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Research Article

Effect of *Myrtus communis* L. Plant Extract as a Drinking Water Supplement on Performance, Some Blood Parameters, Egg Quality and Immune Response of Older Laying Hens

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Abstract

The aim of the present study was to evaluate the effects of myrtle plant extract (MPE) on performance, some blood parameters, egg quality and immune response of older laying hens. A total of 192 laying hens (67 weeks old; initial body weight 1.63±0.17 kg) were used in this study. The MPE was added to the water of the experimental groups (0%, 2.5%, 5.0%, or 10%) for 8 weeks. Liquid chromatography analyses showed that myricetin was the predominant active ingredient (15.34 mg/L) in MPE. In treatment groups, feed consumption, egg mass (P<0.05), egg production (P<0.01) increased (10% MPE) and water consumption ascended (5% MPE) compared to the control birds. Feed conversion ratio did not change. In 2.5% MPE group, greater egg weight (P<0.001) and darker egg yolk (P<0.01) were observed than the control birds. Regarding immunity, the 5% Myrtus group produced higher neutrophil and large amounts of IgG (P<0.05) indicating that significant results were also observed in the immunological response of laying hens vaccinated against the Newcastle virus. Serum alanine aminotransferase level increased (P<0.05) in treatment groups whereas serum glucose levels decreased significantly (P<0.01). Other than that, none of the blood parameters changed. It is concluded that *Myrtus communis* L. plant extract showed positive effects on the egg yolk, some performance parameters and immune system of older laying hens without any adverse effects on egg traits.

Keywords: *Common myrtle, Antibody response, Immunonutrition, Serum profile, Aged hens*

İçme Suyu Katkısı Olarak Kullanılan *Myrtus communis* L. Bitki Özütünün Yaşlı Yumurtacı Tavuklarda Performans, Bazı Kan Parametreleri, Yumurta Kalitesi ve Bağışıklık Tepkisi Üzerine Etkileri

Öz

Bu çalışmanın amacı, mersin bitki özütünün (MPE) yaşlı yumurtacı tavukların içme sularına ilavesinin performans, bazı kan parametreleri, yumurta kalitesi ve bağışıklık tepkisine olan etkilerini değerlendirmektir. Çalışmada 67 haftalık yaşta 192 yumurtacı tavuk kullanılmıştır (Başlangıç ağırlıkları 1.63±0.17 kg). Özüt, deneme gruplarının içme sularına 8 hafta boyunca %0; %2.5; %5 ve %10 düzeylerinde katılmıştır. Sıvı kromatografi analizleri, MPE içerisindeki en baskın aktif bileşenin mirisetin olduğunu göstermiştir (15.34 mg/L). Uygulama gruplarında; yem tüketimi, yumurta kütlesi (P<0.05) ve yumurta verimi (P<0.01) artmış (%10 MPE), su tüketimi de yükselmiştir (%5 MPE). Yemden yararlanma oranı değişmemiştir. Kontrol grubuna göre %2.5 MPE grubunda daha yüksek yumurta ağırlığı (P<0.001) ve daha koyu yumurta sarısı (P<0.01) gözlenmiştir. Newcastle virusuna karşı yapılan aşılamaya verilen immun yanıt açısından değerlendirildiğinde; %5 Myrtus grubunda daha yüksek düzeyde nötrofil sayısı ve IgG düzeyi belirlenmiştir. Serum Alanin Aminotransferaz düzeyi deneme gruplarında yükselirken (P<0.05); serum glikoz düzeyleri düşmüştür (P<0.01). Bunlar haricindeki diğer hiçbir kan parametresi değişmemiştir. Sonuç olarak, *Myrtus communis* L. bitki özütü yaşlı yumurtacı tavukların yumurta kalite özellikleri üzerine herhangi bir yan etki oluşturmadan yumurta sarı rengini, bazı performans parametrelerini ve bağışıklık sistemini olumlu yönde etkilemiştir.

