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The Effects of Dietary Supplementation with *Origanum onites* Essential Oil on Growth Performance, Some Blood Parameters, Jejunal Villus Height, and Meat Quality in Broiler Chickens

Etlik Piliçlerde *Origanum onites* Uçucu Yağlı Diyet Takviyesinin Büyüme Performansı, Bazı Kan Parametreleri, Jejunal Villus Yüksekliği ve Et Kalitesi Üzerine Etkileri

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ABSTRACT

The present study investigated the effects of dietary supplementation with *Origanum onites* essential oil on growth performance, some blood parameters, jejunal villus height, and meat quality in broiler chickens. Two hundred chicks were used and allocated to 4 groups, including a control group and 3 treatment groups, which received 3 different levels of dietary *Origanum onites* essential oil (100 ppm, 200 ppm, and 400 ppm). Each study group consisted of 5 subgroups, each including 10 animals. Feed and water were provided *ad libitum*. Light was provided 24 h/day. The ambient temperature was maintained at an optimum level and adjusted on a weekly basis. Dietary *Origanum onites* essential oil was given on a daily basis. The incorporation of different levels of *Origanum onites* essential oil into the diet did not affect body weight, body weight gain, feed intake, feed conversion rate, and carcass yield ($P > .05$). Similarly, dietary supplementation with *Origanum onites* essential oilhad no significant effect on the antioxidant and serum biochemical parameters investigated ($P > .05$). On the other hand, dietary *Origanum onites* essential oilsignificantly affected the spleen weight, jejunal villus height, and meat color (a*) ($P < .05$). No effect of dietary essential oilwas observed on the meat pH value ($P > .05$). Several studies are required to determine the more effective level of *Origanum onites* in broilers.

Keywords: Broiler, meat quality, *Origanum onites*, performance, serum parameters, villus

ÖZ

Bu çalışmada karma yemlere Origanum onites esasnsiyel yağı (OEY) ilavesinin etlik piliçlerde performans, bazı kan parametreleri, jejenum villus uzunlukları ve et kalitesi üzerine etkileri araştırılmıştır. Deneme, kontrol grubu ve 3 farklı seviyede (100, 200 ve 400 ppm) OEY ilavesi yapılan gruplar olmak üzere toplam 4 gruptan oluşturulmuştur. Denemede, her grup kendi içinde ve her birinde 10 hayvan olacak şekilde 5 alt gruba ayrılmıştır. Denemede toplam 200 adet hayvan kullanılmıştır. Su ve yem ad libitum olarak verilmiştir. Aydınlatma 24 sa/gün olarak ayarlanmıştır. Ortam sıcaklığı haftalık olarak optimum değerlerde tutulmuştur. OEY katkısı günlük olarak uygulanmıştır. Etlik piliç rasyonlarına farklı düzeylerde OEY katkısı; canlı ağırlık, canlı ağırlık artışı, yem tüketimi, yemden yararlanma oranı, karkas randımanı değerlerini etkilememiştir (P>0.05). Aynı şekilde serum biyokimyasal ve antioksidan değerler üzerine de OEY katkısının önemli bir etkisi olmamıştır (P>0.05). OEY ilavesi dalak ağırlıkları, jejenum villus uzunlukları ve et rengi (a*) parametrelerini önemli ölçüde etkilemiştir (P<0.05). Et pH değerleri üzerine OEY'nın herhangi bir etkisi olmamıştır (P>0.05). Etlik piliçlerin besi performansını arttırmak için rasyonlara yem katkı maddesi olarak Origanum onites' in kullanılabileceği, ancak kullanım düzeyini belirlemek için konu ile ilgili daha fazla araştırma yapılması gerektiği sonucuna varılmıştır.

