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Effects of olive leaf extract (oleuropein) on performance, fatty acid levels of breast muscle and some blood parameters in Japanese quail (*Coturnix coturnix Japonica*) reared in different stocking densities

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Summary: This study was performed to determine the effects of olive leaf extract (oleuropein) on performance, fatty acid levels of breast muscle and some blood parameters in quails reared in different stocking densities. In the study, a total of 270 quail chicks (*Coturnix coturnix Japanica*), 15 days-old were used. The birds were randomly assigned according to 2 stocking densities (12, 18 birds/cage) × 3 oleuropein levels (0, 200, 400 ppm) including 6 treatment groups of 3 replicates while keeping the gender (135 female, 135 male) and initial weight balanced. The treatment was completed until 29 days. Stocking densities were arranged to be 100 and 150 cm^2 per quail in cages. Higher stocking density negatively affected live weight, body weight gain and carcass parameters ($P<0.05$). Oleuropein which added to mixed feed increased body weight gain, polyunsaturated fatty acid ratio, omega 3 and 6 fatty acid levels of breast muscle, and improved feed conversion rate ($P<0.05$) while reducing saturated fatty acid level of breast muscle ($P<0.05$).

Consequently, dietary oleuropein supplementation would improve performance and quality of breast muscle lipids by lowering saturated fatty acid proportions and by enhancing contents of polyunsaturated fatty acids in either stocking densities. According to examined parameters, a dose of 400 ppm oleuropein provided preferable results among the groups, especially in higher stocking density.

Key words: Carcass lipids, olive leaf extract, performance, quail, stocking density.

Farklı yerleşim sıklığında yetiştirilen Japon bıldırcınlarında (*Coturnix coturnix Japonica*) zeytin yaprağı ekstraktının (oleuropein) performans, göğüs eti yağ asidi düzeyleri ve bazı kan parametrelerine etkisi

Özet: Bu araştırma, zeytin yaprağı ekstraktının (oleropein) farklı yerleşim sıklığında yetiştirilen bıldırcınlarda performans, bazı kan parametreleri ve göğüs eti yağ asidi düzeylerine etkisini tespit etmek için yapılmıştır. Araştırmada 15 günlük yaşta 270 adet Japon bıldırcını (*Coturnix coturnix Japonica*) kullanılmıştır. Bıldırcınlar iki yerleşim sıklığı (12, 18 bıldırcın/kafes) x 3 oleropein düzeyi (0, 200, 400 ppm) olmak üzere 3 tekerrürlü 6 deneme grubuna ayrılmıştır. Gruplar başlangıç canlı ağırlığı ve cinsiyet (135 dişi, 135 erkek) bakımından dengelenmiştir. Deneme 29 gün sürmüştür. Yerleşim sıklıkları kafeslerde bıldırcın başına 100 ve 150 cm^2 olacak şekilde düzenlenmiştir. Yüksek yerleşim sıklığı canlı ağırlık, canlı ağırlık artışı ve karkas özelliklerini olumsuz etkilemiştir ($P<0.05$). Karma yeme katılan oleropein canlı ağırlık artışı, göğüs eti çoklu doymamış yağ asidi oranı, omega 3 ve 6 yağ asidi düzeylerini yükseltmiş, yemden yararlanma oranını iyileştirmiş ($P<0.05$), göğüs eti doymuş yağ asidi düzeyini düşürmüştür ($P<0.05$). Sonuç olarak, her iki yerleşim sıklığında diyete katılan oleropeinin performansı ve göğüs eti lipit kalitesini; doymuş yağ asidi oranlarını azaltıp, çoklu doymamış yağ asidi düzeylerini artırarak iyileştirdiği sonucuna varılmıştır. İncelenen parametrelere göre, 400 ppm dozundaki oleropeinden özellikle yüksek yerleşim sıklığına sahip gruplarda daha iyi sonuçlar elde edilmiştir.

Anahtar sözcükler: Bıldırcın, karkas lipitleri, performans, yerleşim sıklığı, zeytin yaprağı ekstraktı.

Introduction

Concern about the welfare of intensively reared poultry is a topic of current interest (32). The welfare of poultry is tightly regulated by various intrinsic and extrinsic factors such as management, stress, nutrition, immunosuppression and exposure to disease agents (38). A number of studies have examined the effects of stocking density on the performance and welfare

parameters (13, 30). Stocking density play an important role, especially during summer, in poultry production. Higher mortality, lower meat production, greater incidence of leg disorders, and cannibalism occur at higher stocking densities in poultry (34).

The usage of agricultural by-products in animal feeding is a practice as old as the domestication of animals (19). One of the many waste by-products used nowadays is the olive leaf obtained during the harvesting of the olives, the pruning of olive trees or the cleaning procedures before the extraction of the olive oil. Olive oil is rich of three classes of polyphenols such as hydroxytyrosol, secoiridoid such as oleuropein and lignans, which exhibit remarkable antioxidant actions (23). Oleuropein is the most abundant biophenol and the major bioactive compound in olive leaves. Oleuropein improves lipid metabolism to protect against obesity problems and it seems to be an activator of protein digestion and inhibitor of triacylglycerol absorption (26). Several studies have shown that oleuropein possesses a wide range of pharmacologic and health promoting properties including antioxidative (6), antiatherogenic (9), anticarcinogenic (24, 33), antimicrobial (7), antiviral (22), hypotensive (20), anti-ischemic (2) and antidiabetic effects (17). Many of these properties have been attributed to its antioxidant character of oleuropein (35).