Anahtar sözcükler: *Mersin, Antikor yanıt, Immun besleme, Serum profili, Yaşlı yumurtacı tavuklar*

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INTRODUCTION

Poultry sector plays an important role in the economy of any country and also poultry is one of the most economical and easily available sources in terms of protein. Currently, many studies are being conducted to keep poultry products safer for human health because humans of all ages consume poultry products such as eggs and meat. In the past, many synthetic antibiotics were used to maintain poultry health and production, however, nowadays herbal extracts are explored for their potential as a replacement for synthetic antibiotics without affecting the profitability of poultry farms [1].

Many dietary herbal products (powder, extracts, and essential oils) showed good effects on the performance parameters (eggs production, internal and external egg quality) of laying hens [2]. The chemical components of many plant extracts have antioxidant properties which help to eliminate free radicals in the body to cover stress-related problems and therefore improve the health status of hens [3].

Myrtle *(Myrtus communis* L.) is a medicinal plant and largely used for therapeutic purpose. Myrtle belongs to the family of *Myrtaceae*, which contains more than 5500 species [4]. Its leaves are traditionally used as a disinfectant, hypoglycemic and antiseptic agent [5]. Myrtle is among natural herbal products, which is mostly used in skin lotions for different skin disorders such as sunburn, acute wounds and to accelerate the healing process of the skin. Myrtus plant extract (MPE) is a pure natural, nontoxic, non-steroidal and commonly available herbal extract which is used for different medical treatments such as rashes, allergies, healing wounds, pimples [6]. Researchers observed that MPE has high phenolic content which activates the plants' defense mechanism. Moreover, MPE also contains antioxidants which further avoids oxidative damage in the body [7,8].

Myrtus plant extract contains many active components such as gallic acid, protocatechuic acid (PCA), catechin, myricetin, salicylic acid, rosmarinic acid, quercetin, genistein, and octanoate [9-11]. Even though research on the effects of the dietary MPE is limited in poultry, there are some studies about the aforementioned active ingredients in avian models. For instance, Samuel et al.[12] observed that supplementation of dietary gallic acid improved plasma antioxidant activity in broilers. In another study, it was observed that the survival rate of chickens in the group treated with PCA was 90% against infectious bursal disease virus, therefore, indicating that PCA could improve the immune organ index (spleen, bursa) in chickens [13]. Research shows that the dietary supplementation of broiler feed with genistein overcomes the deleterious effects of heat stress in persistent summer stress. Moreover, genistein also increases the feed conversion ratio (FCR), feed consumption, and body weight of broilers in extremely hot weather [14]. Similarly, positive results have been observed in the sensory score, breast meat quality and oxidative stress of broiler chickens when their diets are supplemented with hesperidin and genistein [14].

Previous evidence suggests that provision of any nutrients or additives as dietary or water supplement may have different effects on birds. Noy and Sklan [15] concluded that carbohydrates supplied with drinking water in the early stages of chicks' life were more effective than carbohydrates provided by diet for body weight gain. Similarly, Ritzi et al.[16] observed that probiotics as a water supplement had a better effect on broiler performance than the same probiotics provided by the diet. Gültepe et al.[17] observed some positive effects of lemon juice as a water supplement on egg production during the late phase production cycle of laying hens. Furthermore, Çetingül et al.[18] reported that supplementation of pomegranate molasses with drinking water to laying hens may affect some quality parameters in eggs after 30 days of storage.

This study was carried out to determine the effect of MPE on production parameters, egg quality, and blood physiology in laying hens. In our knowledge, the novelty of this study is that MPE was used first time in drinking water of older laying hens. The bioavailability of water is very high and also its metabolism is very fast. Therefore, we chose the water route for the supplementation of MPE to the laying hens.

MATERIAL and METHODS

This study was conducted to investigate the effects of Myrtus plant extract (Biyoderm®, ArsArthro Biotechnologies Inc, Ankara, Turkey) on egg production, egg quality and some blood parameters in older laying hens. All experimental procedures were performed at the Animal Research Center of Afyon Kocatepe University, Turkey after the approval of the ethics committee under approval No: 49533702/91; dated: 30/05/2017.

