Effects of Myrtle (*Myrtus communis*) essential oil on growth performance, carcass characteristics, intestinal morphology, immune response and blood parameters in broiler chickens

E.Salehifar[1]*, M.Abbasi[2], R.Bahari-Kashani[3]

[1,2,3]Department of Animal Science, Mashhad Branch, Islamic Azad University, Mashhad, Iran
*Corresponding Author E-mail:E.salehifar@mshdiau.ac.ir, Tel;+989155030386

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Abstract

An experiment was conducted to determine the effects of Myrtle Essential Oil (MEO) on growth performance, carcass characteristics, intestinal morphology, immune response and blood parameters of broiler chickens. A total of 360 Ross 308 broiler chickens were allocated to five dietary treatments with five replicates of 15 birds each. Dietary treatments were prepared by formulating a corn-soybean meal-based diet free of antibiotics (Control) and supplementing the basal diet with three levels of MEO at 150, 300, 450 mg/Kg and antibiotic Virginiamycin (VGN) at 200 mg/Kg. The results showed that diets supplemented with MEO and VGN increased the feed intake, body weight gain and improved the feed conversion ratio compared to the control treatment (P<0.05). The relative carcass weight was significantly increased, whereas the weight of duodenum, jejunum and ilium and liver were decreased in broilers fed MEO (P<0.05). Dietary myrtle essential oil and antibiotic supplementations consistently resulted in significantly longer villus height, lower epithelial thickness of the small intestinal at 42 day of age compared with the control treatment (P<0.05). Supplementing the basal diet with MEO increased the antibody titers against sheep red blood cell (SRBC), although supplementing diet with 300 mg/kg of MEO was more effective (P<0.05). Broilers fed MEO diets especially at the level of 450 mg/Kg had a lower white blood cells count and heterophil, heterophil to lymphocyte ratio, but a higher lymphocyte and red blood cells count (P<0.05). In conclusion, data showed that diet supplemented with MEO improved the growth performance and increased antibody titers against SRBC, especially at the level of 300 mg/Kg, in broiler chickens. Myrtle oil supplementation may be considered a potential natural growth promoter. However, more studies are needed to define the effect of myrtle oil supplementation on the performance of poultry with regard to environmental conditions, effective dosage, active oil substances, dietary ingredients and nutrient density.

Key Words: broilers; immunity; myrtle essential oil; antibiotic; intestinal morphology; blood profile.

Introduction

Use of antibiotic growth promoters to improve animal performance and health has been practiced during the last 50 years. Unfortunately, the bacteria have established resistant strains by transferring resistance to other species especially in shared strains between humans and animals and resulting serious problems in public health and livestock production (Thakar, 2004). Therefore, a number of the antibiotic growth promoters have been banned by the European Union. In recent years, an extensive research has been performed on substances that could be used as an alternative antibiotic replacement in poultry diets. Prebiotics, probiotics and organic acids have the potential to improve the performance of poultry through positive influence on the microflora of the digestive tract. Recently, the use of essential oil has become popular due their antimicrobial properties (Akin et al., 2010). Essential oils may enhance the activities of digestive enzymes and nutrient absorption, and thereby improving the nutritional value of feed. Also, the essential oils may alterthe intestinal functions, for example, villi structure. It is well documented that the structure and morphology of villi play a substantial role in the digestion and absorption of nutrients in the gastrointestinal tract. *Myrtus communis* (from Myrtaceae family and subfamily Myrtoideae) is an annual plant used for medicinal, food, and spice purposes. This aromatic plant grows wildly in the coastal areas of Tunisia, Morocco, Turkey, France, and Iran. The leaves contain tannins, flavonoids such as quercetin, catechin, myricetin derivatives and volatile oils (Romani et al., 2004). Ozek et al. (2000) reported the most important constituents of Myrtle essential oil (MEO) as myrtenol, myrtenol acetate, limonene, linalool, α-pinene, 1,8-cineole, β-caryophyllenein, p-cymene, geraniol, nerol, phenylpropanoid, and methyleugenol (Romani et al., 2004). Essential oils obtained from the leaves of Myrtus communis have antioxidant, antiseptic and anti-inflammatory activities (Al- Hindawi et al., 1989), as well as antibacterial properties (Hayder et al., 2003). Then, the aim of current study was to determine the effects of MEO on growth performance, carcass characteristics, intestinal morphology, immune response and blood parameters of broiler chickens and also making a comparison between its effects and virginiamycin as a growth promoter.

