutrient composition (% as-fed basis, unless otherwise indicated) of the basal diets during different growth periods. 1- Vitamin premix provided the following per kilogram of diet: vitamin A (trans-retinyl acetate), 10000 IU; vitamin D3 (cholecalciferol), 2000 IU; vitamin E (DL- α-tocopherol acetate), 45 IU; vitamin K3 (bisulfate menadione complex), 3 mg; thiamine (thiamine mononitrate), 3 mg; riboflavin, 9 mg; nicotinic acid, 30 mg; pantothenic acid (D-calcium pantothenate), 10 mg; vitamin B6, 4 mg; D-biotin, 0.1 mg; folic acid, 2 mg; vitamin B12 (cyanocobalamin), 0.02 mg and choline (choline chloride), 1000 mg. 2- Mineral premix provided the following per kilogram of diet: iron (FeSO4•7H2O), 55 mg; manganese (MnSO4•H2O), 100 mg; zinc (ZnO), 85 mg; copper (CuSO4•5H2O), 13 mg; iodine (Ca (IO3)2), 1.3 mg; selenium (Na2SeO3), 0.2 mg.

Item	Starter, 0-10 d	Grower, 11-24 d	Finisher, 25-42 d
Ingredient%			
Corn grain	56.49	58.70	61.97
Soybean meal, 44%	36.85	34.48	30.40
Soybean oil	1.97	2.89	3.84
Calcium carbonate	1.17	0.94	0.92
Dicalcium phosphate	2.00	1.75	1.68
Sodium chloride	0.36	0.29	0.29
Sodium bicarbonate	0.00	0.10	0.10
L-Threonine, 99%	0.10	0.03	0.02
DL-Methionine, 99%	0.31	0.23	0.20
L-Lysine monohydrochloride, 78%	0.24	0.09	0.07
Vitamin premix1	0.25	0.25	0.25
Mineral premix2	0.25	0.25	0.25
Total	100	100	100
Calculated composition			
AMEn, kcal/kg	2850	2950	3050
CP	21.0	20.1	18.6
Digestible Arg	1.32	1.25	1.14
Digestible Lys	1.20	1.03	0.92
Digestible Met+Cys	0.89	0.79	0.72
Digestible Thr	0.78	0.68	0.62
Calcium	0.99	0.84	0.81
Available P	0.47	0.42	0.40
Sodium	0.16	0.16	0.16
Potassium	0.87	0.84	0.77
Chloride	0.28	0.23	0.23

combinations of EOs or commercial oregano oil had no effect ($P > 0.05$) on growth performance of broilers in either phase or the entire period of the experiment. However, broilers fed SR supplemented diets tended to have a slightly lower ($P = 0.119$) FCR compared with NC birds from 0 to 42 d of age.

Serum metabolites: As presented in Table 4, blood serum cholesterol of broilers significantly reduced ($P < 0.05$) by feeding each blend of EOs compared with NC, while it was not significantly different between the NC and PC. Serum triglyceride, glucose, and protein concentration were not affected by inclusion of EOs to the diet.

Intestinal microbial populations: Intestinal microflora identified (*E. coli* and Lactobacillus) in each treatment of EOs are summarized in Table 5. Broiler fed SR supplemented diets had the least *E. coli* bacteria count in ileum, which was not sig-

Table 2. Composition (% total volatile) and Retention Indices (RI) of the essential oil of Origanum vulgare, Satureja khuzistanica, Rosemarinus officinalis, Thymus daenensis celak and Foeniculum vulgare. [a] Retention indices on DB-1 capillary column. [b] < 0.5%.