Anahtar Kelimeler: Broyler, et kalitesi, Origanum onites, performans, serum parametreleri, villus

INTRODUCTION

Since the ban placed by the European Union (EU) in 2006 on the use of antibiotic growth factors (AGFs) confirmed to pose a risk to human and animal health on the basis of scientific data, researchers continuously seek new feed additives that can replace AGFs.[1] Several recent studies have focused on the use of essential oils (EOs) as substitutes of AGFs in animal nutrition and have investigated the effects of these EOs. It has been demonstrated that EOs have several positive effects on the health and performance of animals, including increased levels of endogenous enzymes.[2] Some feed additives can serve as alternatives of antibiotic substitutes in poultry nutrition, including enzymes, prebiotics, probiotics, manno-oligosaccharides, symbiotics, and phytobiotics.[3] Phytogenic products, including aromatic plants and essential oils, have been reported to show biological activity, when used in animal nutrition, and are considered to be natural products that offer potential use as antibiotic substitutes.[4] EOs are described as natural and non-residual alternative feed additives, which are derived from aromatic plants in various ways, improve the flavor and palatability of feed, and show digestive stimulant and performance-enhancing effects. Depending on the aromatic plants from which they are derived, EOs contain different types and levels of phenolic compounds and, thereby, show a wide range of activities (antimicrobial, antioxidant, anti-inflammatory, antifungal, etc.).[4,5]

Origanum species, classified under the family Labiatae, are among the several alternative feed additives used as performance enhancers for poultry. The main constituents of Origanum are carvacrol and thymol, but the presence of thymoquinone, *p*-cymene, and γ-terpinene have also been reported in the composition of Origanum from different geographical regions.[6] The more widely known Origanum species in Türkiye are *Origanum minutiflorum, Origanum vulgare*, *Origanum syriacum*, *Origanum majorana L.*, and *Origanum onites L.* Among these species, *Origanum onites L.* referred to as "Turkish thyme" or "Izmir thyme," is a well-known herb with common use in medicine and several other areas. This herb is also used for digestive disorders and upper respiratory infections.[7]

Feizi et al (2013) reported that the incorporation of *Origanum vulgaris* essential oil (200/1000cc) into the feed and drinking water of broiler chickens increased body weight (BW), decreased feed intake (FI), and improved the feed conversion rate (FCR).[8] In another study, oregano EOs were determined to show an inhibitory effect against several bacteria, including *Campylobacter jejuni*, *Salmonella enteritidis*, *Escherichia coli*, *Staphylococcus aureus*, and *Listeria monocytogenes*. It has been reported that the purified constituents of *Origanum onites* essential oil (OEO) inhibit HMG-CoA reductase, an important enzyme that regulates cholesterol synthesis, and thereby show a hypocholesterolemic effect.[9]

This study was carried out in order to report the effects of dietary supplementation with essential oil obtained from Izmir thyme (*Origanum onites*) on growth performance, carcass traits, organ characteristics, serum biochemical and antioxidant status, meat quality, and jejunal villi height.

MATERIALS AND METHODS

Animals and Experimental Design

The study was carried out at the premises of the Poultry Unit of the Livestock Research and Application Centre of Atatürk University, Faculty of Veterinary Medicine, using floor cages (121 × 110 × 108 cm). Two-hundred-day-old male Ross-308 chicks were used in the study. After 1 week, the animals were randomly divided to 4 groups, each of 50 chicks, and each group was further divided into 5 replicates, each of 10 animals. The chicks were randomly distributed into 20 compartments, such that the average BWs of the animals in each compartment were equal. The chicks were raised for a period of 42 days, including a 7-day acclimatization period and a 35-day trial period. Throughout the study, water and feed were provided *ad libitum*. The nutrient composition and Near Infrared Spectroscopy (NIRS) analysis results of the feed rations provided to the animals during the different phases of the study are presented in Table 1. Four study groups, including a control group and 3 treatment groups, were formulated. While the control group did not receive any feed supplement, the treatment groups were dietary supplemented with 100 ppm, 200 ppm, and 400 ppm of OEO, respectively. Each day at 17:00 h, the remainder of the feed provided to the animals on the previous day was collected and weighed. Subsequently, the feed was replenished and OEO was incorporated into the feed offered to the treatment groups.