Material and methods

Animal husbandry and dietary treatment

The experimental protocol was approved by the Animal Care Committee of Islamic Azad University.The study was conducted at Poultry Research Station, Islamic Azad University, Mashhad branch (Mashhad, Iran) from October to December 2015. Three hundred sixty mixed-sex one-day-old broiler chickens (Ross 308) were weighed and randomly assigned to five dietary treatments with five replicates of 15 birds each. To prepare the dietary treatments, an antibiotic-free corn-soybean meal-based diet was formulated according to Ross 308 broiler manual (Table 1). Birds were fed *ad libitum*. The ambient temperature and lighting program was in line with the recommendations given in the broiler management guide (Aviagen, 2009).

Table 1. Feed ingredients and composition of the basal diet

Ingredients (g/kg)	Starter (0-10 d)	Grower (11-24 d)	Finisher (25-42 d)
Corn, yellow	559.50	577.20	630.00
Soybean meal	366.50	342.60	292.50
Soybean oil	27.95	41.70	40.00
Dicalcium phosphate	17.85	14.50	13.50
Calcium carbonate	13.30	11.60	11.40
Common Salt	2.00	2.00	2.90
Vitamin Permix[1]	2.50	2.50	2.50
Mineral Permix[2]	2.50	2.50	2.50
DL-Methionine	3.90	3.25	2.70
L-Lysine HCL	4.00	2.15	2.00
Calculated chemical composition			
ME (Kcal/Kg)	2938	3055	3100
Crude protein (g/kg)	214.80	203.70	185.7
Lysine (g/Kg)	13.90	12.00	10.60
Methionine (g/Kg)	7.20	6.50	5.70
Methionine + Cysteine (g/Kg)	10.20	9.40	8.30
Calcium (g/Kg)	10.11	8.66	8.22
Available phosphorus (g/Kg)	5.05	4.39	4.13
Sodium chloride (g/Kg)	1.70	1.70	1.70

[1]Contained per kilogram; Vitamin A: 5,500,000 IU; Vitamin D3: 1,500,000 IU; Vitamin E: 15,000 mg; Vitamin K: 800 mg; Thiamine: 1000 mg; Riboflavin: 4000 mg; Niacin: 25,000 mg; Biotin: 30 mg; Folic acid: 500 mg; Pantothenic acid: 5000 mg; Pyridoxine: 1500 mg; Vitamin B12: 15 mg; [2]Contained per kilogram; Cu: 12,000 mg; Fe: 35,000 mg; Zn: 25,000 mg; Co: 150 mg; I: 500 mg; Se: 120 mg; Mn: 38,000 mg.

Green leaves of myrtle were gathered from cultivated farms at Mashhad city and then were dried under tree umbrage at ambient temperature. Then, their essential oil was extracted by using Clevenger apparatus via distillation for 4 hours (Kumar & Tripathi, 2011). The myrtle essential oil was analyzed by a gas chromatograph (9-A-Shimadzu) and GC/MS (Varian-3400) column (DB-1, 60 mm×0.25 mm fused silica capillary column, film thickness 0.25 μm) using a temperature program of 40 °C-220 °C at a rate of 4 °C/min, an injector temperature of 260 °C and using the carrier gas helium. The constituents were identified by comparison of their mass spectra with those in the computer library and with authentic compounds.

The experimental treatments were prepared by supplementing the basal diet with 200 mg/Kg of Virginiamycin (VGN), 150, 300, and 450 mg/Kg of Myrtle essential oil (MEO). The myrtle essential oil was mixed in a carrier (soybean oil), which was then added to the basal diet. All diets were prepared freshly every week and diets were in mash form.

Performance traits

Body weight and feed intake were recorded weekly on pen basis and thenfeed: weight gain was calculated.

Carcass characteristics

On d 42, two birds per pen closest to the mean body weight of the corresponding pen selected, killed by cervical dislocation and slaughtered. Carcass, breast, thigh, neck, wings, liver, gizzard, pancreas, different parts of small intestine (duodenum, jejunum, and ileum) and lymphoid organs (spleen and bursa of Fabricius) were weighed.