The aim of this study is to evaluate the effects of dietary olive leaf extract (oleuropein) supplementation on the performance, carcass traits and some blood parameters in Japanese quail reared in different stocking densities.

Materials and Methods

Experimental design and diet regimens: A total of 270 fifteen-days-old Japanese quails (*Coturnix coturnix Japonica*) obtained from a commercial company were used. This study was undertaken after ethical approval of Fırat University (Official form number: 2014/9). The experiment was conducted at the Poultry Unit of Fırat University. The birds were randomly assigned, according to a 2 stocking densities (12, 18 birds/cage) × 3 oleuropein levels (0, 200, 400 ppm) experimental design, to 6 treatment groups of 3 replicates while keeping the gender (135 female, 135 male) and initial weight balanced. They were placed 100 and 150 cm^2/quails in cages. The cage dimensions were 30 cm width x 60 cm length x 40 cm height and temperature was 22±2°C. Basal diet was given to control groups. The birds were fed with the basal diet supplemented with 200 or 400 ppm of oleuropein in the other experimental groups. The treatment was completed until 29 days. The concentration of the volatile components in olive leaf extract was shown at Table 1 and *Fig. 1* (shown as percentage peak areas of GC-MS). Diets and fresh water were offered *ad libitum*. Light was provided continuously (24 h) throughout the experiment. Ingredients and chemical composition of the basal diet were shown at Table 2. The basal diets contained 23.87% CP and 12.13 MJ/kg of ME. The body weights of the birds were measured individually, and feed intakes per pen were recorded at weekly intervals. The weight gains of birds were then calculated. Feed conversion ratio (FCR) was calculated as feed consumed per unit of gain (g feed/g gain).

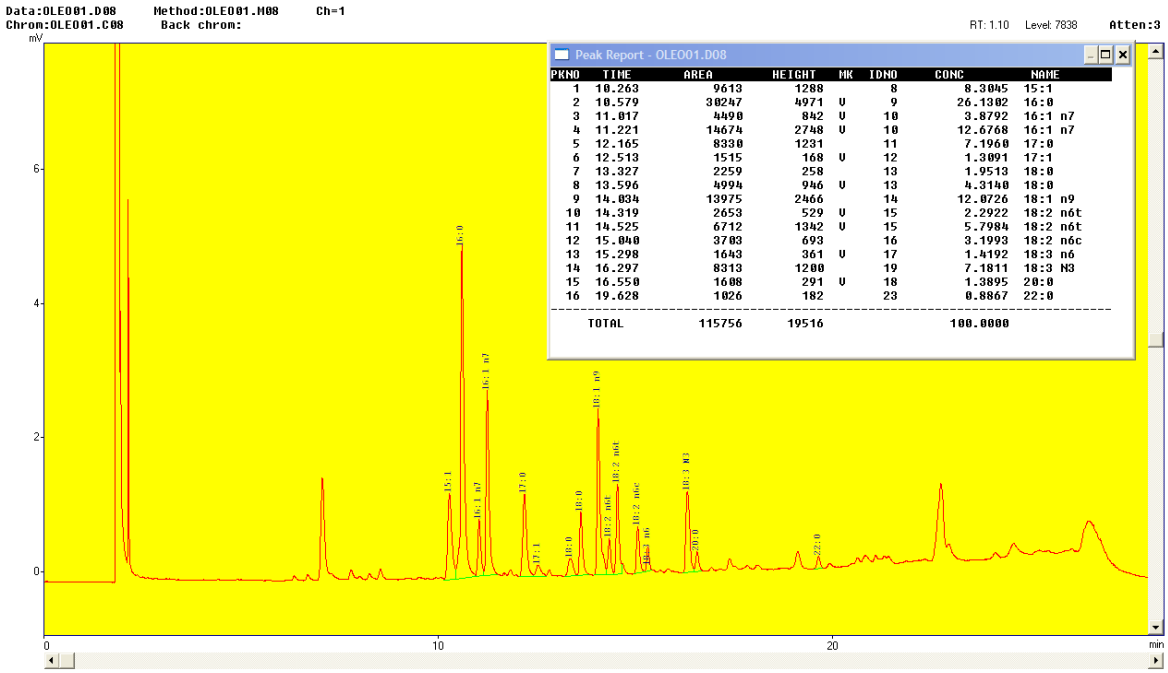

Fig 1. Total ion chromatogram of olive leaf extract.
Şekil 1. Zeytin yaprağı ekstraktının toplam iyon kromatogramı.