RI a	Compound	%				
		Oreganum vulgare	Satureja khuzistanica	Thymus daenensis	Foeniculum vulgare	Rosemarinus officinalis
931	α-thujene	1.01	-b	2.3	4.3	-
938	α-pinene	-	0.78	0.7	-	26.02
950	camphene	-	-	-	-	5.11
977	β-pinene	-	-	-	-	1.01
980	3-octanone	-	-	-	-	3.68
989	β-myrcene	0.96	-	1.6	-	1.72
1008	α -phellandrene	-	-	-	4.6	-
1016	α-terpinene	-	-	1.8	-	-
1024	para-cymene	3.76	1.85	5.4	-	5.18
1035	1,8-cineol	-	-	-	-	19.26
1039	β-phellandrene	-	-	-	1.9	-
1059	γ-terpinene	5.1	-	4.8	0.5	-
1071	fenchone	-	-	-	7.2	-
1101	linalool	-	0.73	-	-	1.96
1150	camphor	-	-	-	-	6.73
1198	α-terpineol	-	-	-	-	2.92
1207	myrtenol	-	-	-	-	0.82
1221	berbenone	-	-	-	-	14.85
1228	estragol	-	-	-	3	-
1282	trans-anethole	-	-	-	75.8	-
1290	endobornyl acetate	-	-	-	-	2.05
1299	thymol	2.42	11.86	72.3	-	-
1311	carvacrol	81.89	80.05	7.1	-	-
1383	β-caryophyllene	-	0.63	2.5	-	-
1495	α-humulene	-	0.59	-	-	-
1575	spathulenol	-	0.66	-	-	-
	Total	95.14	97.15	98.5	97.3	91.31

nificantly different from NC (P> 0.05). In addition, broilers fed either PC or SR in diets had lower (P< 0.05) *Lactobacillus* spp. counts than those fed SRT supplemented diets, however none of them were different from those fed NC or SRTF.

Meat quality: As presented in Table 6, lipid peroxidation, as reflected by the amount of TBARS in thigh meat samples, after 30 and 60 days of frozen storage was significantly lower (P< 0.05) in meats from broilers fed PC diet than those from NC, while the 3 other groups had no significant difference from NC or PC. No significant difference was observed in the pH and moisture of thigh samples among the treatments in different storing time.

Discussion

Literature review has shown variations between chemical compositions of EOs. For example, Farzaneh et al. (2015) determined the main components of the S. khuzistanica oil as carvacrol (48%), p-cymene

Table 3. Average body weight gain (BWG), average daily feed intake (ADFI) and feed conversion ratio (FCR) of broilers fed diets supplemented with blends of essential oils. Positive Control: Oregano EO, 300 mg/kg, SR: Savory+Rosemary, 300 mg/kg, SRT: Savory+Rosemary+Thyme, 300 mg/kg, SRTF: Savory+Rosemary+Thyme+Fennel, 300 mg/kg. SEM = Standard error of means.

	0 to 21 d			22 to 42 d			0 to 42 d		
Treatment	BWG, g/d	ADFI, g/d	FCR, g/g	BWG, g/d	ADFI, g/d	FCR, g/g	BWG, g/d	ADFI, g/d	FCR, g/g
Negative Control	27.1	42.2	1.57	76.8	147.0	1.91	51.9	94.6	1.81
Positive Control	25.4	39.6	1.55	73.5	145.7	1.98	49.5	92.6	1.87
SR, 300 mg/kg	28.4	41.0	1.44	77.6	148.2	1.90	53.0	94.6	1.78
SRT, 300 mg/kg	27.3	42.8	1.57	78.8	152.8	1.93	53.1	97.8	1.84
SRTF, 300 mg/kg	26.7	40.9	1.53	72.0	140.3	1.95	49.2	90.6	1.84
SEM	2.57	2.36	0.076	4.78	6.96	0.080	2.76	4.27	0.044
P-value	0.592	0.379	0.152	0.272	0.208	0.724	0.164	0.235	0.119

Table 4. Blood metabolites (mg/dl) of broilers fed diets supplemented with blends of essential oils. a-b Means within a column with different superscripts are significantly different ($P< 0.05$). Positive Control: Oregano EO, 300 mg/kg, SR: Savory+Rosemary, 300 mg/kg, SRT: Savory+Rosemary+Thyme, 300 mg/kg, SRTF: Savory+Rosemary+Thyme+Fennel, 300 mg/kg. SEM = Standard error of means.