Production and Validation Processes of The Extract

Myrtus plant extract was done with a process containing collection, size reduction, drying, authentication of plant material, filtration, extraction, drying, and reconstitution [19]. Briefly, the dried leaves and barks of plants were used for the extraction. The extraction was water-based and no solvent was used except distilled water (1% MPE, 99% pure water as a carrier). LC-MS/QTOF (Agilent Technologies, Santa Clara, CA, USA) and Orbitrap (Thermo Electron, Bremen, Germany) were used. Moreover, Agilent Poroshell 120 EC-C18 3.0x100 mm, 2.7 µm colon were also used by 10mM Amonyum Format 0.1%, Formic Acid and water with MFB 0.1% Formic Acid MeOH (Gas Temperature 325°C, Gas Flow 10 L/min, Nebuliser 45 psig) for active ingredients determination (Agilent Technologies, Santa Clara, CA, USA).

All the ingredients were reported by negative and positive polarization with both instruments.

Poultry, Management and Experimental Design

A total of 192 Babcock white laying hens (67 weeks old; initial body weight 1.63±0.17 kg) were divided into 4 groups (n=48) with 8 subgroups containing 6 hens in each subgroup. MPE was added to the drinking water of the experimental groups with 0%, 2.5%, 5.0%, and 10% respectively during 8 weeks. For lighting, 16 h light and 8 h dark were applied and feed and water were supplied ad-libitum. All MPE treatment groups including the control group were fed a basal diet prepared to meet the needs of laying hens *(Table 1)* as reported in the NRC [20].

Automatic nipple drinking system was used and each group has separated water tank where the different concentration of *Myrtus communis* L. plant extract (MPE) was added in their water tank. Graduated cylinder glass was used for scaling of Myrtus plant extract. Then, MPE was mixed with water at the ratio of 0 mL/L, 2.5 mL/L, 5 mL/L and 10 mL/L for control, 2.5%, 5%, and 10% groups respectively in 20-liter water box between 1 p.m. to 2 p.m. every day. The product could be solved easily in the water and homogeneity was confirmed visually. During the study, water consumption (l) was measured by total water consumption per each group after 24 h interval.

Data Collection and Analyses

Hens were weighed at the beginning and end of the study to determine their live weights. Egg production and mortality were recorded daily while feed consumption was recorded weekly. Eggs were weighed once per week. Egg mass was calculated as follows:

Egg mass = Percent egg production × average egg weight in grams

Feed conversion ratio (FCR) values were calculated as follows:

FCR = feed consumption (g)/egg mass (g)

Eggs delivered to the laboratory at the end of the 4th week and also at the end of the 8th week to determine egg quality parameters. Eggs were kept for 24 h at room temperature before the egg trait analyses. Egg weight, egg yolk color index, breaking strength, eggshell thickness and Haugh unit were determined. Egg breaking strength was measured by using ORKA Egg Force Reader, EF 0468-2011 (Orka Feed Tech. Ltd., Hong Kong, China) and Haugh Unit were calculated by measuring albumen height (Digital Caliper, CD-15CP, Mitutoya Ltd., UK) according to the method devised by Haugh [21]. Egg yolk color was determined by using Roche Improved Yolk Color Fan and comparing the color of yolks with 15 bands of the color fan (YolkFan™, DSM Nutritional Products AG, Kaiseraugst, Switzerland). Albumen index and yolk index were calculated as follows [22]:

Table 1. *Composition and nutrient ingredients of the basal diet*

Ingredients	**%, As-fed Basis**
Corn grain	54.90
Vegetable oil	0.34
Sunflower meal (32% CP)[1]	16.93
Full fat soybean	10.00
Soybean meal (44% CP)[1]	7.39
Limestone	7.87
Dicalcium phosphate	1.73
Common salt	0.40
Vitamin-mineral premix[2]	0.25
L-lysine hydrochloride	0.10
DL-methionine	0.10
Chemical components[3]	
CP[1],%	17.00
ME[4],kcal/kg	2750
Calcium,%	3.71
Available P,%	0.38
Sodium,%	0.20
Methionine + Cystine, %	0.71
Lysine,%	0.83
Treonin,%	0.61
Triptophane,%	0.20
Linoleic acid,%	2.36
Active ingredients of MPE[5]	**mg/L**
Myricetin	15.34
Catechin	4.80
Quercetin	0.19
Gallic acid	0.13
Salicylic acid	0.06
Rosmarinic acid	0.01

[1] CP: Crude Protein; [2] Provided per kg of diet: Vitamin A: 12.000.000 IU, Vitamin D_3: 3.000.000 IU, Vitamin E: 35.000 IU, Vitamin K_3: 3.500 IU, Vitamin B_1: 2.750 IU, Vitamin B_2: 5.500 IU, Nicotinamide: 30.000 IU, Ca-D-Panthotenate: 10.000 IU, Vitamin B_6: 4.000 IU, Vitamin B_{12}: 15 IU, Folic acid: 1.000 IU, D-Biotin: 50 IU,Choline chloride: 150.000 IU, Manganese: 80.000 mg, Iron: 60.000 mg, Zinc: 60.000 mg, Copper: 5.000 mg, Iodine: 2.000 mg, Cobalt: 500 mg, Selenium: 150 mg, Antioxidant: 15.000 mg; [3] Calculated according to NRC [20]; [4] ME: Metabolisable energy; [5] LC-MS/QTOF analyse [19]

Albumen index = Albumen height (mm)/[Albumen length (mm) + Albumen width (mm)] × 100

Yolk index = Yolk height (mm)/Yolk diameter (mm) × 100

To determine the antibody level against Newcastle disease, all chickens were vaccinated with Newcastle vaccine at the beginning of the study. At the end of the trial, 3 animals were randomly selected from each subgroup and blood was collected directly from the heart. The samples were transferred into two separate tubes (vacutainer tubes without anticoagulant and with EDTA; BD Vacutainer®, Franklin Lakes, NJ, USA). Blood samples immediately

arrived in the laboratory under a cold chain. Samples with anticoagulant were used for the full blood count. The blood count was performed by a compact blood analyzer (BC 2800 Vet, Mindray Medical International Ltd., Shenzhen, China) for determining the numbers of total leukocyte (TLC), lymphocyte (LC), neutrophil (NC), monocyte (MC), and red blood cell (RBC); the levels of Hemoglobin (He), mean corpuscular hemoglobin (MCH), and platelet (PLT); the concentration of mean corpuscular hemoglobin concentration (MCHC); the volume of mean corpuscular (MCV) and mean platelet (MPV). The samples in vacutainer tubes without anticoagulant were centrifuged for 10 min (5 000 g). The supernatants were stored at -20°C till analyses in 2 mL microcentrifuge tubes. The levels of an automated analyzer (Elisys Uno, Human mbH, Wiesbaden, Germany) was used for serum biochemical assays (glucose, total cholesterol - CHO, high density- and low density-lipoprotein - HDL & LDL, aspartate and alanine aminotransferase - AST & ALT), gamma-glutamyl transpeptidase - GGT, phosphorus, calcium and Immunoglobulin G - IgG.

Statistical Analysis

Firstly, Shapiro-Wilk and Levene tests were performed for determining the normal distribution of data and variance homogeneity. One-way analysis of variance was used for comparison of mean between the group and Tukey-Kramer test was chosen for *post-hoc*. The following model was used for data to analyze:

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

where Y_{ij} = the response variable, μ = the general mean, α_i = the effect of water supplements and e_{ij} = the random error. The significance level was determined as $P<0.05$. All data were expressed as mean±SEM in tables. The environment of MedCalc Software (v.18, Oostend, Belgium) was used all data analysis.