Table 1. The Fatty acid composition of the olive leaf extract, %.
Tablo 1. Zeytin yaprağı ekstraktının yağ asidi kompozisyonu, %.

Fatty Acids	%
Saturated Fatty Acid (SFA)	41.87
Monounsaturated Fatty Acid (MUFA)	38.24
Polyunsaturated Fatty Acid (PUFA)	19.89
PUFA / SFA	0.52
ω-6	12.71
ω-3	7.18
Ratio ω-6/ ω-3	1.77

SFA (Saturated Fatty Acid): Fatty Acid 160, 170, 180, 200, 220; MUFA (Monounsaturated Fatty Acid): Fatty Acid 151, 161n7, 171, 181n9; PUFA (Polyunsaturated Fatty Acid): Fatty Acid 182n6, 183n3, 183n6; ω-6 (Omega-6): Fatty Acid 182n6, 183n6; ω-3 (Omega-3): Fatty Acid 183n3
SFA (Doymuş Yağ Asidi): Yağ Asidi 160, 170, 180, 200, 220; MUFA (Tekli Doymamış Yağ Asidi): Yağ Asidi 151, 161n7, 171, 181n9; PUFA (Çoklu Doymamış Yağ Asidi): Yağ Asidi 182n6, 183n3, 183n6; ω-6 (Omega-6): Yağ Asidi 182n6, 183n6; ω-3 (Omega-3): Yağ Asidi 183n3

At the end of the study (43th day) six male and six female quails from each group with a body weight near the group average were slaughtered by cervical dislocation. Blood samples were collected from the jugular vein during slaughter, allowed to clot and centrifuged for 5 min at 2260 × *g* to separate the sera. The sera samples were stored (-20 °C) until analyzed. Following slaughtering, hot and cold carcass (+4^{o}C during 24 hours) yields were evaluated according to the criteria of Institute of Turkish Standards (3).

Hot carcass yield: [Hot carcass weight (g)/ Slaughter weight (g)] x 100

Cold carcass yield: [Cold carcass weight (g) / Slaughter weight (g)] x 100

For the fatty acid analyses of chicken meat, *M. pectoralis profundus* of breast were obtained and stored (-20 °C).

Chemical analyses: Serum total cholesterol, HDL and LDL cholesterol and glucose concentrations were measured using a biochemical analyzer (Olympus AU-600) at University of Fırat, Faculty of Medicine, Department of Biochemistry. Chemical composition of

Table 2. Ingredients and chemical composition of basal diet.
Tablo 2. Bazal karma yemin bileşimi ve kimyasal kompozisyonu.

Feed ingredients	%	Nutritional composition	%
Maize	29.03	Dry matter	88.25
Wheat	26.00	Crude protein	23.87
Soybean meal (44 CP)	34.29	Crude fibre	2.55
Corn Gluten	4.10	Ether extract	4.75
Vegetable oil	2.92	Ash	5.45
Dicalcium phosphate	2.02	Calcium ***	1.00
Ground limestone	0.87	Available phosphorus***	0.79
$NaHCO_3$	0.12	Methionine ***	0.40
Salt	0.28	Lysine ***	1.18
DL-Metiyonin	0.02	ME, Kcal/kg***	2897
Vitamin mix *	0.25		
Mineral mix**	0.10		

*Vitamin premix supplied per 2.5 kg; Vitamin A 12.000.000 IU; vitamin D3 2.000.000 IU; vitamin E 35.000 mg; vitamin K3 4.000 mg; vitamin B1 3.000 mg; vitamin B2 7.000 mg; Niacine 20.000 mg; Calcium D-pantotenat 10.000 mg; vitamin B6 5.000 mg; vitamin B12 15 mg; Folik Asit 1.000 mg; D-Biotin 45 mg; vitamin C 50.000 mg; Choline chloride 125.000 mg; Canthaxanthin 2.500 mg; Apo Karotenoik Acid Ester 500 mg
**Mineral premix supplied per kg; Mn 80.000 mg; Fe 60.000 mg; Zn 60.000 mg; Cu 5.000 mg; Co 200 mg; I 1.000 mg; Se 150 mg
*****:** Calculated. ME (kcal/kg) = 53+38 B used formula. B= (% Crude protein) + (2.25) (%Ether extract) + (1.1) (% Starch) + (% Sugar)
*****Vitamin karması: Her 2.5 kg'lık karışımda; A vitamini 12.000.000 IU; D3 vitamini 2.000.000 IU; E vitamini 35.000 mg; K3 vitamini 4.000 mg; B1 vitamini 3.000 mg; B2 vitamini 7.000 mg; Niasin 20.000 mg; Kalsiyum D-pantotenat 10.000 mg; B6 vitamini 5.000 mg; B12 vitamini 15 mg; Folik Asit 1.000 mg; D-Biotin 45 mg; C vitamini 50.000 mg; Kolin Klorit 125.000 mg; Kantaksantin 2.500 mg; Apo Karotenoik Asit Ester 500 mg bulunmaktadır.
******Mineral karması: Her 1 kg'lık karışımda; manganez 80.000 mg; demir 60.000 mg; çinko 60.000 mg; bakır 5.000 mg; kobalt 200 mg; iyot 1.000 mg; selenyum 150 mg bulunmaktadır.
*****:** Hesaplama yolu ile tespit edilmiştir. ME (kcal/kg) = 53+38 B formülü kullanıldı. B= (% ham protein) + (2.25) (% ham yağ) + (1.1) (% nişasta) + (% şeker)

