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Growth Performance, Carcass Characteristics, Antibody Titer and Blood Parameters in Broiler Chickens Fed Dietary Myrtle (*Myrtus communis*) Essential Oil as an Alternative to Antibiotic Growth Promoter

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Abstract

This experiment was conducted to determine the effects of Myrtle Essential Oil (MEO) on growth performance, carcass characteristics, antibody titer and blood parameters of broiler chickens. A total of 200 Ross 308 broiler chickens were allocated to five dietary treatments with four replicates of 10 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 100, 200, 300 mg/Kg or antibiotic Flavophospholipol (FPL) at 600 mg/Kg. The results showed that diets supplemented with MEO and FPL 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 gastrointestinal tract and liver were decreased in broilers fed MEO (*P<0.05*). Supplementing the basal diet with MEO increased the antibody titers against Avian Influenza Virus (AIV) and Newcastle disease Virus (NDV), although supplementing diet with 200 mg/Kg of MEO was more effective (*P<0.05*). Broilers fed MEO diets especially at the level of 300 mg/Kg had a lower white blood cells count and heterophil, heterophil to lymphocyte ratio, mean corpuscular volume and mean corpuscular hemoglobin, 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 AIV and NDV, especially at the level of 200 mg/Kg, in broiler chickens and could be an adequate alternative to antibiotics.

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Introduction

Antibiotics have been used for more than half a century to improve animal performance and decreasing the pathogenic bacteria populations (Gollnisch *et al.*, 2001). Unfortunately, the bacteria have established resistant strains by transferring resistance to other species especially in shared strains between humans and animals, and resulted in serious problems in public health and livestock production (Thakar, 2004). Therefore, a number of the antibiotic growth promoters have been banned by European Union (Neu, 1992). In recent years, an extensive research has been performed on substances that could be used as an alternative antibiotic replacement in poultry diets. Prebiotics (Ashraf *et al.,* 2013), probiotics (Sohail *et al*., 2012), and organic acids (Ricke, 2003) have the potential to improve the performance of poultry through microbial balance in the gastrointestinal tract.

Recently, the use of essential oil has become popular due to 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 (Di Pasqua *et al.,* 2007). *Myrtus communis* (from Myrtaceae family and subfamily Myrtoideae) is an annual plant used for medicinal, food, and spice purposes (Baytop, 1999). This aromatic plant grows wildly in the coastal areas of Tunisia, Morocco, Turkey, France, and Iran (Romani *et al.,* 2004). The fruits are mostly composed of volatile oils, tannins, sugars, flavonoids, and organic acids such as citric and malic acids (Martin *et al*., 1999). The leaves contain tannins, flavonoids such as quercetin, catechin, myricetin derivatives and volatile oils (Baytop, 1999; Romani *et al.*, 2004). The essential oil can be obtained from the leaves of *Myrtus communis* by steam distillation (Baytop, 1999). Ozek *et al.* (2000) reported the most important constituents of MEO as myrtenol, myrtenol acetate, limonene, linalool, α-pinene, 1,8-cineole, β-caryophyllenein, p-cymene, geraniol, nerol, phenylpropanoid and methyleugenol. Different parts of *Myrtus communis* essential oils have been used for different purposes. The antioxidative property of flavonoid derivatives from *Myrtus communis* had a protective effect against cardiovascular diseases (Romani *et al.,* 2004). The antimicrobial effects of the essential oils against *Escherichia coli*, *Staphylococcus aureus,* and *Candida albicans* have also been demonstrated by Yadegarinia *et al.* (2006). Essential oils obtained from the leaves of *Myrtus communis* have also 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 chemical composition of MEO and making a comparison between its effects and Flavophospholipol (FPL) as a growth promoter on the performance, carcass characteristics, and blood parameters in broiler chickens.

Materials and Methods

Essential Oil Composition

The essential oil was analyzed by a gas chromatograph (9-A-Shimadzu, Australasia) and GC/MS (Varian-3400, Australasia) column (DB-1, 60 mm × 0.25 mm fused silica capillary column, film thickness 0.25 μm) using a temperature program of 40-220ºC at a rate of 4ºC /min, an injector temperature of 260ºC and with helium as the carrier gas. The constituents were identified by the comparison of their mass spectra with those in the computer library and with authentic compounds. The identifications were confirmed by the comparison of their retention indices with those of authentic compounds or with literature.