RESULTS

All active ingredients of MPE were determined as follow: Gallic acid 0.13 mg/L, Catechin 4.8 mg/L, Myricetin 15.34 mg/L, Salicylic acid 0.06 mg/L, Rosmarinic acid 0.015 mg/L, Quercetin 0.197 mg/L).

The result of the recent study revealed that hen-day egg production was significantly ($P<0.01$) increased by 10% of the MPE group as compared to the control group. Similarly, feed consumption and egg mass were also significantly ($P<0.05$) increased in 10% MPE group. For egg weight, only 2.5% MPE group showed significant ($P<0.01$) results. None of the groups manifested any impact on FCR. Moreover, water consumption significantly increased ($P<0.01$) in 5% MPE groups as compared with the control group *(Table 2)*.

The group supplemented with 2.5% of MPE showed significant ($P<0.01$) results in terms of egg yolk and produced darker yellow egg yolks. However, the results for other egg quality parameters such as egg-breaking strength, shell thickness, albumin index, yolk index, and Haugh unit remained insignificant ($P>0.05$) during the whole study period *(Table 3)*.

For serological examinations, quadratic decreases ($P<0.01$) were observed for serum glucose level in all MPE treatment groups compared to the control group. However, serum ALT level significantly ($P<0.05$) increased in the group supplemented with 2.5% MPE group. Other serological parameters such as serum CHO, HDL, LDL, AST, GGT, calcium, and phosphorus levels did not show any significant ($P>0.05$) results. Regarding immunity, serum IgG level significantly ($P<0.05$) increased in the group supplemented with 5% of MPE compared to the other treatment and control groups *(Table 4)*.

For hematological parameters, NC increased significantly ($P<0.05$) in 5% and 10% MPE treatment groups compared to the control group. However other parameters such as TLC, LC, MC, RBC, He, MCV, MCH, MCHC, PLT, and MPV did not show any positive ($P>0.05$) response *(Table 5)*.

DISCUSSION

Many studies have manifested that dietary phytogenic extracts had some positive effect on the performance of laying birds [23]. Until now, most of the phytogenic research in avian models focused on dietary supplementation route. However, the comparative studies showed that dietary supplementation or water supplementation of similar products can cause a different response to performance in laying hens and broilers [15,16]. Although the research on water supplement of phytogenic extracts and herbal

Table 2. *Effects of graded levels of Myrtus plant extract on performance parameters of laying hens for 8 weeks (Mean±SEM; n=48)*

Group	Feed Consumption (g/hen/day)	Egg Production (%/day)	FCR	Egg Weight (g/hen/day)	Egg Mass (g/hen/day)	Water Consumption (L/hen/day)
Control	108.51±1.59^{a}	83.36±0.74^{a}	2.03±0.05	64.14±0.27^{b}	53.48±0.97^{a}	8.88±0.14^{a}
2.5 %	113.65±1.76^{ab}	83.98±0.75^{a}	2.10±0.04	65.27±0.29^{a}	54.22±0.55^{ab}	8.65±0.11^{a}
5 %	113.77±2.02^{ab}	84.92±0.72^{a}	2.08±0.05	64.76±0.29^{ab}	54.74±0.79^{ab}	9.92±0.14^{b}
10 %	115.30±1.57^{b}	90.32±0.66^{b}	2.02±0.06	63.40±0.26^{b}	57.08±0.99^{b}	9.22±0.15^{ab}
P value	0.024	0.001	0.705	0.001	0.038	0.001

Values with different superscripts in the same column differ significantly ($P<0.05$); FCR Feed conversion ratio; FCR = feed consumption (g)/egg mass (g)

Table 3. *Effects of graded levels of Myrtus plant extract on egg quality parameters of laying hens for 8 weeks (Mean±SEM; n=48)*