basal feed ingredients (dry matter, crude protein, ash, and ether extract) were analyzed according to the AOAC (4) procedures and crude fiber was determined by the methods of Crampton and Maynard (11). Extraction of lipids from the tissue specimens was performed according to the method of Hara and Radin (14). For this purpose, a 1-g tissue specimen was homogenized in 10 mL of 3:2 (vol/vol) hexane:isoproropanol mixture for 30 s. Tissue homogenate was centrifuged at 2,260 × *g* for 10 min; the supernatant was taken for analysis.

Preparation of fatty acid methyl esters; For the preparation of methyl esters, lipid extract in a hexane:isopropanol phase was placed in 30-mL experiment tubes. Five milliliters of 2% methanolic sulfuric acid was added and the mixture was vortexed. This mixture was left to methylate in a 50°C incubation for 15 h. It was then cooled at room temperature, and 5 mL of 5% sodium chloride was added and mixed. The fatty acid methyl esters that were produced were extracted with 5 mL of hexane. The hexane phase was then removed with a pipette and treated with 5 mL of 2% KHCO3. The solvent in the methyl ester-containing mixture was evaporated at 45°C with a nitrogen flow and dissolved with 1 mL of hexane. All the mixture containing the solvent and hexane was then placed in 2-mL closed autosampler vials and analyzed (10).

Gas chromatographic analysis of fatty acid methyl esters: The fatty acid methyl esters were analyzed in a gas chromatograph with a Machery-Nagel capillary column. During the analysis, column heat was maintained at 120 to 220°C, injection heat was maintained at 240°C, and detector heat was maintained at 280°C. The column heat program was regulated to 220°C from 120°C; the heat increase was set to 5°C/min until reaching 200°C, to 4°C/min from 200 to 220°C, and held at 220°C for 8 min. Nitrogen was the carrier gas and the detector was a flame-ionization detector. Before analysis of the fatty acid methyl esters in the samples, standard fatty acid methyl esters and residence times of each fatty acid were determined. After this determination, the necessary program analysis was made and fatty acid methyl esters mixtures were analyzed.

Statistical analysis: Data were subjected to two-way Anova by using GLM (General Linear Model) procedure. Significant differences were further subjected to Duncan's multiple range test (31). The results were considered as significant when $p \leq 0.05$.

Results

Table 3 shows that in cases of high stocking density, body weight at 43d ($p<0.01$) and body weight gain ($p<0.01$) were significantly reduced. Feed intake and feed conversion ratio were not affected substantially from stocking densities ($p>0.05$). Oleuropein, which added to mixed feed with a dose of 400 ppm, were identified as a factor that increases body weight gain

Table 3. Effects of oleuropein supplementation in diet on performance, hot and cold carcass yield in Japanese quails under stocking density stress.
Tablo 3. Yerleşim sıklığı stresi altındaki Japon bıldırcınlarda karma yeme ilave edilen oleuropeinin performans, sıcak ve soğuk karkas randımanı üzerine etkileri.

Number of Animals in Groups	12			18			P		
Traits	Oleuropein, ppm			Oleuropein, ppm			Oleuropein (O)	Stocking Density (SD)	O x SD
	C	200	400	C	200	400			
Body Weight at 15d, g	64.83±1.67	64.58±2.65	63.92±2.25	64.19±3.27	64.03±3.55	64.23±2.78	0.990	0.886	0.981
Body Weight at 43d, g	212.58±4.84	215.17±5.48	222.63±5.04	200.06±5.99	207.31±5.98	209.31±4.96	0.093	0.006	0.840
Body Weight Gain, g/bird/day	5.10 ± 0.01^{b}	5.19 ± 0.10^{b}	5.48 ± 0.03^{a}	4.69±0.10	4.94±0.10	5.00±0.01	0.010	0.001	0.184
Feed Intake, g/bird/day	21.75±0.48	21.37±0.58	20.64±0.15	19.76±0.31	20.62±0.33	19.95±0.83	0.579	0.118	0.832
Feed Conversion Ratio, g feed/g gain	4.27 ± 0.04^{a}	4.12 ± 0.28^{ab}	3.77 ± 0.01^{b}	4.22 ± 0.14^{A}	4.18 ± 0.01^{A}	3.99 ± 0.17^{B}	0.050	0.815	0.824
Hot Carcass, %	64.96±2.66	67.96±1.55	69.68±1.40	64.52±0.77	65.76±1.77	67.52±2.10	0.623	0.027	0.691
Cold Carcass, %	63.48±0.77	66.01±2.06	68.28±1.40	63.36±2.60	64.82±1.73	65.03±1.56	0.520	0.036	0.973