Animal husbandry and dietary treatment

The study was conducted at Poultry Research Station, University of Tehran (Aburaihan Campus, Pakdasht, Tehran, Iran) from February to April 2012. Two hundred mixed-sex day-old broiler chickens (Ross 308) were weighed and randomly assigned to five dietary treatments with four replicates of 10 birds each. To prepare the dietary treatments, an antibiotic-free corn soybean meal-based diet was formulated according to Ross 308 broiler r manual (Aviagen, 2009; Table 1).

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)
Maize, 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 Premix[1]	2.50	2.50	2.50
Mineral Premix[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.

The experimental treatments were prepared by supplementing the basal diet with 600 mg/Kg of Flavophospholipol (FLP) either 100, 200, or 300 mg/Kg of Myrtle essential oil (MEO). Myrtle essential oil was obtained from Zardband Pharmaceutical Company, (Tehran, Iran). The diets were in mash form and prepared every week. Birds were reared on deep litter floor pens and had free access to the feed and water throughout the trial. The ambient temperature was gradually decreased from 33 to 20°C according to Ross 308 broiler manual (Aviagen, 2009). The lighting program consisted of 23 hrs light and 1 h darkness during the study.

Performance traits

Body weight and feed intake were recorded weekly on pen basis and feed conversion ratio (feed intake to body weight gain) was then calculated.

Carcass characteristics

On d 42, one bird per pen closest to the mean body weight of the corresponding pen was slaughtered and carcass, breast, thigh, abdominal fat pad, liver, heart, gastrointestinal, and lymphoid organs (spleen and bursa of Fabricius) were weighed.

Antibody titer

All chickens were vaccinated against Avian Influenza Viruses (AIV, H9N2; Intervet Co., Boxmeer, Netherlands) intramuscularly and Newcastle Disease Viruses (NDV-IV strain vaccine, Intervet Co., Boxmeer, Netherlands) via drinking water on d 18. At 28 days of age two birds per replicate were randomly chosen and blood samples were collected from the brachial vein. Blood samples centrifuged at 2000×g for 15 minutes to obtain the serum. Antibody titers against NDV and AIV were measured using Hemagglutination Inhibition (HI) test according to Brugh *et al*. (1978).

Blood parameters

At d 42, one bird per replicate was selected and blood samples were collected for determination of white blood cell (WBC), red blood cell (RBC), hematocrit (HCT), and hemoglobin (Hb). For heterophil to lymphocyte ratio, smears were prepared and stained by Giemsa method (Toghyani *et al*., 2010). One hundred leukocytes per slide were counted by heterophil to lymphocyte separation by light microscopy and then heterophil to lymphocyte ratio was calculated (Gross and Sigel, 1983). The RBC and WBC counts were determined by a hemocytometer using Natt-Herrick solution (Toghyani *et al*., 2010). Hematocrit and hemoglobin values were measured by microhematocrit and cyanmethemoglobin methods, respectively (Kececi *et al*., 1998). The mean corpuscular volume (MCV), mean

corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were computed according to Campbell (1995).

Statistical analysis

Data were analyzed in a completely randomized design using the General Linear Models (GLM) procedure of SAS (2001). Significant differences among treatment means were separated using Duncan's multiple range test. Statements of statistical significance are based on *P<0.05*.

Results

Chemical composition of Myrtle essential oil

The chemical composition of MEO obtained by GC and GC–MS analysis has been presented in Table 2. The major components of MEO were α-Pinene (30.1%), Limonene (20.4%), 1,8-Cineole (18.1%), Linalool (9.8%), Linalyl acetate (4.2%), and α-Terpineole (3.3%).

Table 2. Chemical composition of MEO[1]

No	Compound	Retention index	%	No	Compound	Retention index	%
1	Isobutyl isobutyrate	890	0.8	13	α-Terpineole	1179	3.3
2	α-Thujene	920	0.24	14	Trans-Carveole	1213	0.5
3	α-Pinene	932	30.1	15	Cis-Carveole	1218	0.1
4	Myrcene	981	0.2	16	Geraniol	1244	1.0
5	ρ-Cymene	1019	0.5	17	Linalyl acetate	1249	4.2
6	Limonene	1023	20.4	18	Methyl geranate	1312	0.2
7	1,8-Cineole	1027	18.1	19	α-terpinyl acetate	1342	1.2
8	γ-terpinene	1057	0.5	20	Methyl eugenol	1370	1.7
9	Terpinolene	1084	0.4	21	β-Caryophyllene	1432	0.3
10	Linalool	1087	9.8	22	α-humulene	1464	0.1
11	α–Campholenal	1120	0.3	23	Spathulenol	1564	0.1
12	Terpinene-4-ol	1168	0.4	24	Caryophylleneb epoxide	1588	0.3

[1]Myrtle Essential Oil.