Origanum onites essential oil was stored at +4°C in dark-colored bottles and was incorporated into feed manually, on a daily basis, starting from the lowest supplementation dose. The chemical composition of the essential oil was determined by the supplier (Mahan Cosmetics, Hatay/Türkiye) with an automatic gas chromatograph system equipped with a mass spectrometer and a flame ionization detector (GC-MS/FID) (Table 2). The pen, in which the animals were housed, was illuminated with tungsten bulbs, and light was provided 24 h/day. The temperature of the pen was maintained at 33°C for the first 2 days and was progressively decreased to 24°C by the end of the study. Wood shavings were used as the cage bedding material.

Determination of Performance Parameters

Chicks from each experimental group were weighed on a weekly basis, on the same days and at the same hour, and their BWs were recorded. The difference between 2 consecutive weightings was

Table 1. Nutritional Composition of Feeds and NIRS Analysis Results

Raw Materials	Starter (%) 1–-14 Days	Grower (%) 15-28 Days	Finisher (%) 29-42 Days
Maize	55.48	70.25	62.62
Soybean meal (44%)	22.55	5.05	10.60
Corn gluten (60%)	16.20	20.12	20.55
Limestone	2.35	1.15	2.45
DCP	1.90	1.80	2.10
Salt	0.24	0.23	0.25
Vitamin (K3-A)	0.14	0.15	0.14
Soda	0.10	0.09	0.09
Vitamin E	0.64	0.64	0.65
Lysine	0.35	0.52	0.50
Methionine	0.05	–	0.05
Analysis			
ME (kcal/kg)	3020	3145	3215
Dry matter (%)	89.00	88.00	88.00
Crude protein (%)	24	21.15	20.3
Crude fat (%)	3.45	6.40	3.15
Ash (%)	3.25	2,22	5.12
Methionine (%)	0.78	0.65	0.48
Lysine (%)	1.24	1.13	1.07

DCP, dicalcium phosphate; ME, metabolizable energy.

Table 2. Chemical Composition of *Origanum onites*

No	Component	Quantity (%)	No	Component	Quantity (%)
1	α-pinene	0.56	9	Terpinene-4-ol	0.66
2	α-tujene	0.47	10	Trans-caryophyllene	2.56
3	Myrcene	1.38	11	Borneol	1.08
4	α-terpinene	1.45	12	β-bisabolene	0.53
5	γ-terpinene	7.19	13	Caryophyllene oxide	0.43
6	Cymene	6.12	14	Thymol	5.54
7	1-octen-3-ol	0.43	15	Carvacrol	70.13
8	Linalol	1.47			

recorded as the body weight gain (BWG). Feed was provided to the animals at 17:00 hours each day, in an amount 20% greater than that they could consume. The daily FI of the animals was determined by weighing the leftover feed on the next day and subtracting this value from the amount of feed initially provided. The average daily FI per animal was calculated by dividing the daily FI by the number of animals included in the group. The FCR was calculated by dividing the average FI of the animals in-between 2 consecutive weightings by the average BWG of the animals in the same group in-between the same 2 consecutive weightings.

Determination of Slaughter and Carcass Characteristics

On the last day of the study (day 42), the slaughter weights of the animals were recorded. In total 40 animals, 10 per group (2 animals per subgroup), were randomly selected for slaughter. At slaughter, the chickens were decapitated, plucked, and eviscerated, and their legs were also cut. The carcasses were first weighed to record the hot carcass weight and were weighed for a second time after being kept at +4°C for 24 hours to record the cold carcass weight. The carcass yield was calculated by dividing the carcass weight by the preslaughter weight and multiplying the quotient by 100. The extracted visceral organs were also weighed and their weights were recorded. The weight percentages of the visceral organs were determined by dividing their weights by the BW of the animal and multiplying the quotient by 100.