Data were given as Mean ± SEM., C: Control, P<0.05: Difference between groups is important; a,b,A,B: The difference among the means represented by letters in the same row is important. O: Oleuropein; SD: Stocking Density O*SD: Interaction between oleuropein and stocking density.
Bulgular ortalama±standart hata olarak verildi, C: Kontrol, P<0.05:Gruplar arası farklılıklar önemlidir: a,b,A,B: Aynı satırdaki farklı harfleri taşıyan ortalamalar arasındaki farklılıklar önemlidir. O: Oleuropein; SD: Yerleşim sıklığı; O*SD: Oleuropein ile yerleşim sıklığı arasındaki interaksiyon

Table 4. Effects of oleuropein supplementation in diet on serum glucose and lipid levels in Japanese quails under stocking density stress.
Tablo 4. Yerleşim sıklığı stresi altındaki Japon bıldırcınlarda karma yeme ilave edilen oleuropeinin serum glikoz ve lipit seviyeleri üzerine etkileri.

Number of Animals in Groups	12			18			P		
Traits, mg/dl	Oleuropein, ppm			Oleuropein, ppm			Oleuropein (O)	Stocking Density (SD)	O x SD
	C	200	400	C	200	400			
Glucose	248.67±9.62	244.17±9.64	250.50±12.18	275.00±4.48	247.17±11.42	252.17±5.28	0.223	0.181	0.335
Total Cholesterol	167.33±18.13	164.67±18.03	168.50±22.48	194.67±19.08	171.83±20.81	175.83±18.42	0.790	0.375	0.839
HDL Cholesterol	72.00±10.07	82.83±9.78	80.50±4.69	73.33±3.96	90.00±8.58	82.17±3.47	0.161	0.561	0.904
LDL Cholesterol	96.00±13.63	87.50±13.28	79.83±9.95	109.17±11.63	102.50±20.03	104.17±10.48	0.711	0.113	0.907

Data were given as Mean ± SEM., C: Control, P>0.05: Difference between groups is not significant; O: Oleuropein; SD: Stocking Density O*SD: Interaction between oleuropein and stocking density.
Bulgular ortalama±standart hata olarak verildi, C: Kontrol, P>0.05:Gruplar arası farklılıklar önemsizdir, O: Oleuropein; SD: Yerleşim sıklığı; O*SD: Oleuropein ile yerleşim sıklığı arasındaki interaksiyon

Table 5. Effects of oleuropein supplementation in diet on fatty acid composition of musculus pectoralis in Japanese quails under stocking density stress.
Tablo 5. Yerleşim sıklığı stresi altındaki Japon bıldırcınlarında karma yeme ilave edilen oleuropeinin göğüs kası yağ asidi kompozisyonu üzerine etkileri.

Number of Animals in Groups	12			18			P		
Traits, %	Oleuropein, ppm			Oleuropein, ppm			Oleuropein (O)	Stocking Density (SD)	O x SD
	C	200	400	C	200	400			
Saturated Fatty Acid (SFA)	20.28±0.32	20.07±0.32	18.94±0.70	$20.82±0.20^{A}$	$19.68±0.016^{A}$	$18.85±0.033^{B}$	0.031	0.952	0.009
Mono Unsaturated Fatty Acid (MUFA)	20.08±0.25	19.17±0.15	20.11±0.44	20.44±0.21	17.75±1.13	19.50±0.42	0.280	0.277	0.009
Poly Unsaturated Fatty Acid (PUFA)	$57.43±0.49^{b}$	$59.41±0.33^{a}$	$60.01±0.92^{a}$	$56.20±0.35^{B}$	$60.45±1.24^{A}$	$62.21±0.60^{A}$	0.047	0.425	0.001
Others	0.38±0.06	0.22±0.03	0.13±0.31	0.24±0.06	0.57±0.33	0.08±0.01	0.693	0.724	0.184
ω-3	$2.81±0.16^{b}$	$3.32±0.22^{a}$	$3.42±0.28^{a}$	$2.54±0.35^{B}$	$2.93±0.32^{AB}$	$3.41±0.38^{A}$	0.017	0.184	0.001
ω-6	54.62±0.65	56.09±1.27	56.59±0.53	$53.66±0.36^{B}$	$57.52±0.45^{A}$	$58.80±1.29^{A}$	0.042	0.214	0.001