Growth performance

The results related to feed intake are presented in Table 3. Broiler chickens were fed MEO and FPL treatments had a lower feed intake during 8-14 days of age as compared to the control treatment (*P<0.05*). Supplementing of MEO to the basal diet decreased the feed intake during 22-28 days of age (*P<0.05*), but it reversely increased during 29-35 and 36-42 days of age compare to the control treatment (*P<0.05*). MEO at the level of 300 mg/Kg and also FPL supplementation were more effective in increasing feed intake throughout the trial (1-42 days of age) (*P<0.05*).

The effects of MEO and FPL supplementation on body weight gain of broiler chickens are shown in Table 4. Birds were fed diets supplemented with MEO and FPL had a higher body weight gain in all weeks and it was significant, except during 0-7 and 7-14 days of age, compared to the control treatment (*P<0.05*). As

shown in Table 5, a beneficial effect of supplementing MEO and FPL to the broiler chickens diet on feed conversion ratio was observed during all weeks, 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 FPL[2] on feed intake in broiler chickens

Treatment	Feed intake (g)						
	0-7 d	8-14 d	15-21 d	22-28 d	29-35 d	36-42 d	1-42 d
Control	128.0	400.9^{a}	686.3	1127.7^{a}	976.0^{b}	746.6^{c}	4065.5^{b}
MEO (100 mg/Kg)	113.5	292.5^{b}	751.3	1020.7^{bc}	1140.4^{a}	839.1^{b}	4157.5^{ab}
MEO (200 mg/Kg)	116.6	317.9^{b}	743.3	968.3^{c}	1106.5^{a}	881.1^{b}	4133.7^{ab}
MEO (300 mg/Kg)	118.2	299.9^{b}	811.1	1035.5^{bc}	1113.8^{a}	958.9^{a}	4337.4^{a}
FPL	121.0	293.1^{b}	777.5	1100.2^{ab}	1128.1^{a}	865.5^{b}	4285.4^{a}
SEM[3]	8.20	17.41	30.65	28.21	35.97	19.35	66.37
P value	0.770	0.002	0.110	0.008	0.034	<0.001	0.05

[1]Myrtle Essential Oil; [2]Flavophospholipol; [3] Standard Error of Means.
[a-c]Means within a column having different superscripts are significantly different (*P<0.05*).

Table 4. Effect of dietary MEO[1] and FPL[2] on body weight gain in broiler chickens

Treatment	Body weight gain (g)						
	0-7 d	8-14 d	15-21 d	22-28 d	29-35 d	36-42 d	1-42 d
Control	71.8	161.9	300.2^{b}	518.9^{b}	416.5^{b}	387.7^{c}	1857^{b}
MEO (100 mg/Kg)	72.6	181.3	385.9^{a}	747.7^{a}	584.4^{a}	431.4^{bc}	2403.3^{a}
MEO (200 mg/Kg)	74.0	179.9	405.1^{a}	766.1^{a}	546.9^{a}	458.8^{ab}	2430.8^{a}
MEO (300 mg/Kg)	74.6	184.6	407.9^{a}	768.6^{a}	552.8^{a}	504.7^{a}	2493.2^{a}
FPL	72.4	183.6	373.7^{a}	770.9^{a}	572.0^{a}	455.2^{ab}	2427.8^{a}
SEM[3]	2.5	10.2	15.7	25.5	20.9	20.3	45.88
P value	0.9	0.5	0.001	<0.0001	0.0003	0.015	<0.001

[1]Myrtle Essential Oil; [2]Flavophospholipol; [3] Standard Error of Means.
[a-c]Means within a column having different superscripts are significantly different (*P<0.05*).

Table 5. Effect of dietary MEO[1] and FPL[2] on feed conversion ratio in broiler chickens

Treatment	Feed conversion ratio						
	0-7 d	8-14 d	15-21 d	22-28 d	29-35 d	36-42 d	1-42 d
Control	1.78	2.50^{a}	2.3^{a}	2.17^{a}	2.37	1.92	2.19^{a}
MEO (100 mg/Kg)	1.57	1.61^{b}	1.95^{b}	1.37^{bc}	1.96	1.96	1.73^{b}
MEO (200 mg/Kg)	1.58	1.77^{b}	1.85^{b}	1.27^{c}	2.03	1.93	1.70^{b}
MEO (300 mg/Kg)	1.58	1.63^{b}	2.00^{ab}	1.35^{bc}	2.02	1.90	1.74^{b}
FPL	1.67	1.65^{b}	2.10^{ab}	1.43^{b}	2.00	1.91	1.77^{b}
SEM[3]	0.10	0.16	0.10	0.04	0.14	0.08	0.03
P value	0.50	0.004	0.049	<0.001	0.24	0.97	<0.001

[1]Myrtle Essential Oil; [2]Flavophospholipol; [3] Standard error of means.
[a-c]Means within a column having different superscripts are significantly different (*P<0.05*).