Determination of Serum Biochemical Parameters

Blood samples were collected from the slaughtered chickens into individually numbered tubes and were centrifuged at 3000 rpm for 10 minutes. The extracted sera were transferred into Eppendorf tubes and stored at −20°C until being analyzed. The biochemical parameters of the samples were determined spectrophotometrically using commercial test kits (Roche) and a Cobas-8000 autoanalyzer.

Determination of Serum Antioxidant Parameters

Serum malondialdehyde (MDA) levels were determined as described by Placer et al (1966) and serum glutathione (GSH) levels were determined as described by Sedlak and Lindsay (1968).[10,11]

Determination of Jejunal Villi Height

At the necropsy of the slaughtered chickens, tissue samples were collected from the jejunum and were subjected to histopathological examination, during which the height of the jejunal villi was determined.[12,13]

Determination of Breast Meat pH and Color Parameters

The pH value of the breast meat samples was determined by placing 10 g of breast meat in 100 mL distilled water and homogenizing the mixture on a homogenizer. The pH value of the homogenates was measured with a pH-meter (SCHOTT L 6880, Lab Star). The color intensities (L*, a*, and b*) of the breast meat samples were analyzed with a colorimeter device (Minolta CR-400).[14]

Statistical Analysis

The data obtained in the present study were analyzed with 1-way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) 18.0 software (SPSS Inc.; Chicago, IL, USA). Duncan's multiple comparison test was used for the comparison of the differences detected between the study groups. Statistical significance was set at $P < .05$.[15]

RESULTS

No statistically significant difference was detected for the performance parameters, daily FI, and FCR ($P > .05$). Dietary OEO supplementation caused only numerical alterations in the performance parameters (Table 3). The liveability percentages of the control group and the treatment groups, which received 100 ppm, 200 ppm, and 400 ppm of dietary OEO, were 88%, 90%, 88%, and 96%, respectively.

As shown in Table 4, no difference was determined for the slaughter and carcass characteristics investigated ($P > .05$). Except for the spleen percentage, no statistically significant difference was found for the visceral organ percentages.

No significant differences were detected for the serum total protein, cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), glucose, and triglyceride levels among the experimental groups ($P > .05$) (Table 5).

The serum MDA and GSH levels, which are oxidative stress parameters directly related to the life span of cells, and serum antioxidant parameters are shown in Table 6. No statistically significant differences were shown among the study groups for the serum MDA and GSH levels, thus, dietary OEO supplementation was determined to have not affected these parameters ($P > .05$).

Histological analysis demonstrated significant differences for the height of the jejunal villi ($P < .05$) (Table 7). The height of the jejunal villi had increased in the groups that received 200 ppm and

Table 3. Performance Parameters of Broilers as Affected by the Different Levels of *Origanum onites* Essential Oil Ddietary Supplementation

	AFC (g/day)	ADLWG (g/day)	FCR (g/g)	7-42 TLWG (g)	TFI (g)
Groups	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Control	120.79 ± 0.97	61.08 ± 0.86	1.98 ± 0.31	2137.78 ± 30.01	4227.58 ± 33.81
OEO 100	118.97 ± 1.32	61.92 ± 0.98	1.92 ± 0.30	2167.07 ± 34.52	4163.85 ± 46.20
OEO 200	121.45 ± 0.79	62.79 ± 1.38	1.93 ± 0.33	2197.72 ± 48.52	4250.66 ± 27.54
OEO 400	119.22 ± 0.60	64.01 ± 0.33	1.86 ± 0.20	2240.35 ± 11.36	4172.63 ± 21.11
P	0.114	0.070	0.094	0.070	0.114

The difference between the means is significant at the $P < .05$ level. OEO 100, 100 ppm *Origanum onites;* OEO 200, 200 ppm *Origanum onites*; OEO 400, 400 ppm *Origanum onites*. ADLWG, average daily live weight gain; AFC, average feed consumption; FCR, feed conversion rate; TFI, total feed intake; TLWG, total live weight gain.