Data were given as Mean ± SEM., C: Control, P<0.05: Difference between groups is important; ^{a,b,A,B}: The difference among the means represented by letters in the same row is important. O: Oleuropein; SD: Stocking Density O*SD: Interaction between oleuropein and stocking density.
Bulgular ortalama±standart hata olarak verildi, C: Kontrol, P<0.05:Gruplar arası farklılıklar önemlidir: a,b,A,B: Aynı satırdaki farklı harfleri taşıyan ortalamalar arasındaki farklılıklar önemlidir. O: Oleuropein; SD: Yerleşim sıklığı; O*SD: Oleuropein ile yerleşim sıklığı arasındaki interaksiyon
SFA (Saturated Fatty Acid): Fatty Acid 140, 160, 180, 240; MUFA (Monounsaturated Fatty Acid): Fatty Acid 151, 161n7, 181n9, 201n9; PUFA (Polyunsaturated Fatty Acid): Fatty Acid 182n6, 183n3, 183n6, 203n6, 204n6, 225, 226n3; ω-6 (Omega-6): Fatty Acid 182n6, 183n6, 203n6, 204n6; ω-3 (Omega-3): Fatty Acid 183n3, 226n3
SFA (Doymuş Yağ Asidi): Yağ Asidi 140, 160, 180, 240; MUFA (Tekli Doymamış Yağ Asidi): Yağ Asidi 151, 161n7, 181n9, 201n9; PUFA (Çoklu Doymamış Yağ Asidi): Yağ Asidi 182n6, 183n3, 183n6, 203n6, 204n6, 225, 226n3; ω-6 (Omega-6): Yağ Asidi 182n6, 183n6, 203n6, 204n6; ω-3 (Omega-3): Yağ Asidi 183n, 226n3

($p<0.01$) and that improves feed conversion ratio ($p\leq0.05$). There was no interaction between stocking density and oleuropein on body weight, body weight gain, feed intake and feed conversion ratio. There wasn't any mortality during the treatment period.

At the inspection of the carcass characteristics (Table 3), there was significant effect of stocking density on this parameter. Supplementation of oleuropein to diet didn't affect the carcass yields at the present study. There wasn't any interaction between stocking density and oleuropein on carcass yields.

As seen in Table 4, stocking densities and addition of oleuropein were found to have no effect on blood glucose, total cholesterol, HDL and LDL cholesterol ($p>0.05$). Between stocking density and oleuropein, there was no interaction on parameters of blood glucose, total cholesterol, HDL and LDL cholesterol.

Based on Table 5, stocking densities had no effect on fatty acids of breast meat; oleuropein which added to mixed feed decreased proportion of total SFA in high stocking density group and increased total PUFA, omega 3 and 6 fatty acids in both stocking densities. There were significant interactions between stocking density and oleuropein on level of SFA, PUFA, MUFA, omega 3 and 6 fatty acids.

Discussion and Conclusion

In the study, by analyzing the body weight at 43 d, effects of stocking densities were found to be statistically significant. This condition may be associated with rather large numbers of animals per unit area which caused stress and the numerical reduction in feed intake. Thus Şimşek at al. (30) in their study was performed to determine the effects of stocking density on performance, carcass traits and bone mineralization of broiler chickens. They have concluded that the feed consumption was adversely affected by stocking density in poor growth and bone mineralization. There are some studies that include impairment of protein synthesis in poultries which grown under stress (29). While high stocking densities reduced body weight gain, with the effect of addition of oleuropein, an increase in body weight gains in both stocking density groups was determined. This situation can be explained by many studies (15) reporting that addition of essential oils to mixed feed regulates gastrointestinal tract of animals, increases feed intake by stimulating appetitezing digestive extract juices and acts as protective agents to bacterial diseases of animals and, as a result, improves yield performance of animals. In some studies in accordance with the present study, addition of essential oil to mixed feed had positive effects on live weight (1, 5, 18), while in others reported insignificant results (8, 36). Moreover, Erener et al. (12), in their study with broilers, were created research scheme of mixed feed with negative control without any additives, antibiotics (500 ppm chlortetracycline, positive control), vitamin E (200 ppm α-tocopherol acetate) and olive oil extract (75, 150, 300 and 600 ppm oleuropein), as a result of study, 600 ppm of oleuropein group had better body weight gain values than any other participating group.

Stocking densities and use of oleuropein had no statistically significant effect on feed intake. Although, depending on stocking densities, a reduction in feed intake was detected, this decrease was not statistically significant. Wilson et al. (37) reported small and insignificant increase in feed intake with increased cage floor area in their study in accordance with findings of the present study. Effects of stocking densities to feed conversion ratio was found insignificant, while effects of oleuropein was determined as statistically significant. This situation may be explained by positive effects of vegetable oils on digestive system. Moreover in studies, vegetable oils cause an increase in protein, fat and fiber digestion and activities of lipase and amilase (16).

At the inspection of the carcass characteristics, significant effect of stocking density on this parameter was detected. In stressed conditions, elevated concentrations of gluco-corticoids exert catabolic effects. This demolition decreases the rate of muscle synthesis and thus results in muscle wasting and retardation in growth. Supplementation of oleuropein to diet did not affect the carcass yields at the present study.

Effects of stocking densities and oleuropein on serum blood parameters were statistically insignificant. Similarly, in a study conducted by Seven et al. (28), examining the effects of different stocking densities on performance of Japanese quails, there were no significant effects of stocking densities on serum glucose, HDL, LDL and total cholesterol; also there was no effect of used additives on these parameters.