Intestinal morphology

At 42 days of age, one bird from each pen (closest to the mean pen body weight) was selected. The birds were sacrificed by cervical dislocation and the digestive tract was carefully excised. After removing the intestinal contents, approximately 3 cm lengths of duodenum (midpoint of the pancreatic loop), jejunum (midpoint of jejunum) and ileum (5 cm after Meckel’s diverticulum) were removed for gut morphological measurements. Intestinal samples from each section were immersed in formaldehyde, before fixation in Bouin’s solution and paraffin embedding. Histological examinations were carried out according to the method of Iji et al. (2001). Paraffin sections at 6 μm thickness were made from each sample, stained with hematoxylin and eosin, and examined by light microscopy. Villus height, crypt depth, goblet cell number and epithelial thickness were analyzed from each preparation. The length of the intestinal villi and the depth of the intestinal crypt were measured with linear scaled graticule.

Sampling and analytical procedure

On days 21 and 35, two chicks in each pen were inoculated with 0.1 mL of SRBC (0.5%) in the pectoral muscle. On day 27 and 41, 2 mL of blood was collected from the brachial vein of SRBC-inoculated chicks with EDTA as the anticoagulant to measure antibody titers using the microtiter hemagglutination procedure (Wegmann & Smithies, 1966). Titers were expressed as the log2 of the reciprocal of the last dilution in which there was agglutination. At d 42, two birds per replicate were selected and blood samples were collected for determination of white blood cell (WBC), red blood cell (RBC), hematocrit (HCT), total protein and cholesterol. For heterophil to lymphocyte ratio, smears were prepared and stained by Giemsa method (Gross & Sigel, 1983; Toghyani et al., 2010). Hematocrit values were measured by microhematocrit method (Kececi et al., 1998).

Statistical analysis

Treatments were analyzed as a completely randomized design under the general model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where Y_{ij} is the dependent variable, μ is the general mean, T_i is the treatment, and e_{ij} is the experimental error calculated using the GLM procedure of SAS for Windows version 9.1 (SAS Institute Inc., Cary, NC). Statistical significance was considered at $p<0.05$. The significance of differences between means was estimated by Duncan’s test.

Results

The major components were α-Pinene (30.1%), Limonene (20.5%), 1, 8-cineole (18.0%), Linalool (9.8%), Linalyl acetate (4.2%) and α-terpineole (3.3%). Retention index were 932, 1024, 1027, 1087, 1249 and 1179 respectively.

The results related to performance are presented in Table 3. Broiler chickens were fed MEO and VGN treatments had a lower feed intake during 1-14 days of age as compared to the control treatment ($P<0.05$). Supplementing of MEO to the basal diet decreased the feed intake during 15-28 days of age ($P<0.05$), but it reversely increased during 29-42 days of age compare to the control treatment ($P<0.05$). Control, MEO2, MEO3 and VGN had high feed intake during 1-42 days of age ($P<0.05$).

Birds were fed diets supplemented with MEO and VGN had a higher body weight gain in all weeks and it was significant, except during 0-14 days of age, compared to the control treatment (P<0.05).

Table 2. Chemical composition of the essential oil of *Myrtus communis*

Components	%
α-pinene	30.1
1,8-cineole	18
Limonene	20.5
Linalool	9.8
α-terpineol/ α-terpinyl acetate	4.5
Linalyl acetate	4.2
Geraniol	1.0
β-caryophyllene	0.3

As shown in Table 3, a beneficial effect of supplementing MEO and VGN to the broiler chickens diet on feed conversion ratio was observed during 15-28 days of age and 1-42 days of age, especially for the whole experimental period (1-42 days of age) which was significantly lower than control treatment (P<0.05).