Table 4 Slaughter and Carcass Parameters of Broilers as Affected by the Different Levels of *Origanum onites* Essential Oil Dietary Supplementation

	Slaughter Weight (g)	Hot Carcass Ratio (%)	Cold Carcass Ratio (%)	Heart (%)	Gizzard (%)	Liver (%)	Spleen (%)
Groups	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Control	2283.00 ± 33.63	72.50 ± 0.52	71.26 ± 0.55	0.53 ± 0.20	0.97 ± 0.06	2.03 ± 0.04	0.13 ± 0.01[b]
OEO 100	2314.00 ± 36.83	71.77 ± 0.21	70.70 ± 0.28	0.53 ± 0.20	1.00 ± 0.06	1.97 ± 0.06	0.15 ± 0.01[a]
OEO 200	2067.00 ± 39.28	72.47 ± 0.32	71.21 ± 0.31	0.53 ± 0.20	1.07 ± 0.08	1.98 ± 0.10	0.16 ± 0.01[a]
OEO 400	2102.00 ± 24.42	71.96 ± 0.29	70.77 ± 0.24	0.56 ± 0.10	0.95 ± 0.02	1.92 ± 0.05	0.12 ± 0.01[b]
P	.544	.197	.330	.188	.182	.284	.050

The difference between the means is significant at the $P < .05$ level. OEO 100, 100 ppm *Origanum onites;* OEO 200, 200 ppm *Origanum onites*; OEO 400, 400 ppm *Origanum onites*.
a,b: Superscripts in a row showed significant differences.

Table 5. Serum Biochemical Parameters (mg/dL) of Broilers as Affected by the Different Levels of *Origanum onites* Essential Oil Dietary Supplementation

	Total Protein	Cholesterol	HDL	Glucose	LDL	TG
Groups	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Control	2.58 ± 0.16	99.10 ± 5.85	67.58 ± 3.68	194.20 ± 11.70	20.00 ± 3.07	57.60 ± 3.94
OEO 100	2.51 ± 0.81	86.60 ± 4.61	62.00 ± 2.90	190.40 ± 8.73	13.42 ± 1.52	55.90 ± 5.82
OEO 200	2.39 ± 0.09	88.50 ± 5.42	62.80 ± 3.56	210.80 ± 13.86	14.07 ± 2.26	58.20 ± 4.79
OEO 400	2.49 ± 0.16	100.30 ± 4.91	69.72 ± 0.11	202.80 ± 13.22	17.72 ± 1.50	64.30 ± 5.37
P	.071	.097	.144	.283	.057	.290

OEO 100, 100 ppm *Origanum onites*; OEO 200, 200 ppm *Origanum onites;* OEO 400, 400 ppm *Origanum onites*. HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides.

400 ppm of dietary OEO, compared to the control group and the group supplemented with 100 ppm of dietary OEO. Jejunal villi height was greatest in the group supplemented with 400 ppm of dietary OEO (994.92 µm).

The results of the color and pH analyses of the breast meat samples are presented in Table 8. As indicated in Table 8, dietary supplementation with OEO did not cause any difference for the L*, b*, and pH values, excluding a* ($P > .05$). The assessment of the color parameters revealed that the a* value had increased in the group that received 400 ppm of dietary OEO, in comparison to the other study groups ($P < .05$).