Group	Egg Breaking Strength (kg/cm)	Egg Shell Thickness (mm)	Haugh Unit	Egg Yolk Colour	Albumen Index (%)	Yolk Index (%)
Control	32.04±1.57	0.364±0.002	81.07±0.52	11.40±0.07^{a}	5.78±0.14	42.41±0.42
2.5%	31.52±1.86	0.365±0.002	82.32±0.57	11.72±0.06^{b}	6.02±0.14	42.29±0.44
5%	29.45±2.15	0.361±0.002	82.79±1.68	11.54±0.06^{ab}	6.62±0.95	43.18±0.39
10%	30.48±2.05	0.368±0.002	80.74±0.58	11.42±0.06^{a}	5.63±0.13	43.01±0.79
P value	0.656	0.242	0.369	0.001	0.548	0.559

Values with different superscripts in the same column differ significantly (P<0.05)

Table 4. *Effects of graded levels of Myrtus plant extract on serum biochemical parameters of laying hens for 8 weeks (Mean±SEM; n=48)*

Group	Glucose (mg/dL)	CHO (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	AST (U/L)	ALT (U/L)	GGT (U/L)	P (mg/dL)	Ca (mg/dL)	IgG (mg/dL)
Control	215.06±2.85^{a}	136.00±8.42	26.5±1.33	36.50±3.14	176.63±10.10	3.50±0.74^{b}	28.69±1.07	5.66±0.30	23.91±0.96	14.21±1.23^{b}
2.5%	193.19±5.08^{b}	156.13±11.84	23.88±0.44	45.81±4.41	161.00±6.71	8.19±0.89^{a}	26.81±0.75	6.43±0.34	25.69±0.91	12.32±1.73^{b}
5%	196.38±2.48^{b}	123.19±7.61	25.81±0.84	33.44±2.89	175.44±7.11	6.50±0.88^{ab}	28.44±1.35	5.42±0.27	23.73±0.89	17.75±0.66^{a}
10%	201.06±2.58^{b}	130.19±9.47	24.69±0.57	35.44±3.41	158.31±9.35	5.44±0.97^{ab}	28.31±1.03	5.44±0.38	23.73±0.62	14.53±1.36^{b}
P value	0.001	0.141	0.169	0.186	0.215	0.002	0.568	0.125	0.402	0.048

Values with different superscripts in the same column differ significantly (P<0.05); CHO: Total cholesterol; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transpeptidase; P: Phosphorus; Ca: Calcium

Table 5. *Effects of graded levels of Myrtus plant extract on hematological parameters of laying hens for 8 weeks (Mean±SEM; n=48)*

Group	TLC (10^9/L)	LC (10^9/L)	NC (10^9/L)	MC (10^9/L)	RBC (10^{12}/L)	He (g/L)	MCV (fL)	MCH (pg)	MCHC (g/L)	PLT (10^9/L)	MPV (pg)
Control	2.86±0.14	1.39±0.07	0.68±0.07^{a}	0.220±0.019	2.42±0.08	11.00±0.23	108.94±0.52	29.39±0.50	30.17±0.39	27.11±0.29	6.49±0.08
2.5%	2.99±0.14	1.59±0.09	0.88±0.08^{ab}	0.266±0.021	2.39±0.07	11.59±0.22	109.38±0.35	28.29±0.43	31.33±0.38	27.23±0.26	6.47±0.07
5%	2.98±0.17	1.53±0.10	1.01±0.10^{b}	0.263±0.020	2.46±0.07	11.76±0.18	109.05±0.48	29.14±0.52	31.23±0.35	26.83±0.25	6.48±0.08
10%	3.11±0.15	1.51±0.09	0.93±0.07^{b}	0.246±0.020	2.57±0.08	11.48±0.24	110.06±0.31	29.78±0.36	31.03±0.45	26.80±0.30	6.71±0.18
P	0.742	0.521	0.020	0.319	0.385	0.102	0.242	0.132	0.152	0.619	0.398