Table 3. Effect of dietary MEO[1] and VGN[2] on performance in broiler chickens

Item	Control	MEO1[1]	MEO2[2]	MEO3[3]	VGN[4]	SEM[5]	P value
Feed intake (g)							
0-14 d	33.23^{a}	30.10^{b}	32.78^{b}	31.15^{b}	30.16^{b}	5.20	0.002
15-28 d	110.05^{a}	105.25^{bc}	102.03^{c}	106.36^{bc}	108.23^{ab}	15.79	0.08
29-42 d	153.59^{a}	155.05^{b}	160.35^{b}	166.26^{bc}	158.36^{b}	16.59	0.03
1-42 d	296.87^{ab}	290.4^{b}	295.16^{ab}	303.77^{a}	296.75^{ab}	30.4	0.04
Body weight gain (g)							
0-14 d	268.20	285.26	278.14	293.17	290.17	3.39	0.9
15-28 d	617.00^{b}	788.54^{a}	816.84^{a}	853.20^{a}	855.88^{a}	18.33	0.001
29-42 d	950.74^{c}	984.14^{bc}	1010.16^{ab}	1049.00^{a}	1004.15^{ab}	32.78	0.003
1-42 d	1835.94^{b}	2057.94^{a}	2105.14^{a}	2195.37^{a}	2150.2^{a}	30.4	0.04
Feed conversion ratio							
0-14 d	1.78	1.57	1.58	1.58	1.67	0.10	0.5
15-28 d	2.17^{a}	1.37^{bc}	1.27^{c}	1.35^{bc}	1.43^{b}	0.16	0.004
29-42 d	1.92	1.96	1.93	1.90	1.91	0.14	0.24
1-42 d	1.95^{a}	1.63^{b}	1.59^{b}	1.61^{b}	1.67^{b}	0.08	0.04

[1]Myrtle Essential oil (150 mg/Kg); [2]Myrtle Essential oil (300 mg/Kg); [3]Myrtle Essential oil (450 mg/Kg); [4]Virginiamycin; [5]Standard Error of Means.

[a-c]Means within a row having different superscripts are significantly different (P < 0.05).

The effects of MEO and VGN on carcass characteristics of broiler chickens are shown in Table 4. The relative weight of breast, thigh, gizzard, liver and pancreas were not affected by MEO and VGN supplementation (P > 0.05). Relative carcass weight of broiler chickens was significantly increased by VGN and MEO supplementation at 450 mg/Kg (P<0.05). The relative jejunum and ileum weights were significantly lower in broiler chickens fed VGN (P<0.05) compared with the control treatment (P<0.05). Broiler chicks in control had lower proventriculus weight relative to other treatments (P<0.05).

Table 4. Effect of dietary MEO[1] and VGN[2] on carcass composition in broiler chickens

Treatment	Carcass composition									
	Carcass	Breast	Thigh	Liver	Duodenum	Jejunum	Ileum	proventriculus	Gizzard	Pancreas
Control	68.49^{c}	34.00	28.23	2.44	8.84^{b}	13.13^{a}	11.96^{a}	2.27^{c}	14.48	2.52
MEO1[1]	70.69^{bc}	35.26	29.88	2.36	7.44^{a}	12.59^{a}	11.21^{a}	3.61^{ab}	16.26	2.35
MEO2[2]	70.80^{bc}	34.42	29.43	2.42	6.98^{a}	12.16^{a}	11.18^{ab}	3.59^{ab}	15.95	2.30
MEO3[3]	71.73^{ab}	34.30	30.08	2.32	6.22^{a}	10.76^{ab}	11.15^{a}	4.10^{a}	16.70	2.85
VGN[4]	72.01^{a}	35.69	29.70	1.94	6.95^{a}	8.13^{b}	9.41^{b}	3.12^{b}	14.96	2.28
SEM[5]	1.39	1.29	0.85	1.77	0.81	1.15	0.99	0.25	1.24	0.15
P value	0.01	0.38	0.58	0.6	0.03	0.04	0.04	0.02	0.1	0.2

[1]Myrtle Essential oil (150 mg/Kg); [2]Myrtle Essential oil (300 mg/Kg); [3]Myrtle Essential oil (450 mg/Kg); [4]Virginiamycin; [5]Standard Error of Means.[a-c]Means within a column having different superscripts are significantly different (P < 0.05)

Morphometric measurements made in the small intestinal (duodenum, jejunum, and ileum) of broiler chicken are presented in table 5. It was found that dietary myrtle essential oil and antibiotic supplementations consistently resulted in significantly longer villus height of the jejunum at 42 days of age compared with the control treatment (P<0.05). No differences were found in villus width and villus height to crypt depth ratio of small intestinal at 42 days of age by the inclusion of myrtle essential oil and antibiotic supplementations in the diet. Some Myrtle essential oil and antibiotic supplementations significantly decreased the epithelial thickness of small intestinal (P<0.05) at 42 days of age compared with the control treatment.