DISCUSSION

In the present study, the performance values of the animals, based on weekly weightings, did not differ between the study groups. The performance results found in this study are in agreement with those reported in previous research on the effects of various essential oils and organic acids on the performance of broiler chickens (i.e., BW and BWG).[16,17]

In their study on the effects of dietary supplementation with thymol (0.2 g/kg, 0.4 g/kg, 0.8 g/kg) and thymol essential oil (2 mL/kg and 4 mL/kg) on the performance of broiler chickens, Hoffman–Pennesi and Wu (2010) found no effect of dietary supplementation with thymol essential oil on BW.[18] On the other hand, Modeva and Profirov (2003) reported that the incorporation of a commercial plant extract, containing 5% of oregano essential oil, at rates of 0.025% and 0.050% into feed resulted in increased BWG.[19] Similarly, Windisch et al (2008) suggested that phytogenic components could improve nutrient absorption and BWG by increasing the activity of digestive enzymes.[20]

In their investigation on the effects of thyme oil and garlic oil, when administered alone and together, on the performance of broiler chickens, Kırkıpınar et al (2011) determined that, in comparison to the control group, dietary oregano essential oil altered neither FI nor the FCR.[21] In another study on the effects of Origanum essential oil on the performance and immunity of broiler chickens, Mansoub (2011) reported that Origanum essential oil increased FI and improved the FCR, in comparison to the control group.[22]

The results achieved with essential oils are attributed to their appetizing effect, and it is considered that even if they do not increase FI, they may show a positive effect by increasing BWG.[23] It is indicated that Origanum plants increase the FCR by regulating the intestinal microflora and increasing the activation of endogenous digestive enzymes.

Comparison of the carcass characteristics of the animals slaughtered on the 42nd day of the study demonstrated that there was no difference among the 4 experimental groups. The results obtained in the present study for the carcass parameters investigated are similar to those obtained in previous research on the effect of essential oils on performance values.[24,25] In their study on the incorporation of an essential oil blend (thyme, clove, aniseed),

Table 6. Serum Antioxidant Parameters (nmol/mL) of Broilers as Affected by the Different Levels of *Origanum onites* Essential Oil Dietary Supplementation

	MDA	GSH
Groups	Mean ± SEM	Mean ± SEM
Control	2.13 ± 0.19	0.14 ± 0.003
OEO 100	2.39 ± 0.31	0.16 ± 0.003
OEO 200	2.73 ± 0.31	0.16 ± 0.009
OEO 400	2.59 ± 0.24	0.15 ± 0.003
P	.155	.055

OEO 100, 100 ppm *Origanum onites*; OEO 200, 200 ppm *Origanum onites;* OEO 400, 400 ppm *Origanum onites*. GSH, glutathione; MDA, malondialdehyde.

Table 7. Jejunal Villus Heights (µm) of Broilers as Affected by the Different Levels of *Origanum onites* Essential Oil Dietary Supplementation

Groups	Mean ± SEM
Control	858.97 ± 5.27[c]
OEO 100	836.86 ± 14.38[c]
OEO 200	954.73 ± 8.66[b]
OEO 400	994.92 ± 8.78[a]
P	.000

The difference between the means is significant at the $P < .05$ level. OEO 100, 100 ppm *Origanum onites*; OEO 200, 200 ppm *Origanum onites*; OEO 400, 400 ppm *Origanum onites*. SEM, standard error of the mean.
a,b: Superscripts in a row showed significant differences.

Table 8. Breast Meat pH and Color Intensities of Broilers as Affected by the Different Levels of *Origanum onites* Essential Oil Dietary Supplementation

	pH	L*	a*	b*
Groups	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Control	5.89 ± 0.26	52.15 ± 0.36	4.16 ± 0.31[b]	15.66 ± 0.35
OEO 100	5.91 ± 0.20	52.60 ± 0.41	3.88 ± 0.20[b]	15.28 ± 0.34
OEO 200	5.92 ± 0.28	52.48 ± 0.45	4.12 ± 0.19[b]	14.73 ± 0.43
OEO 400	5.88 ± 0.24	52.69 ± 0.44	5.01 ± 0.30[a]	15.69 ± 0.41
P	.283	.408	.048	.111