Values with different superscripts in the same column differ significantly (P<0.05); TLC: Total leukocyte count; LC: Lymphocyte count; NC: Neutrophil count; MC: Monocyte count; RBC: Red blood cell count; He: Hemoglobin; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; PLT: Platelet; MPV: Mean platelet volume

products are limited, the findings of the aforementioned studies are various. Karadağoğlu et al.[24] reported no beneficial effect of water supplementation of an herbal blend (peppermint, thyme, and anise oil) on growth performance, meat quality and intestinal development of quails. However, Gültepe et al.[17] reported a positive effect of lemon juice on the performance of older laying hens. Additionally, Çetingül et al.[18] reported that providing pomegranate molasses in drinking water to laying hens had no major effect on egg quality after 30 days storage at 4°C, although it had some minor effects in earlier storage days. In our knowledge, this is the first report on the effects of water supplementation of MPE on the performance of laying hens. Bülbül et al.[25] observed that dietary myrtle oil increased egg production in laying quails after 8 weeks in agreement with the present study. Although Çabuk et al.[26] reported a greater number of eggs in laying hens fed with an essential oil blend (myrtle leaf, oregano, laurel leaf, sage leaf, fennel seed, and citrus peel oil) than the layers in control group after 140 days, they observed no significant effect of oil blend on egg production percentage of the layers. The incompatible results of the aforementioned study from the results of the present study and Bülbül et al.[25] can be due to the potential interaction between myrtle leaf oil and the others in the blend. Also, the presence of heat stress in the experiment of Çabuk et al.[26] may have limited the potential effects of myrtle oil.

A lot of research has been conducted on the effects of dietary supplementation of herbal products on egg traits of avian models. Christaki et al.[23] observed no major effect on egg traits of the quails fed with oregano, anise and olive leaves. Additionally, Freitas et al.[27] observed no positive effect of *Syzygium cumini* which is a herb belongs the same family with myrtle *(Myrtaceae)* on egg weight and egg mass of laying hens. Yet in the present study, we found that the supplementation of laying hens with 2.5% of MPE improved egg weight. The differences in observed findings from previous reports may be caused by the purity of MPE used in the present study.

Abdel-Wareth and Lohakare [28] observed that dietary herbs and plant extracts improved some performance parameters, such as feed consumption, egg mass, and FCR, in laying hens during the late phase of the production cycle. However, other studies reported that the addition of herbal extracts had no impact on the FCR of quails [29]. In the present study, we found that feed consumption and egg mass increased significantly ($P<0.05$) in the group supplemented with 10% of MPE while no effects were seen on FCR among any of the treatment groups. Bülbül et al.[25] showed that supplementations of 5% dietary Myrtus oil did not alter the feed consumption of laying quails. Similarly, in the present study, there was also no significant effect observed on the feed consumption in control, 2.5%, and 5% groups. Recently, Liu et al.[30] observed that the taste sense of birds may be more sophisticated and developed than previously thought. Unlike the dietary supplementation, the highest dose (10% MPE) of myrtle oil in the present study may change the water flavor due to high concentrated essential oil content and fresh supplementation method. Increase in the feed and water consumption of the layers in the highest dose MPE groups may be a result of mentioned taste improvement.

Researchers observed that the increase in egg production had a direct correlation with water consumption. Increase in egg production will increase water consumption [31]. Our result was consistent with the finding of Medway and Kare [31], we also observed a significant increase in both egg production and water consumption.

Previous evidence of the research suggests that the effects of essential oils on egg quality parameters are inconsistent. Bölükbaşı et al.[32] reported that dietary thyme oil, sage oil, and rosemary oil had no effect on albumen proportion of egg while it affected Haugh unit, yolk and eggshell proportion of egg in laying hens. Çabuk et al.[26] observed that no effect of dietary essential oil blend (myrtle leaf, oregano, laurel leaf, sage leaf, fennel seed, and citrus peel oil) on egg weight of laying hens suffering from heat stress. Olgun [33] reported a quadratic response to supplementation of a dietary essential oil blend on eggshell thickness while the supplement had no effect on egg breaking strength and the specific gravity of eggs in laying hens. Also, Ding et al.[34] recently reported no effects of an essential oil blend on egg quality parameters in laying hens. In the present study, egg yolk color significantly ($P<0.01$) increase in 2.5% MPE group while other egg quality parameters such as egg-breaking strength, shell thickness, Haugh unit, albumen index, and yolk index remained unchanged ($P>0.05$). The darker egg yolks may be caused by antioxidant properties of myricetin [35], which is the predominant active ingredient in MPE according to the ingredient analyze in the present study.