Carcass characteristics and lymphoid organs

The effects of MEO and FPL on carcass characteristics of broiler chickens are shown in Table 6. The relative weight of breast, thigh, abdominal fat, liver, heart, and gastrointestinal tract were not affected by MEO and FPL supplementation. Relative carcass weight of broiler chickens was significantly increased by FPL and MEO supplementation at 300 mg/Kg (*P<0.05*). The relative gastrointestinal weight was significantly lower in broiler chickens fed FPL (*P<0.05*) compared to the control treatment (*P<0.05*). Differences in the relative lymphoid organs weights were not statistically significant among the dietary treatments (*P>0.05*).

Table 6. Effect of dietary MEO[1] and FPL[2] on carcass composition and lymphoid organs in broiler chickens (% live body weight)

Treatment	Carcass composition							Lymphoid organs	
	Carcass	Breast	Thigh	AF[3]	Liver	Heart	GI[4]	Spleen	BF[5]
Control	67.70^{c}	22.74	10.27	1.96	2.44	0.67	10.38^{a}	0.12	0.21
MEO (100 mg/Kg)	68.05^{bc}	23.11	11.09	1.69	2.36	0.60	10.48^{a}	0.10	0.19
MEO (200 mg/Kg)	68.17^{bc}	23.44	10.58	1.73	2.42	0.60	10.18^{a}	0.15	0.22
MEO (300 mg/Kg)	69.47^{ab}	24.44	10.82	1.29	2.32	0.57	9.53^{ab}	0.11	0.18
FPL	70.08^{a}	23.78	10.65	1.55	1.94	0.57	8.98^{b}	0.10	0.25
SEM[6]	0.47	0.78	0.2	0.19	0.12	0.03	0.36	0.01	0.03
P value	0.01	0.58	0.13	0.2	0.08	0.38	0.05	0.14	0.76

[1]Myrtle Essential Oil; [2]Flavophospholipol; [3]Abdominal fat; [4]Gastrointestinal; [5]Bursa of Fabricius; [6]Standard Error of Means.

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

Antibody response and blood parameters

The effects of MEO and FPL on antibody response and blood parameters are shown in Table 7. The results indicated that MEO supplementation at 200 mg/Kg feed was more effective to increase the antibody titers against NDV and AIV (*P<0.05*). Broiler chickens fed diet supplemented with 300 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.

Dietary supplementation with MEO and FPL did not influence hemoglobin, hematocrit, and MCHC (*P>0.05*). Broiler chickens fed diets supplemented with MEO or FPL had significantly higher RBC and lower MCV as well as MCH rather than those birds were fed the control diet (*P<0.05*).

Table 7. Effect of dietary MEO[1] and FPL[2] on antibody response and blood parameters in broiler chickens

Treatments	Control	MEO (g/Kg)			FLP	SEM[3]	*P value*
		100	200	300			
Antibody response:							
AIV[4]	3.75[b]	4.00[b]	4.75[a]	4.00[b]	4.00[b]	0.158	0.005
NDV[5]	4.00[b]	4.75[ab]	5.50[a]	4.75[ab]	4.25[b]	0.290	0.02
Blood parameters:							
WBC[6] ($\times 10^3/\mu L$)	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]	0.104[a]	0.077[ab]	0.093[a]	0.036[b]	0.097[a]	0.01	0.02
RBC[8] ($\times 10^6/\mu L$)	1.78[d]	1.93[c]	2.16[b]	3.38[a]	2.25[ab]	0.04	<0.001
Hemoglobin (g/dL)	9.70	8.92	9.72	9.70	9.25	0.30	0.28
Hematocrit (%)	35.00	34.43	34.92	35.42	33.12	0.61	0.13
MCV[9] (fL)	195.8[a]	178.6[b]	161.7[c]	149.1[d]	147.3[d]	3.62	<0.001
MCH[10] (pg)	54.28[a]	46.20[b]	45.01[b]	40.75[c]	41.12[c]	1.16	<0.001
MCHC[11] (%)	27.73	25.91	27.83	27.36	27.93	0.67	0.23

[1]Myrtle Essential Oil; [2]Flavophospholipol; [3]Standard Error of Means; [4]Avian Influenza Virus; [5]Newcastle Disease Virus; [6]White Blood Cell; [7] Heterophil/Lymphocyte Ratio; [8]Red Blood Cell; [9]Mean Corpuscular Volume (Mean Red Blood Cell Volume); [10]Mean Corpuscular Hemoglobin; [11]Mean Corpuscular Hemoglobin Concentration.