When composition of fatty acids of breast muscle examined in the present study, in both placement groups, lowest saturated fatty acids (SFA), highest polyunsaturated fatty acids (PUFA), omega 3 and 6 fatty acids levels were detected in 400 ppm oleuropein group. Moreover, there were significant interactions between stocking density and dose of oleuropein on the breast muscle fatty acids. This situation can be explained by bonding with metal ions including iron and copper and thus due to this bonding that causes preventing the formation of free radicals by suppressing inflammatory activity of enzymes such as lipoxygenase as suggested by Malayoğlu and Aktaş (21). In the other hand, activities of desaturase enzymes, which involved in synthesis of omega 3 and 6 fatty acids which referred as essential fatty acids, inhibited by increased synthesis of glucocorticoids under stress. Moreover, Özdemir and Azman (25) conducted a

study to determine the effects of addition of olive leaf extract to quail mixed feeds on yield performance and found that the lowest saturated fatty acid ratios in egg yolk were determined in oleuropein added group. In the another study Sarıca and Toptaş (27) reported that supplemented with oleuropein at the levels of 150 or 200 mg/kg had significantly the highest polyunsaturated fatty acid and omega-3 fatty acid contents in thigh meat, and levels of the oleuropein supplementation didn't affect performence parameters in quails.

For the purpose of avoiding drawbacks caused by stocking densities while increasing body weight gain, feed conversion ratio, polyunsaturated fatty acid and omega 3 and 6 fatty acid ratios, oleuropein were added to mixed feeds. In conclusion, addition of oleuropein to mixed feeds improved performance and reduced saturated fatty acid ratios. In this context, we believe oleuropein can be used to prevent negative effects of stress especially under the stressed conditions and to obtain meat with rich polyunsaturated fatty acids.

References

1. **Alçiçek A, Bozkurt M, Çabuk M** (2003): *The effect of essential oil combination derived from selected herbs growing wild in Turkey on broiler performance.* South Afr J Anim Sci, **33,** 89-94.
2. **Andreadou I, Iliodromitis EK, Mikros E, Constantinou M, Agalias A, Magiatis P, Skaltsounis AL, Kamber E, Tsantili-Kakoulidou A, Kremastinos DT** (2006): *The olive constituent oleuropein exhibits anti-ischemic, antioxidative, and hypolipidemic effects in anesthetized rabbits.* J Nutr, **136,** 2213-2219.
3. **Anonymous** (1989): *Turk standartları-tavuk govde eti parcalama kuralları TSE*.
4. **AOAC** (2000): *Official Methods of Analysis Association of AOAC International. 17th ed.,* (AOAC International Maryland).
5. **Bassett BR** (2000): *Oreganos positive impact on poultry production.* World Poultry Elsevier, **16,** 31-34.
6. **Benavente-Garcia O, Castillo J, Lorente J, Ortuno A, Del Rio JA** (2000): *Antioxidant activity of phenolics extracted from Olea europaea leaves.* Food Chem, **68,** 457-462.
7. **Bisingnano G, Tomaino A, Lo Cascio R, Crisafi G, Uccella N, Saija A** (1999): *On the in vitro antimicrobial activity of oleuropein and hydroxtyrosol.* J Pharm Pharmacol, **51,** 971-974.
8. **Botsoglou NA, Florou-Paner P, Christaki E, Fletouris DJ, Spais AB** (2002): *Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues.* Br Poult Sci, **43,** 223-230.
9. **Carluccio MA, Siculella L, Ancora MA, Massaro M, Scoditti E, Storelli C, Visioli F, Distante A, De Caterina R** (2003): *Olive oil and red wine antioxidant polyphenols inhibit endothelial activation: antiatherogenic properties of Mediterranean diet phytochemicals.* Arterioscler Thromb Vasc Biol, **23,** 622-629.
10. **Christie WW** (1992): *Gas chromatography and lipids.* The Oil Press, pp, 302, Glaskow.
11. **Crampton EW, Maynard LA** (1983): *The Relation of cellulose and lignin content to nutritive value of animal feeds.* J Nutr, **15,** 383-395.
12. **Erener G, Ocak N, Öztürk E, Çankaya S, Özkanca R, Altop A** (2009): *Zeytin yaprağı ekstraktının etlik piliçlerde performans, bazı kan parametreleri ve körbağırsak mikroflorası üzerine etkileri.* TUBITAK, TOVAG-107O820, Proje Kesin Rapor Özeti, Samsun.
13. **Feddes JJ, Emmanuel EJ, Zuidhof MJ** (2002): *Broiler performance, body weight variance, feed and water intake, and carcass quality at different stocking densities.* Poult Sci, **81,** 774-779.
14. **Hara AR, Radin NS** (1978): *Lipid extraction of tissues with a low-toxicity solvent.* Anal Biochem, **90,** 420-426.
15. **Hernandez F, Madrid J, Garcia V, Orengo J, Megias MD** (2004): *Influence of two plant extracts on broilers performance, digestibility, and digestive organ size.* Poult Sci, **83,** 169-174.
16. **Jamroz D, Kamel C** (2002): *Plant extracts enhance broiler performance. In non ruminant nutrition: Antimicrobial agents and plant extracts on immunity, health and performance.* J Anim Sci, **80,** 41.
17. **Jemai H, El Feki A, Sayadi S** (2009): *Antidiabetic and antioxidant effects of hydroxytyrosol and oleuropein from olive leaves in alloxan-diabetic rats.* J Agric Food Chem, **57,** 8798-8804.
18. **Kamel C** (2001): *Tracing modes of action and the roles of plant extracts in non-ruminants.* **In: Garnsworthy PC, Wiseman J.** (Editors). *Recent Advances in Animal Nutrition.* Nottingham: Nottingham University Press, 135-150.
19. **Keser O, Bilal T** (2010): *The use of olive-by products in animal nutrition.* J Anim Prod, **51,** 64-72.
20. **Khayyal MT, El-Ghazaly MA, Abdallah DM, Nassar NN, Okpanyi SN, Kreuter MH** (2002): *Blood pressure lowering effect of an olive leaf extract (Olea europaea) in L-NAME induced hypertension in rats.* Arzneimittelforschung, **52,** 797–802.
21. **Malayoğlu HB, Aktaş B** (2011): *Antioxidant and antimicrobial activities of olive leaf and olive mill wastewater from olive oil processing by-products.* J Anim Prod, **52,** 49-58.
22. **Micol V, Caturla N, Perez-Fons L, Mas V, Perez L, Estepa A** (2005): *The olive leaf extract exhibits antiviral activity against viral haemorrhagic septicaemia rhabdovirus (VHSV).* Antiviral Res, **66,** 129-136.
23. **Owen RW, Giacosa A, Hull WE, Haubner R, Wurtele G, Spiegelhalder B, Bartsch H** (2000a): *Olive-oil consumption and health: the possible role of antioxidants.* Lancet Oncol, **1,** 107-112.
24. **Owen RW, Giacosa A, Hull WE, Haubner R, Spiegelhalder B, Bartsch H (**2000b): *The antioxidant/ anticancer potential of phenolic compounds isolated from olive oil.* Eur J Cancer, **36,** 1235–1247.
25. **Özdemir A, Azman MA** (2013): *The effects of supplemental olive leaf extract in diet on performance of quails.* Fırat Univ Vet J Health Sci, **27,** 141-147.
26. **Polzonetti V, Egidi D, Vita A, Vincenzetti S, Natalini P** (2004): *Involvement of oleuropein in (some) digestive metabolic pathways.* Food Chem, **88,** 11–15.