In the present study, some serological parameters such as serum glucose, ALT, and IgG showed significant ($P<0.01$) results such as the decrease in glucose level in a quadratic manner compared to the control group. In contrast, serum ALT concentration significantly ($P<0.01$) increased in treatment groups. However other serological parameters such as CHO, HDL, LDL, CHO, AST, GGT, serum phosphorus, and calcium did not show any significant results ($P>0.05$). In the present study, LC-MS analyses showed that myricetin is the most abundant active ingredient in MPE. Myricetin is a hexahydroxyflavone and significant quantities of myricetin are absorbed in the gut of monogastric animals [35]. Ong and Khoo [36] investigated the effects of more than 30 bioflavonoids on lipogenesis and only myricetin had a stimulatory effect. They found that myricetin enhanced the glucose transport in rat adipocytes without any effect of insulin receptor function and Glucose Transporter Type 4 (GLUT-4) translocation. In a consecutive study, Ong and Khoo [37] concluded that treatment of diabetic rats with myricetin resulted in the lowering of hyperglycemia. In the present study, the significantly low serum glucose level in the treatment groups might be explained by the hypoglycemic effect of myricetin.

For immunity, it was observed that active components of onion juice had positive effects on lymphoid organs [38] and produced a large number of antibodies in chickens [39]. The effects of garlic and onion juice on immunoglobulin have been found to be similar to that of antibiotics [40]. In the present study, we observed that the serum IgG level increased in the group supplemented with 5% of MPE compared to the other treatment and control groups. Lee et al.[41] founded that myricetin inhibited both Cyclo-oxygenase-2 (COX-2) and nuclear factor kappa B (NF-kappaB) trans-activation in phorbol ester-treated JB6 P+ cells. The COX-2 is an inducible isoform member of cyclooxygenase enzyme family, which is regulated by growth factors and different cytokines such as IL1β, IL6, or TNFα, therefore overexpressed during inflammation [42]. The NF-kappaB is a family of inducible transcription factors that play a key role in the immune system. Transcription of genes which are responsibly regulating the inflammation, proliferation, and differentiation of immune cells are induced by primary activation of the NF-κB pathway [43]. Hence, myricetin could have anti-inflammatory as well as antioxidant effects through COX-2 inhibition pathway like nonsteroidal anti-inflammatory drugs. In the present study, the significant effect of myrtus on serum IgG levels might be explained by these complex mechanisms. Further studies are needed to evaluate both antioxidant and anti-inflammatory potential of MPE.

Unlike serum biochemical parameters, the research findings of the effects of MPE on hematology are limited in laying hens. Çetin et al.[44] reported no effect of propolis (bee glue), which is an antioxidant flavonoid, on most of the hematological parameters such as total leucocyte, hemoglobin and hematocrit although they observed improvement on serum IgG and IgM levels in laying hens. In the present study, NC increased quadratically however

other parameters such as TLC, LC, MC, RBC, He, MCV, MCH, MCHC, PLT, and MPV remained unaffected in agreement with Çetin et al.[44].

In conclusion, MPE could be used as a potential drinking water supplement and has shown positive effects on performance, egg production, egg quality and immunity without any adverse effects on the egg traits in laying hens. It is recommended to conduct more research during prolonged storage at higher temperatures to explore further effects of MPE on egg quality parameters.

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Conflict of Interest

This study was partially-funded by ArsArthro Biotechnologies Inc. (Ankara, Turkey). The funder provided financial support for expenses of serum biochemical analyses and hematology analyses, but did not have any additional role in the study design, data collection and analysis, perform the laboratory analysis, decision to publish, or preparation of the manuscript.

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