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

Discussion

In the present study, the major components of MEO were α-Pinene (30.1%), Limonene (20.4%), 1,8-Cineole (18.1%), Linalool (9.8%), Linalyl acetate (4.2%), and α-Terpineole (3.3%). Rasooli *et al.* (2002) reported that the major components of MEO were α-Pinene (29.4%), Limonene (21.2%), 1,8-Cineole (18%), Linalool (10.6%), Linalyl acetate (4.6%), and α-Terpineole (3.1%). Gardeli *et al.* (2008) reported that the major components of MEO were myrtenyl acetate (39%), 1,8 cineole (13.5%), α-pinene (10.9%), and linalyl acetate (3.6%). However, Akin *et al.* (2010) did not find α-pinene and 1,8-cineole in myrtle oil. Romani *et al.* (2004) reported that environmental factors such as geography, temperature, day length, and nutrients may modify the chemical composition of the myrtle oil. These factors influence the plant biosynthesis pathways and consequently, the relative proportions of the main characteristic compounds (Biricik *et al.*, 2012).

Although MEO and FPL supplementation decreased the feed intake at the lower ages, but increased it at the end weeks and throughout the trial. This finding is not in agreement with the findings of Lee *et al.* (2003), Botsoglou *et al.* (2004) and Hernandez *et al.* (2004) who reported that addition of plant extracts or essential oils to broiler diets had no effect on feed intake. In this study, MEO and FPL significantly increased body weight gain compared to the control treatment. It is expected that the use of essential oils influence the population of the gut microflora

and their impact on digestive enzyme secretion. Similarly, Jang *et al.* (2007) reported that feeding commercial mixed herbs to broiler chickens during the starter period increased daily weight gain compared with the control group. Also, Hernandez *et al.* (2004) reported that the addition of plant extracts to feed mixtures in starter period generates reasonably 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 FPL could be related to a more efficient use of nutrients. Hernandez *et al.* (2004) and also Madrid *et al.* (2003) 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. Cross *et al.* (2007) reported that essential oils derived from herbs improve growth in poultry by stimulating digestive, microbial ecosystem balance and increasing internal secretion of the enzymes. It was suggested that dietary essential oils improve birds 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*., 2003; Lee *et al.*, 2004).

In this study, relative carcass weight was increased by supplementing FPL and MEO especially at 300 mg/Kg and also relative gastrointestinal weight 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 so 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).

Myrtle essential oil (MEO) at 200 mg/Kg was more effective in increasing antibody titers against NDV and AIV. Also, broiler chickens fed MEO at 300 mg/Kg had lower white blood cells, heterophils, heterophil to lymphocyte ratio and higher lymphocytes compared with the other treatments. 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 antibody levels were improved in the coneflower group. Al–Ankari *et al.* (2004) found that the use of herbal mint (*Mentha longifolia*) in broiler chicken diets increases antibody titers against NDV, which suggested that essential oil stimulates the immune system. 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 immune system in this experiment showed that MEO was most effective in the immune system improvement. Suggested 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).

Red blood cell value was increased and MCV and MCH values were decreased in MEO and FPL 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. Al-Kassie *et al.* (2008) showed that broilers fed with oil extracts derived from thyme and cinnamons exhibited significantly higher hemoglobin concentrations and hematocrit percentages. 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 decreasing the erythrocyte susceptibility to hemolysis.

Conclusion

The results of current study suggested that supplementary MEO, at least the level of 100 mg/Kg, improves body weight gain and feed conversion ratio in broiler chickens. Myrtle essential oil (MEO) at the level of 200 mg/Kg improves antibody titers against NDV and AIV in broiler chickens. However, taking into consideration the environmental conditions, dosages used, active oil substances, dietary ingredients and nutrient density, more studies are needed to elucidate the other effects of MEO supplementation on the performance and immune system in poultry.

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