27. **Sarıca S, Toptaş S** (2014): Effects of dietary oleuropein supplementation on growth performance, serum lipid concentrations and lipid oxidation of Japanese quails. J Anim Physiol Anim Nutr, **98,** 1176-1186.
28. **Seven I, Seven PT, Aslan AS, Yıldız N** (2011): *The effects of dietary bee pollen on performance and some blood parameters in japanese quails (Coturnix coturnix Japonica) breeding under different stocking densities.* J Fac Vet Med Univ Erciyes, **8,** 173-180.
29. **Seven PT, Seven I, Yılmaz M, Şimşek UG** (2008): *The effects of Turkish propolis on growth and carcass characteristics in broilers under heat stres.* Anim Feed Sci Tech, **146,** 137-148.
30. **Şimşek UG Çiftçi M, Çerci IH, Bayraktar M, Dalkılıç B, Arslan O, Balcı TA** (2011): *Impact of Stocking Density and Feeding Regimen on Broilers: Performance, Carcass Traits and Bone Mineralization.* J App Anim Res, **39,** 230-233.
31. **SPSS**. *Inc. SPSS for Windows Release11.5 (6 Sep. 2002), Standard Version, Copyright SPSS Inc., Chicago.*
32. **Thomas DG, Ravindran V, Thomas DV, Camden BJ, Cottam YH, Morel PCH, Cook CJ** (2004): *Influence of stocking density on the performance, carcass characteristics and selected welfare indicators of broiler chickens.* New Zeal Vet J, **52,** 76-81.
33. **Tripoli E, Giammanco M, Tabacch G, Di Majo D, Giammanco S, La Guardia M** (2005): *The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health.* Nutr Res Rev, **18,** 98–112.
34. **Türkyılmaz MK** (2008): *The effect of stocking density on stress reaction in broiler chickens during summer.* Turk J Vet Anim Sci, **32,** 31-36.
35. **Visioli F, Poli A, Gall C** (2002): *Antioxidant and other biological activities of phenols from olives and olive oil.* Med Res Rev, **22,** 65–75.
36. **Vogt H, Rauch HW** (1991): *Der einsatz einzelner ätherischer öle im geflügelmast futter.* Lanbauforschung Völkenrode, **41,** 94-97.
37. **Wilson HR, Douglas CR, Miller ER** (1987): *Floor space for brooding bobwhite quail.* Poult Sci, **57,** 1499-1502.
38. **Yakubu A, Gwaska JA, Salako AE** (2009): *Strain and placement density effects on welfare, haematological and serum biochemical indices of broilers in North Central Nigeria.* Acta Agric Slov, **94,** 153-158.

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