Treatment	Cholesterol	Triglyceride	Glucose	Protein
Negative Control	121.1^{a}	96.3	238.6	3.96
Positive Control	111.6^{ab}	94.1	247.2	3.87
SR, 300 mg/kg	107.3^{b}	107.0	249.5	3.67
SRT, 300 mg/kg	99.0^{b}	94.5	251.2	3.80
SRTF, 300 mg/kg	107.0^{b}	97.2	247.2	3.65
SEM	12.48	25.42	15.06	0.388
P-value	0.022	0.845	0.515	0.460

(18.5%) and γ-terpinene (11%). The composition of EO depends on the interaction of many factors that deal mainly with the plant raw material and the EO production process. For instance, factors such as the plant species and growth stage, the environment, the agricultural practices and the geography will affect the EO content and composition of the plant raw material. As a result, the plant raw materials for EO production vary considerably and the same applies to the resulting EO products (Russo et al., 1998). This could be one of the potential reasons for inconsistency between results of different studies on EOs in broiler nutrition.

In the current study, growth performance of broilers was not affected by supplementation of any combination of EOs or commercial oregano oil to the corn-soybean meal based diets at 0 to 42 d. This finding is consistent with results of our previous research (Mohiti-Asli and Ghenaatparast-Rashti, 2017) that have demonstrated adding the commercial oregano oil at 300 mg/kg to diet had no effect on BWG, ADFI and FCR of broilers from 0 to 42 days of age. Amad et al. (2011) reported no difference in BWG and ADFI of broilers fed diets supplemented with a commercial blend of EOs, with thymol and anethole as major active components, than those fed the control diet during grower phase and the entire period. In contrast, Hashemipour et al. (2013) reported that supplementation of thymol and carvacrol from a commercial blend added at the rate of 200 ppm in broiler diets en-

Table 5. Log10 bacterial number per gram of ileum contents from broilers fed diets supplemented with blends of essential oils. [a-b] Means within a column with different superscripts are significantly different ($P< 0.05$). Positive Control: Oregano EO, 300 mg/kg, SR: Savory+Rosemary, 300 mg/kg, SRT: Savory+Rosemary+Thyme, 300 mg/kg, SRTF: Savory+Rosemary+Thyme+Fennel, 300 mg/kg. SEM = Standard error of means.

Treatment	*Lactobacillus* spp.	*Escherichia coli*
Negative Control	7.46[ab]	6.70
Positive Control	7.35[b]	6.56
SR, 300 mg/kg	7.29[b]	6.43
SRT, 300 mg/kg	7.59[a]	6.55
SRTF, 300 mg/kg	7.47[ab]	6.66
SEM	0.174	0.227
P-value	0.023	0.173

Table 6. Moisture, pH, and lipid peroxidation (ng of thiobarbituric acid reactive substances, TBARS, per g of meat) of stored thigh meat samples obtained from broilers fed diets supplemented with blends of essential oils. [a-b] Means within a column with different superscripts are significantly different ($P< 0.05$). Positive Control: Oregano EO, 300 mg/kg, SR: Savory+Rosemary, 300 mg/kg, SRT: Savory+Rosemary+Thyme, 300 mg/kg, SRTF: Savory+Rosemary+Thyme+Fennel, 300 mg/kg. SEM = Standard error of means.