The difference between the means is significant at the $P < .05$ level. OEO 100, 100 ppm *Origanum onites;* OEO 200 200 ppm *Origanum onites;* OEO 400, 400 ppm *Origanum onites*.
L*, brightness, a*, redness, b*, yellowness. a,b: Superscripts in a row showed significant differences.

at levels of 100 ppm, 200 ppm, and 400 ppm, into the mixed feed of broiler chickens, Şimşek et al (2005) reported to have observed no difference among the carcass parameters with dietary supplementation, when compared to the control group.[26]

In contrast to the present study, several reports have pointed out that dietary EO supplements can improve carcass parameters.[27,28]

In a study on the effects of dietary supplementation with *Thymus vulgaris* powder on the growth and carcass yield values of broiler chickens, El-Ghousein and Al-Beitawi (2009) reported that, when compared to the control group, all treatment groups that received dietary Origanum (at levels of 0.5%, 1%, 1.5%, and 2%) displayed improved carcass yields with the highest yield obtained in the group provided with 2% of *Thymus vulgaris* powder.[29]

In their research on the effects of dietary supplementation with essential oils in broiler chickens, Küçükyılmaz et al (2012) reported hepatic values similar to and splenic values different from those determined in the present study.[30] Liver and gizzard values reported by Çabuk et al (2006) are similar to our values.[31] Furthermore, while the liver, gizzard and heart weights reported by Eleroğlu et al (2016) are similar to those determined in the present study, the spleen weights reported by these researchers differ from those measured in this study.[32] The increase detected in the weight of the spleen in the groups that received 100 ppm and 200 ppm of OEO, when compared to the other study groups, was attributed to the immune system having developed faster, and in return having increased the weight of the spleen in these 2 groups.

Dietary supplementation with OEO having not caused any adverse effect on the serum biochemical parameters investigated in the present study suggests that OEO can be safely used as a feed supplement. In their research on the effects of rosemary, thyme and fennel essential oils on broiler chickens, Belenli et al (2015) determined that thyme essential oil did not alter triglyceride and glucose levels, while it decreased cholesterol and total protein levels.[33]

In the present study, the numerical decrease observed in the cholesterol levels of the groups that received 100 ppm and 200 ppm of dietary OEO can be explained by thymol and carvacrol, both of which are the main constituents of oregano, inhibiting the cholesterol synthesis enzyme HMG-CoA reductase, and thereby, although to a limited level, showing a cholesterol-reducing activity.[34] Gümüş et al (2017) reported that the incorporation of thyme essential oil into quail mixed feed did not affect total protein, glucose, triglyceride, and HDL levels, but decreased cholesterol and LDL levels.[35] In their study on the effects of different levels of dietary pennyroyal essential oil (0.5%, 1%, 1.5%, and 2%) on performance and blood biochemical parameters in broiler chickens,

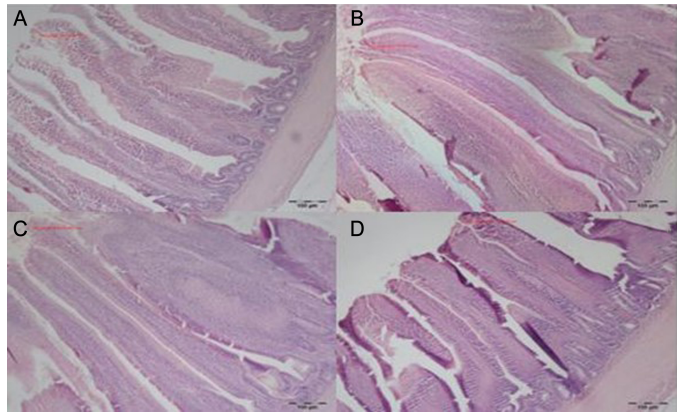

Figure 1. Jejunum hematoxylin and eosin, bar: 100 µm (Control: A; OEO 100: B; OEO 200: C; OEO 400: D).