Table 5. Effect of dietary MEO and VGN on small intestine morphology in broiler chickens

Item	Control	MEO1[1]	MEO2[2]	MEO3[3]	VGN[4]	SEM[5]	P value
Duodenom							
Villous height (µm)	965.00	980.70	914.00	895.30	900.70	23.53	0.55
Villous width (µm)	103.33	116.00	118.67	120.33	119.100	2.14	0.1
Crypt depth (µm)	305.33[b]	326.33[a]	315.67[ab]	326.33[a]	324.00[a]	25.98	0.04
Villous height to crypt depth ratio	3.16	3.00	2.89	2.74	2.77	0.18	0.23
Epithelial thickness (µm)	49.2[a]	43[b]	45[ab]	46.5[ab]	43[b]	1.37	0.02
Jejunum							
Villous height (µm)	872.7[b]	887.00[a]	892.3[a]	888.7[a]	888.7[a]	10.78	0.02
Villous width (µm)	96.67	110.67	108.67	106.67	155.33	20.30	0.6
Crypt depth (µm)	312.67	320.00	320.00	322.00	315.67	19.79	0.7
Villous height to crypt depth ratio	2.79	2.77	2.78	2.75	2.81	0.12	0.44
Epithelial thickness (µm)	41[a]	38.7[ab]	37[abc]	35.2[bc]	34[c]	1.31	0.01
Ileum							
Villous height (µm)	790.33[b]	810.33[a]	806.00[a]	805.67[ab]	803.00[ab]	68.80	0.04
Villous width (µm)	92.66	103.33	100.00	98.00	112.00	4.84	0.43
Crypt depth (µm)	300.67	306.67	311.00	311.00	312.67	19.64	0.68
Villous height to crypt depth ratio	2.62	2.64	2.59	2.59	2.56	0.34	0.5
Epithelial thickness (µm)	37.7[a]	32.5[ab]	27.7[bc]	26.2[bc]	25.7[c]	2.5	0.004

[1]Myrtle Essential oil (150 mg/Kg); [2]Myrtle Essential oil (300 mg/Kg); [3]Myrtle Essential oil (450 mg/Kg); [4]Virginiamycin; [5]Standard Error of Means.
[a-c]Means within a column having different superscripts are significantly different (P < 0.05).

Table 6 shows the antibody response of broiler chicks after immunization with SRBC and lymphoid organs. No significant differences were observed in antibody titer against SRBC at the first time after immunization (P>0.05). The effect of treatments on secondary antibody response was significant (P<0.05). All of the experimental groups showed lower immunity response in comparison with control and immunity response of antibiotic and myrtle at 300 and 450 mg/Kg showed the significant decrease in comparison to other experimental groups. Differences in the relative lymphoid organs weights were not statistically significant among the dietary treatments (P>0.05).

Table 6. Effect of dietary MEO and VGN on antibody response and lymphoid organs in broiler chickens

Treatment	Antibody response and lymphoid organs			
	Primary immunization	Second immunization	spleen	BF[6]
Control	2.00	5.50[a]	0.12	0.21
MEO1[1]	2.75	4.25[ab]	0.10	0.11
MEO2[2]	2.75	3.75[bc]	0.15	0.22
MEO3[3]	2.25	2.75[c]	0.11	0.18
VGN[4]	3.00	2.75[c]	0.10	0.25
SEM[5]	0.46	2.01	0.01	0.03
P value	0.6	0.005	0.14	0.76

[1]Myrtle Essential oil (150 mg/Kg); [2]Myrtle Essential oil (300 mg/Kg); [3]Myrtle Essential oil (450 mg/Kg); [4]Virginiamycin; [5]Standard Error of Means; [4] Bursa of Fabricius.
[a-c]Means within a column having different superscripts are significantly different (P < 0.05).