	30 d			60 d		
Treatment	moisture, %	pH	TBARS, ng/g	moisture, %	pH	TBARS, ng/g
Negative Control	75.3	6.18	2.55[a]	74.6	6.45	2.71a
Positive Control	77.1	6.18	1.66[b]	74.2	6.36	1.80b
SR, 300 mg/kg	74.2	6.20	2.07[ab]	74.1	6.35	2.16ab
SRT, 300 mg/kg	74.5	6.32	1.93[ab]	73.7	6.43	2.08ab
SRTF, 300 mg/kg	75.9	6.04	2.30[ab]	75.3	6.16	2.40ab
SEM	2.70	0.196	0.532	2.59	0.202	0.565
P-value	0.257	0.140	0.030	0.770	0.070	0.044

hanced BWG, and decreased ADFI from day 25 to 42. Moreover, Alp et al. (2012) reported that broilers fed the oregano oil in diets consumed less feed and had better FCR from day 21 to 42 and from day 0 to 42 than those fed control diet. It has been reported that blends of EOs have improved broiler performance when given as dietary supplements (Hosseini et al., 2018). Similarly, Jamroz et al. (2003) reported that a blended supplement containing carvacrol, capsaicin and cinnamaldehyde improved both body weight and FCR in broilers at 21 d of age.

Previous studies performed on the effect of EOs on growth performance of broilers exhibited conflicting results, and do not permit a generalized conclusion on the efficacy of such feed additives. In a study on broilers feeds supplemented with oregano no effect was found on performance (Botsoglou et al., 2002), whereas Mohiti-Asli and Ghenaatparast-Rasht (2015) reported that BWG from 22 to 28 day of age has been increased in broilers with mild coccidiosis challenge fed Orego-Stim, the commercial supplement based on oregano used in the current study, in diets. A potential reason for inconsistent results of previous experiments that studied the growth performance of broiler fed EO may be due to the content of active substances in EO samples. Indeed, the inadequate amounts of the terpenes in the diets could be a probable reason for lack of effect on performance of growing chickens in previous studies. In the current experiment, the

concentration of pure EOs included per kg of the diets was 15 mg (5%×300mg) which was around 100-times lower than dietary inclusion rate of EOs in previous study of Cross et al. (2007). However, these authors reported that dietary inclusion of 1 g/kg oregano oil in broilers diet could not affect the performance, it was therefore suggested that although oregano oil is a potent antimicrobial, it may not improve performance, and may even restrict growth rate of broiler chicks. Jang et al. (2007) reported that BWG, ADFI and FCR were not different among broilers fed the basal diet and those fed diet supplemented with either low (25 mg/kg) or high (50 mg/kg) level of a commercial blend of EO. However, a probable reason for lack of effect observed in many studies on growth performance might be attributed to the variability in the concentrations of EO used in the diet and the composition of the blend, the environmental factors also should be noted. It has been documented that well-nourished healthy chicks may not positively respond to growth promoting supplements when they are housed under clean conditions without any challenge.

Results of the current experiment indicate that serum metabolites concentration were not affected by EOs supplementation to the diets, except for a reduction in serum cholesterol of broilers fed a blend of EOs compared with NC. Limited research investigated the effects of EOs on blood lipids, glucose, and protein in broiler chickens. Kirkpinar et al. (2014) reported that addition of oregano and garlic EOs in broiler diet did not affect serum cholesterol and triglyceride. Some studies indicated that rosemary has hypolipidemic and hypoglycemic activities. Manafi et al. (2014) reported that inclusion of rosemary oil in diet decreased blood cholesterol and triglyceride in broilers. It has been proposed that the hypocholesterolemic effect of EOs is due to compounds in EO that have the ability to inhibit hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, a key regulatory enzyme in cholesterol synthesis (Case et al., 1995). Case et al. (1995) reported that dietary carvacrol and thymol at 150 mg/kg of diet reduced serum cholesterol concentrations in cockerels.