Nobakht et al (2011) determined no effect on cholesterol, triglyceride and total protein levels, but a decrease in glucose levels with the use of 0.5% and 2% of pennyroyal and an increase in glucose levels with the use of 1% and 1.5% of pennyroyal.[36]

In the present study, according to the results obtained for the antioxidant parameters investigated, dietary supplementation with OEO caused only numerical changes in the GSH and MDA levels. On the other hand, Abdel-Ghaney et al (2017) reported that *Thymus vulgaris* leaves decreased MDA levels and increased GSH levels in broiler chickens.[37] Furthermore, Ölmez et al (2020) determined that dietary supplementation with resveratrol decreased serum MDA levels, but did not affect GSH levels.[5]

The comparative assessment of the jejunal villi heights measured in the present study revealed statistically significant differences among the study groups. It was observed that, when compared to the control group, the height of the jejunal villi had significantly increased values in the groups that received 200 ppm and 400 ppm of dietary OEO ($P < .05$). The rate of increase detected in the height of the jejunal villi in the groups, which received 200 ppm and 400 ppm of OEO, was 11.15% and 15.83%, respectively. It has been reported that, owing to the functional hydroxyl groups found in their composition and the high redox potential of these groups, thymol and carvacrol disrupt the cell wall of pathogenic microorganisms, and thereby, inhibit them with an eventual positive effect on the morphology of the small intestines and significant improvement in the height of the intestinal villi.[34]

The results obtained in the present study for intestinal villus height are in agreement with those reported to have been achieved by Hong et al (2012) with the use of 125 ppm of an essential oil blend (thyme, aniseed, and citrus peel)[38] and by Garcia et al (2007) with the use of 5000 ppm of a plant extract (origanum, rosemary, and sage).[39] On the other hand, Silva et al (2009) determined that dietary supplementation with 0.5 g/kg and 1 g/kg of origanum essential oil did not affect intestinal villus height in broiler chickens.[40]

The myoglobin concentration and hemoglobin level of muscles both affect the color of meat. While the color of meat varies with the amount of these pigments it contains, changes in the pH level of muscles also cause meat color differences. It is reported that post-slaughter meat pH levels are higher in animals exposed to stress reported that the dietary supplementation of broiler chickens with oregano powder (150 mg/kg) did not cause any

statistically significant difference in meat quality parameters.[14,37] The results of the present study are not in accordance with those reported by Aksu et al (2006), suggesting that the incorporation of probiotics into mixed feed decreased the redness (a*) value and increased the yellowness (b*) value of meat.[41] Pirmohamammadi et al (2016) determined that the combined incorporation of thyme (0.5%) and mint (0.5%) into the diet of broiler chickens increased meat pH level.[42]

Higher pH levels increase the color intensity and water holding capacity of poultry meat.[43] Poultry meat is pale, leaky, and soft when the pH level is ≤ 5.8, standard at a pH range of 5.9-6.2 and dark colored, hard and dry when the pH level is ≥6.3. It is also known that the pH level of meat directly affects its shelf life. High pH levels pose the risk of microbial growth in meat, and thus, shorten its shelf life.[44]

Differences between the results of this study and previous studies for meat color intensity (L*: lightness, a*: redness, b*: yellowness) and pH levels are attributed to differences in the type of feedstuff and feed supplements used, the season during which the studies were conducted, the length of the study period, the housing methods applied, and the environmental conditions that prevailed.

In conclusion, no significant improvement was observed in the performance parameters of the broiler chickens that received OEO in their diet. No significant effect having been detected on the performance parameters investigated could be related to either the amount of OEO incorporated into the diet or the absence of stress factors, given that antioxidant feed supplements show a more distinct effect in the presence of stress-inducing conditions.

Based on the results found in this study and in light of those reported in previous research, future studies are required to determine the more effective level of *Origanum onites* toward use as a feed additive in broilers.

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