Broiler chickens fed diet supplemented with 450 mg/Kg MEO had lower white blood cell, heterophil, heterophil to lymphocyte ratio and higher lymphocyte count (P<0.05) compared to the other treatments (Table 7). Dietary supplementation with MEO and VGN did not influence hematocrit (P>0.05). Broiler chickens fed diets supplemented with MEO or VGN had significantly higher RBC rather than those birds were fed the control diet (P<0.05). Significant increase in the serum total protein concentration observed in myrtle group at 300 and 450 mg/Kg compared to the other groups (P<0.05). The density of cholesterol at first and second blood sampling (27 and 41 days of age) showed significant differences among experimental groups (P<0.05). The highest and lowest density of cholesterol at each of two steps was observed in antibiotic and myrtle at 450 mg/Kg treatments, respectively.

Table 7 Effect of dietary MEO and VGN on blood parameters in broiler chickens

Item	Control	MEO1[1]	MEO2[2]	MEO3[3]	VGN[4]	SEM[5]	P value
WBC[6] (× 103×µl)	14.37^{a}	13.65^{a}	11.57^{b}	8.20^{d}	10.10^{c}	0.48	0.001
Lymphocyte (%)	90.50^{b}	90.50^{b}	90.75^{b}	96.00^{a}	89.75^{b}	1.12	0.008
Heterophil (%)	9.25^{a}	7.00^{a}	8.50^{a}	3.50^{b}	8.75^{a}	1.15	0.01
H/L[7]	0.10^{a}	0.077^{ab}	0.093^{a}	0.036^{b}	0.097^{a}	0.01	0.02
RBC[8] (× 106×µl)	1.78^{d}	1.93^{c}	2.16^{b}	3.38^{a}	2.25^{ab}	0.04	0.001
Hematocrit	35.00	34.43	34.92	35.42	32.12	0.61	0.13
Serum cholesterol (mg/dl)							
27 day old	111.9^{b}	110.5^{b}	108.7^{b}	104.9^{c}	117.5^{a}	5.5	0.03
41 day old	110.1^{b}	106.2^{bc}	103.4^{bc}	99.5^{c}	120.4^{a}	7.5	0.04
Total protein (gr/dl)	2.30^{b}	3.83^{b}	4.00^{a}	4.98^{a}	3.70^{b}	0.12	0.04

[1]Myrtle Essential oil (150 mg/Kg); [2]Myrtle Essential oil (300 mg/Kg); [3]Myrtle Essential oil (450 mg/Kg);
[4]Virginiamycin; [5]Standard Error of Means; [6] White Blood Cell; [7]Heterophil to Lymphocyte ratio; [8] Red Blood Cell.
[a-c]Means within a column having different superscripts are significantly different (P < 0.05).

Discussion

The major components were α-Pinene (30.1%), Limonene (20.5%), 1, 8-cineole (18.0%), Linalool (9.8%), Linalyl acetate (4.2%) and α-terpineole (3.3%). Rasooli et al. (2002) reported that the major components of *Myryus communis*. Essential oil are α-pinene (29.4%), Limonene (21.2%), 1, 8-cineole (18%), Linalool (10.6%), Linalyl acetate (4.6%) and α-terpineole (3.1%). Romani et al. (2004) have previously reported this composition but they havealso suggested that environmental factors such as geography, temperature, day length and nutrients, may modify the chemicalcomposition of the myrtle oil. These factors influence plant biosynthesis pathways and consequently, the relative proportions of the main characteristic compounds.

Although MEO and VGN supplementation decreased the feed intake in younger birds but increased it at the end weeks and throughout the trial. This finding is not in agreement with the findings of Hernandez et al. (2004) who reported that addition of plant extracts or essential oils to broiler diets had no effect on feed intake. Also, on the contrary of our results, Çabuk et al. (2006) showed that supplementation of essential oils caused a significant decrease of feed intake of broilers. The differences in food consumption may be caused by various tastes and odours of the medicinal herbs.

In this study, MEO and VGN significantly increased body weight gain compared to the control treatment. Studies on the use of essential oils or essential oil combinations (EOC) have yielded inconsistent results. Hernandez et al. (2004) reported that the addition of plant extracts to feed mixtures in the starter period generates higher weights in broiler chickens. Alçiçek et al. (2003) found that supplementation of essential oil complex (containing 6 different oils including oregano oil, laurel leaf oil, sage leaf oil, myrtle leaf oil, fennel seed oil and citrus peel oil) at the concentrations of 24, 48 and 72 mg/Kg increased the body weight in broilers.