The intestine of chickens harbors a complex microbiome, which is almost exclusively composed of bacteria, predominantly *E. coli* and lactobacillus species. Lactobacilli strains ferment carbohydrates present in poultry feed and produce lactic acid, which reduces the pH in the surrounding environment and inhibits the growth of certain pathogens such as *E. coli*. Adverse and pathogenic bacteria in the gastrointestinal tract of chicken, such as *E. coli* compete with the host for nutrients and might also damage the intestinal epithelium, which adversely affects the digestion and absorption function of the host. In the current study, supplementation of SR to diets tended to reduce (but not significantly) *E. coli* count in ileum of broilers compared with those fed NC diet. Broilers fed either SRT or SRTF in diets had higher *Lactobacillus* spp. counts than those fed PC or SR. This effect may be attributed to terpene fraction of these blended EOs which is probably composed of more antibacterial compounds. Jamroz et al. (2005) reported that supplementation of a commercial blend of EOs containing carvacrol, cinnamaldehyde and capsaicin in diet reduced *E. coli* and increased *Lactobacillus* spp. levels in the small intestine of broilers. In an in vitro study, Mathlouthi et al. (2012) reported that EO of rosemary,

oregano, and their combination had antibacterial activity against *E. coli* without affecting *Lactobacillus* spp., whereas Michiels et al. (2009) reported that carvacrol had antimicrobial effects against Lactobacilli. Burt et al. (2007) indicated that *E. coli* cells can grow in the presence of a sub-lethal concentration of carvacrol.

Antimicrobial activity has been recognized as the major beneficial effect of EO on poultry production, although the exact antimicrobial mechanism has not been fully revealed. Numerous in vitro studies confirmed that EO including thymol, carvacrol, etc., displayed antimicrobial activity against intestinal microbes such as *E. coli*, although limited information is available regarding EO effects on beneficial probiotic bacteria, such as Lactobacillus. Only slight or no inhibition against Lactobacillus has been reported for carvacrol at 300 μg/mL (Si et al., 2009). Du et al. (2015) reported that *E. coli* was more sensitive to thymol (MIC; minimum inhibitory concentration and MBC; minimum bactericidal concentration 187.5 and 375 μg/mL, respectively), whereas antibacterial activity of thymol and carvacrol was noticed against Lactobacillus strains at concentrations higher than for pathogenic bacteria (MIC and MBC, 1500 and 3000 μg/mL, respectively). According to in vitro experiments, thymol and carvacrol have additive antibacterial effects. Thymol is structurally analogous to carvacrol, but the locations of the hydroxyl groups differ between the two molecules. Thymol and carvacrol have similar antimicrobial effects but have different mechanisms of action against Gram-positive and Gram-negative bacteria. Thymol and carvacrol have prominent outer membrane disintegrating properties. These compounds are also capable of increasing the permeability of the cytoplasmic membrane to ATP. p-Cymene is the precursor of carvacrol and is a monoterpene with a benzene ring without any functional groups on its side chains. It has been reported that p-cymene not only has bactericidal effect, but also can enhance the antimicrobial activity of other compounds, such as its derivative carvacrol (Marchese et al., 2017). In the current study, the highest count of *Lactobacillus* spp. was found in SRT fed broilers which contained carvacrol, α-pinene, berbenone, 1,8-cineol, and thymol. The synergistic effect of phenols such as carvacrol and thymol, could probably reduce pathogenic bacteria and increase beneficial bacteria such as Lactobacillus. Broilers fed diet supplemented with EO of oregano or SR had the lowest *Lactobacillus* spp. counts in ileum compared with the other combinations of EO which suggests carvacrol in high amount could exert suppressing effect on this Gram-positive bacteria. Broilers fed SR in diets had numerically lower, but not significantly, *E. coli* count than the other blends, suggesting higher antibacterial action R. officinalis EO than T. daenensis celak or F. vulgare. The R. officinalis EO tested in the current study proved to have mainly α-pinene, which exhibited a broad antibacterial spectrum against Gram-negative and Gram-positive bacteria, and 1,8-cineole which displayed antibacterial activity against Gram-negative pathogenic bacteria by inducing cell membrane disruption (Ojeda-Sana et al., 2013).

