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Evaluation of olive leaf extract as a growth promoter on the performance, blood biochemical parameters, and caecal microflora of broiler chickens

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ABSTRACT - The objective of the study was to examine the effects of an alcoholic liquid olive leaf extract (OLE) obtained from fresh leaves on the growth performance, carcass weight, caecal microflora, and some plasma variables, such as triglycerides, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol in broiler chickens. A total of 375 one-day-old male broilers (Ross 308) were randomly divided into five treatments with five replicate pens (15 birds each) per treatment. The birds were fed either a basal diet with no supplement (control), with 75 (OLE75), 150 (OLE150), 300 (OLE300), and 600 (OLE600) mg kg^{-1} oleuropein, provided by 0.66, 1.33, 2.65, and 5.32 g kg^{-1} of the OLE. The dietary supplementation of OLE linearly increased daily body weight gain (BWG), feed intake (FI), and carcass weight and improved feed conversion ratio (FCR). Although the OLE600 broilers had the highest daily FI among the treatments, there were significant increases in their BWG and improvements in FCR compared with the control, OLE75, and OLE150 birds. The carcass weights of OLE150, OLE300, and OLE600 birds were higher than those of the control group. The OLE600 diet increased the dressing percentage compared with the OLE75, OLE150, and OLE300 diets. The abdominal fat weight and the plasma HDL concentration of the control broilers were lower than those of all OLE birds. All doses of OLE supplementation decreased the caecal *E. coli* content. A growth-promoting effect is obtained from the dietary supplementation of 600 mg kg^{-1} oleuropein due to its beneficial effect on growth performance and caecal microflora populations of broilers.

Keywords: antimicrobial, feed additive, oleuropein, olive leaves, plant extract, poultry

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Introduction

There has been an increasing awareness since 2006 that the secondary metabolites (phytogenic products) and essential oils obtained from indigenous plants as growth-promoting or antimicrobial agents may have multiple health and nutritional benefits for poultry (Zeng et al., 2015). Leaves of the olive tree (*Olea europaea* L., Oleaceae) or its metabolites, such as oleuropein, have antimicrobial properties both *in vitro* and *in vivo*. It has been shown that olive leaf extract (OLE) might exert an

antimicrobial action against human pathogenic microorganisms (Markin et al., 2003) including those in the gastric flora (Sudjana et al., 2009). Furthermore, OLE has been found to act effectively on the microbial load of raw peeled undeveined shrimp (Ahmed et al., 2014) and against pathogenic caecal bacteria in broiler chickens (Jabri et al., 2017). Accordingly, oleuropein can potentially be implemented as an interesting feed additive; however, its efficacy, mode of action, and antimicrobial capability as a growth promoter need to be evaluated. Active secondary metabolites of olive leaves could, therefore, be considered as an alternative for modulating or altering the intestinal microbial population as well as for promoting growth and dressing percentage. However, there is no information about whether oleuropein has a protective effect on health in poultry.

Only a limited number of studies could be found in the literature on the effects of oleuropein on the productive performance of quail (Sarica and Toptas, 2014; Bahsi et al., 2016), broilers (El-Damrawy et al., 2013; Jabri et al., 2017), and laying hens (Cayan and Erener, 2015; Ahmed et al., 2017). However, the results of feeding diets enriched with oleuropein to these poultry species are contradictory, perhaps because the levels and forms of dietary oleuropein supplementation as well as the animal breeds and species in these studies differ. Sarica and Topbas (2014) reported that a dietary inclusion rate of 200 mg kg^{-1} oleuropein did not affect growth performance and weights of the digestive organs in quail. In another study, dietary oleuropein supplementation (400 ppm) improved the performance and quality of breast muscle lipids in quail (Bahsi et al., 2016). El-Damrawy et al. (2013) showed that olive leaf powder supplementation (2%) in diets of Mandarah chicks improved body weight and feed conversion, as well as most immunological and blood biochemical traits. Aqueous OLE supplementation through drinking water (10 mL L^{-1}) has been found to promote the growth performance by inducing antimicrobial activity at the local level of broiler chickens (Jabri et al., 2017). Cayan and Erener (2015) reported that up to 3% olive leaf powder had no effect on feed intake, egg weight, egg yield, and feed conversion ratio, but it did increase the final body weight of hens. Inversely, it has been found that different levels of oleuropein (50, 100, and 150 mg kg^{-1} diet) improved egg production, egg mass, and feed conversion ratio (Ahmed et al., 2017).

Considering the above benefits, we hypothesize that oleuropein provided by an alcoholic liquid extract obtained from fresh olive leaves may have positive effects on poultry in terms of promoting growth and regulating the intestinal microbial population and blood parameters that directly relate to animal health. Accordingly, the objective of this study was to determine the effects of oleuropein in an alcoholic liquid OLE on growth performance, carcass weight, some caecal microflora, and blood parameters of male broilers.

Material and Methods

This study was conducted in Samsun city, Turkey (41°21'40" N and 36°11'00" E, and altitude of 224 m above sea level). The study was approved by the local Ethics Committee for Experimental Animals, which ascertained that the experiment was not an unnecessary repetition of previous experiments (case number HADYEK/04).

Broiler chicks were obtained from a commercial hatchery (Ross Breeders, Anadolu, Turkey) and raised in pens (1.2×2.0 m) in a deep litter system under standard hygienic conditions. Animals received the experimental diets for six consecutive weeks (42 days). The OLE was extracted from fresh olive leaves using an ethanol (35%) distillation extraction method (Karkim, Karadeniz Chemical, Samsun, Turkey). The secondary plant metabolites (phenolic composition) of OLE were obtained via HPLC analysis (Altiok et al., 2008).