In the current study, the improvement of feed conversion ratio in broiler chickens fed MEO and VGN could be related to a more efficient use of nutrients. Hernandez et al. (2004) reported the positive effects of essential oil mixtures on nutrient digestibility, improving the feed efficiency. Biricik et al. (2012) reported that using myrtle at 500 and 2000 mg/kg of diet improved feed conversion ratio as compared to the control treatment. It was suggested that dietary essential oils improve bird performance because these substances stimulate the secretion of endogenous digestive enzymes which then increases nutrient digestion, gut passage rate or feed intake (Lee et al., 2004).

In this study, relative carcass weight was increased by supplementing MEO and VGN especially at 450 mg/Kg and VGN treatments and also relative weight of duodenum, jejunum, ileum, gizzard and pancreas tended to decrease. The decrease in the relative weight of gastrointestinal tract in birds consuming antibiotics may be related to the decrease in epithelial thickness of the gut. Rahimi et al. (2011) reported that antibiotics are effective in decreasing the small intestinal weight in broiler chickens. The findings about carcass traits are also in accordance with the findings of Hernandez et al. (2004) who observed no difference in the weights of gizzard, liver, and pancreas in broiler chickens fed wheat-soybean meal based diets supplemented with two plant extracts (an essential oil extract from oregano, cinnamon, and pepper and an essential oil extract from sage, thyme, and rosemary). Herbs and herbal extracts have variable effects on performance parameters. Therefore, further researches are needed to identify the more efficient essential oils in poultry feeding.

Similar observations were reported by Garcia et al. (2007), who showed that using medicinal plants in feed increase villus height in chickens. They suggest that medicinal plants decrease the total harmful bacteria in the intestinal wall and cause a reduction in production of toxic compounds and therefore reduce damage to intestinal epithelial cells such as shorter villi and deeper crypts. This function could lead to a conversion in intestinal morphology. Giannenas et al. (2010) reported a shorter villus and a deeper crypt when the counts of pathogenic bacteria increase in the gastrointestinal tract, which results in fewer absorptive and more secretory cells. The size and height of the villi are important for intestinal function and diet is one of the important factors that could alter the morphology of intestinal villi.

In this experiment, results shows that villi height increased significantly in birds given diet containing myrtle essential oil and this is an important factor in the efficiency of digestion and absorption of nutrients. The height of the villi may reflect the surface area. The increase in villi height suggests an increase in the absorptive surface area and greater absorption of available nutrients and thus, feed efficiency will be improved.

In this study, higher villus height could be related to the antibacterial property of antibiotic and myrtle oil. The mucus layer in the small intestine plays an important role in the protection of the small intestinal epithelial cells and in transport between the lumen and the brush border membrane and thus, the ontogeny of its development has extensive implications for intestinal function. Myrtle essential oil and antibiotic supplementations significantly influenced epithelial thickness in this study may suggest an enhanced rate of nutrient absorption. In this experiment, the results showed that the use of myrtle essential oil improved intestinal morphology characteristics such as longer villus height and lower epithelial thickness, which this reaction can lead to increase feed utilization and improve performance.

Generally, the essential oil possesses the strongest antibacterial properties against pathogens. The essential oil contains a high percentage of phenolic compounds such as carvacrol, eugenol, and thymol (Brenes & Roura, 2010). Improvements in treatments containing myrtle essential oil probably are due to the myrtle oil antibacterial properties.

In poultry production, it is very important to improve immunity to prevent infectious diseases. A variety of factors such as vaccination failure, infection by the immune suppressive diseases and abuse of the antibiotics can induce immune deficiency. Utilization of immune stimulants is a solution to improve the immunity of animals and decrease their vulnerability to the infectious disease (Liu, 1999). The study of the immune system in this experiment showed that MEO was most effective in the immune system improvement. It is suggested that essential oils that are rich in such flavonoids as thyme extend the activity of vitamin C, act as antioxidants and may, therefore, enhance the immune function (Rahimi et al., 2011).

The decrease of secondary immunity response in the experimental group including myrtle at 450 mg/Kg (with respect to antimicrobial traits of these materials and level of myrtle used) may be due to the effect of these materials on the decrease of gut microflora (decrease of secondary immunity response also observed in treatment including antibiotic). Differences in relative lymphoid organs (bursa of Fabricius and spleen) weights were not statistically significant. The results were in agreement with the findings of Rahimi et al. (2011) that found the relative weight of spleen was unaffected by the coneflower group. They reported that the immunity system statuses were improved in the coneflower group.