Results of the current study indicate that oregano oil supplementation to the diets is effective in delaying lipid peroxidation in thigh meat during frozen storage for 30 and 60 days as compared with NC group, whereas the other blends of EOs had no

significant difference from NC or PC. Neither pH nor moisture of stored thigh samples was affected by addition of any EOs in diets. It has been proposed that dietary supplementations of EOs have a beneficial effect on stored meat quality, an effect related to the antioxidant property of EOs, in terms of reducing or delaying lipid oxidation. TBARS is one of the most frequently used indicators of lipid peroxidation. Loetscher et al. (2013) reported that addition of rosemary to broiler diet decreased TBARS formation in breast meat during 9 d of storage. Yesilbag et al. (2011) found the higher antioxidative protection for the lower rosemary supplementation level which demonstrates a potentially better antioxidant activity of rosemary at lower dosages. Kirkpinar et al. (2014) reported that addition of EOs of oregano or garlic to the diet at 300 mg/kg had no effect on pH and oxidative stability of breast meat during the storage period. Botsoglou et al. (2002) found lower TBARS values in meat from broilers fed diets enriched with oregano oil. They attributed this effect to the antioxidant compounds present in oregano oil which enter the circulatory system and are distributed and retained in muscle and other tissues. In the current study, inclusion of oregano EO to broiler diets presented the best antioxidant activity, since the corresponded thigh samples had the lowest TBARS values. Moreover, lipid peroxidation in thigh meat of broilers fed SRT in diet was numerically lower than the other blend of EOs, suggesting that carvacrol and thymol, the main components of EOs in this blend, had the most antioxidant effect among the other components. Carvacrol and thymol are two phenolic compounds with known antioxidant activity, composed 80% and 72% of EOs of S. khuzistanica and T. daenensis celak, respectively. However, the main components of R. officinalis EO are α-pinene, 1,8-cineole and camphor which totally composed almost 50% of the EO, whereas F. vulgare EO predominantly constituted by 75% trans-anethole. Ojeda-Sana et al. (2013) determined chemical composition of EOs from two phenotypes of rosemary growing in Argentina and studied the antioxidant and antibacterial activities of EOs. They concluded that the rosemary EO rich in myrcene had the highest antioxidant activity, while the rosemary EO rich in α-pinene had the highest antibacterial activity. This can partly explain the relatively less antioxidative activity of SR, since R. officinalis EO tested in our study mainly contained α-pinene. Youdim et al. (2002) reported that antioxidant activity of EO components ranks as follows: thymol, carvacrol, γ-terpinene, myrcene, linalool, p-cymene, limonene, 1,8-cineole, and α-pinene. This phenolic compound of EO has a hydrogen-donating ability which can act as a chain breaking antioxidant in free radical chain reactions, converting lipid radicals to more stable products, thus extending the shelf life of meat.

Conclusion: The lowest ileal *Lactobacillus* spp. counts achieved in this experiment with supplementation of EO of oregano or SR in diets can be related to the high carvacrol contents of these EOs with suppressive activity on growth and proliferation of this Gram-positive bacteria. Only EOs as combinations were effective in reducing serum cholesterol concentration, suggesting other bioactive compounds except carvacrol as hypocholesterolemic agents in EOs blend. The inclusion of EOs in diets increased meat oxidation stability, with the highest amount achieved by oregano EO-fed broilers.

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Conflicts of interest

The author declared no conflict of interest.

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مجله طب دامی ایران، ۱۳۹۸، دوره ۱۳، شماره ۲، ۲۱۵-۱۹۹

اثر مخلوط اسانس‌های گیاهی بر عملکرد رشد، متابولیت‌های خونی، فلور میکروبی روده و کیفیت گوشت جوجه‌های گوشتی

مازیار محیطی اصلی[۱]، میلاد خدمتگزار[۱]، حسن درمانی کوهی[۱]، محسن فرزانه[۲]

[۱]گروه علوم دامی، دانشکده کشاورزی، دانشگاه گیلان، رشت، ایران

[۲]پژوهشکده گیاهان و مواد اولیه دارویی، دانشگاه شهید بهشتی، تهران، ایران

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چکیده

زمینه مطالعه: استفاده از افزودنی‌های گیاهی به صورت اسانس‌های گیاهی توجه بسیاری را در صنعت طیور جلب نموده است.

هدف: این آزمایش برای بررسی اثرات مخلوط اسانس‌های گیاهی استخراج شده از برخی گیاهان دارویی شامل مرزه، آویشن، رازیانه و رزماری بر عملکرد، متابولیت‌های خونی، میکروفلور روده و کیفیت گوشت جوجه‌های گوشتی انجام شد.

روش کار: تعداد ۲۰۰ قطعه جوجه گوشتی یکروزه به طور تصادفی به ۵ تیمار و ۴ تکرار و ۱۰ قطعه جوجه در هر تکرار تقسیم شدند. تیمارها شامل افزودن ۱) شاهد منفی (بدون اسانس)، ۲) شاهد مثبت (۳۰۰ میلی‌گرم اسانس مرزن‌جوش در هر کیلوگرم)، ۳) مرزه و رزماری (۳۰۰ میلی‌گرم در کیلوگرم)، ۴) مرزه، رزماری و آویشن (۳۰۰ میلی‌گرم در کیلوگرم) و ۵) مرزه، رزماری، آویشن و رازیانه (۳۰۰ میلی‌گرم در کیلوگرم) به جیره بودند.

نتایج: شاهد مثبت و مخلوط اسانس‌ها هیچ کدام بر عملکرد رشد جوجه‌ها اثری نداشتند. کلسترول سرم جوجه‌های تغذیه شده با مخلوط اسانس ها در مقایسه با شاهد منفی کاهش یافت ($P<۰/۰۵$). جوجه‌های تغذیه شده با اسانس مرزن‌جوش یا مرزه و رزماری کمترین شمار لاکتوباسیل را در ایلئوم داشتند($P<۰/۰۵$). مکمل سازی جیره با اسانس مرزن‌جوش سبب کاهش پراکسیداسیون چربی‌ها در گوشت ران نگهداری شده به مدت ۳۰ و ۶۰ روز در فریزر در مقایسه با شاهد منفی شد($P<۰/۰۵$).

نتیجه گیری نهایی: جوجه‌های گوشتی که با اسانس گیاهی مرزن‌جوش یا مرزه و رزماری تغذیه شدند کمترین شمار لاکتوباسیلوس را در ایلئوم داشتند که احتمالاً به دلیل مقادیر زیاد کارواکرول در آن‌هاست که رشد و تکثیر این باکتری گرم مثبت را کاهش می‌دهد. بر خلاف مخلوط اسانس‌های گیاهی، اسانس مرزن‌جوش غلظت کلسترول سرم را کاهش نداد این مسأله می‌تواند بیانگر اثرات کاهنده کلسترول برای سایر ترکیبات غیر از کارواکرول باشد. جوجه‌های تغذیه شده با اسانس مرزن‌جوش بیشترین پایداری اکسیداتیو گوشت را داشتند. این اثر آنتی‌اکسیدانی در سایر تیمارهایی که مقدار کارواکرول کمتری داشتند، کاهش یافت.

واژه‌های کلیدی:

جوجه گوشتی، اسانس‌های گیاهی، میکروفلور روده، کیفیت گوشت، عملکرد

نویسنده مسؤول: تلفن: ۳۳۶۹۰۲۷۴ (۱۳)۹۸+ نمابر: ۳۳۶۹۰۲۸۱ (۱۳)۹۸+ Email: mmohiti@guilan.ac.ir