Red blood cell value was increased in MEO and VGN treatments compared to the control. It can be deduced from these findings that MEO has a favorable effect on hematopoiesis. Biricik et al. (2012) observed a positive effect of adding myrtle oil to broiler chicken diets at doses above 1000 mg/Kg on hematocrit. Toghyani et al. (2010) observed a significant increase in hemoglobin concentration and hematocrit percentage in quails fed with Black seeds (Nigella sativa). They concluded that Black seeds have a favorable effect on hematopoiesis. In another study, the highest hematocrit value was related to the broiler chickens fed diets supplemented with oil extracts derived from clove and cinnamon, but not with thyme (Najafi & Torki, 2010). The myrtle oil antioxidant activity may be provided mainly through myricetin-3-o-galactoside and myricetin-3-o-rhamnoside, which prevents lipid oxidation in muscular cells but also in other cells such as erythrocytes (Romani et al., 2004). Consequently, the reduction in lipid oxidation in erythrocytes may contribute to the strengthening of the cell membrane stability and decrease the erythrocyte susceptibility to hemolysis. The highest amount of blood cholesterol at both steps at which blood was drawn observed at birds that consumed antibiotic. With respect to the role of lactobacillus and bifidobacteria in the decrease of blood cholesterol, and also the role of virginiamycin antibiotic in harness of gram-positive bacteria (such as lactobacillus and bifidobacteria), it can be deduced that increase of blood cholesterol at experimental group consumed antibiotic was due to antibacterial role of virginiamycin against gram-positive bacteria. Also, it is reported that after antibiotic consumption, immunity response decreases that is due to the decrease of gut microbial load. It is likely that in the absence of stimulating the immunity system, need for energy in order to immunization decrease. In this case, extra available energy (likely in the form of acetyl-CoA) will shift to increase the synthesis of lipid and cholesterol that will cause to increase of abdominal fat pad and blood cholesterol. In experimental birds fed myrtle, the amount of blood cholesterol was lowest in both steps of drawing blood. Studies showed that different kinds of EO compounds like granule, cineol and borneol cause in the decrease of S-3-Hydroxy-3-methylglutaryl-CoA (HMG-COA) reductase activity in liver (Middleton et al., 1979). Studies showed that for each 5 percent harness of HMG-COA reductase (a key enzyme in cholesterol synthesis), the amount of cholesterol synthesis decreases 2 percent (Case et al., 1995). Results achieved from this study agree with AL-Kassie (2009) who reported that when 200 ppm of essential oil derived from *C. verum* were added to a standard diet of broiler chicks for 42 days; a significant increase in total proteins was observed, compared to a control group. Also, it agrees with Abbas (2010) who found that dietary *T. foenum-graecum* seeds at 3 g/kg of the diet for 42 days, increased numerically the total protein in the chicks. From this case, one can suggest that elevated serum TP level may be due to high level of protein and other nutrients in *T. foenum-graecum* L seeds. This could be supported by the idea of Hoffman (1966) who cited that, serum protein levels are sensitive to nutritional influences. Over and above, Tollba et al. (2010) found that mixture of volatile oils including thyme, oregano, C.verum and capsicum added to the two groups of chicks diets at 1 or 2 g /kg feed in the experimental period which lasted at 12 wks of age, increased significantly total protein as well as albumin and globulin comparing to unsupplemented control group. In some cases increased serum TP may accompany acute inflammatory states, dehydration, or secondary to certain types of tissue damage. In this study, no adverse clinical signs were observed. From findings and observations in this work, it can suggest that elevated serum TP levels in myrtle treated groups may be due to the nutritional potential effect of the treated diets and an increased body weight gain.

In conclusion, data showed that diet supplemented with Myrtus improved the growth performance, increased antibody titers against SRBC, especially at the level of 300 mg/Kg, significantly increased the serum total proteins at 450 mg/Kg and improved morphological status of small intestine in broiler chickens. Myrtle oil supplementation may be considered a potential natural growth promoter. However, more studies are needed to define the effect of myrtle oil supplementation on the performance of poultry with regard to environmental conditions, effective dosage, active oil substances, dietary ingredients and nutrient density.

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