A total of 375 one-day-old Ross 308 male broiler chicks (average weight 42.0±0.07 g) were individually weighed and randomly assigned to five groups (75 per group) with five replicates (15 broilers each). The birds were fed either a basal diet with no supplement (control) or with 75 (OLE75), 150 (OLE150), 300 (OLE300), or 600 (OLE600) mg kg^{-1} oleuropein, provided by OLE. The OLE at four different dietary levels (0.66, 1.33, 2.65, and 5.32 g kg^{-1}) was sprayed onto a small amount (approximately 0.5 g) of feed and this premix was added to a sufficient amount of feed to achieve the desired final concentration.

All birds were fed *ad libitum* (Table 1) from day one to day 21 of age (starter), from day 22 to day 35 of age (grower), and from day 36 to day 42 of age (finisher). All diets, formulated according to NRC (1994), were provided as mash. All groups were subjected to similar management practices (lighting, feeding, and watering) throughout the experiment. Broilers were housed in floor pens placed within a commercial farm, where they were reared with their commercial contemporaries (Ozturk et al., 2010). The birds were provided with feed in cylindrical hanging feeders and with water in hanging drinkers *ad libitum*. Feeder and drinker spaces were 2 cm per bird. Lighting was provided for 23 h/day throughout the experimental period by two fluorescent bulbs. Ambient temperature was gradually decreased from 33 °C on day 7 to 21 °C on day 21 and was then kept constant (Ozturk et al., 2010).

Performance parameter measurements included initial body weight (BW), daily body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR). Therefore, the BW and FI of birds were recorded pen-wise on a weekly basis. However, average BWG and FI were calculated on a daily basis. All pens were checked for health status of the birds and mortality twice a day. The FI was corrected for mortality, which was recorded on a daily basis. The FCR was estimated as daily FI divided by daily BWG (g feed:g gain).

On day 42, eight broilers per replicate (40 birds per treatment) were randomly selected, had undergone 8 h of feed withdrawal and then weighed and slaughtered to complete bleeding at the slaughterhouse of the Agricultural Faculty. The carcasses of three broilers slaughtered per replicate (15 birds per treatment) were plucked, eviscerated, and weighed to determine carcass weight and yield, expressed as dressing percentage ([dressed carcass weight/BW] × 100). The whole gastrointestinal tract (gut) and edible inner organs (gizzard + heart + liver) were separated, individually weighed, and the weights were recorded in grams using a sensitive electronic balance. The abdominal fat pad, comprising the leaf fat surrounding the cloaca and abdominal muscles and excluding fat surrounding the gizzard, was separated and similarly weighed and recorded. The length of the whole gut, from the oesophagus to the cloaca were recorded in centimetres using a calibrated scale and thread. Then, the relative weights

Table 1 - Diet formulation and calculated chemical composition of the basal ration

	Starter	Grower	Finisher
Feed ingredient (g kg^{-1} as fed)			
Yellow corn	519.6	559.9	584.4
Soybean meal (480 g kg^{-1} CP)	299.5	264.0	233.6
Full fat soybean	135.0	124.0	134.0
Vegetable oil	8.0	20.0	20.0
Limestone	24.2	20.2	19.1
Dicalcium phosphate[1]	1.5	2.0	1.8
Salt	3.3	3.3	3.3
Vitamin and mineral premix[2]	3.5	3.5	3.5
L-lysine hydrochloride	1.7	0.4	0.3
DL-methionine	3.5	2.7	-
L-threonine, %99	0.2	-	-
Chemical composition (g kg^{-1} on DM basis)			
Metabolizable energy (Mcal kg^{-1})	3.0	3.1	3.2
Crude protein (CP)	240.0	220.0	200.0
Lysine	14.3	12.4	10.9
Methionine	5.1	4.5	4.1
Threonine	9.4	8.3	7.4
Methionine + cysteine	10.7	9.5	8.6
Calcium	10.5	9.0	8.5
Available P	4.5	4.5	3.5

Diets were mixed with different levels of olive leaf extract, except for the control.
[1] Each kilogram contained: Ca, 240 g; P, 175 g.
[2] Supplied per kg diet: trans-retinyl acetate, 12,000 IU; cholecalciferol, 2,400 IU; DL-α-tocopheryl acetate, 40 mg; menadione, 4 mg; thiamine, 3 mg; riboflavin, 6 mg; niacin, 25 mg; folic acid, 1 mg; pantothenic acid, 10 mg; pyridoxine, 5 mg; cyanocobalamin, 0.03 mg; biotin, 0.05 mg; choline chloride, 200 mg; Mn, 80 mg; Zn, 60 mg; Fe, 60 mg; Cu, 5 mg; Co, 0.2 mg; I, 1 mg, Se, 0.15 mg.

and lengths (when appropriate) of these organs and the abdominal fat pad were calculated using the BW just before slaughter of the broilers ([organ weight or length/BW] × 100) and thus expressed as a percentage of BW just before slaughter (g or cm/100 g BW).

Blood samples of another three birds per replicate (15 birds per treatment) were collected (about 4 mL) from the brachial wing vein in Vacutainer sterilised tubes (BD Biosciences, Franklin Lakes, NJ) containing heparin. Plasma was separated from the blood cells by centrifugation at 4,000 rpm for 10 min. Plasma was frozen and stored at −20 °C. Frozen plasma samples were thawed at 4 °C before processing and were then analysed for triglycerides, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol using an automatic analyser (AIRONE 200 RA, Roma, Italy) according to the recommendations of the manufacturer (Biolab, Maizy, France). In addition, the LDL:HDL cholesterol ratio for each treatment was calculated by dividing the mean LDL value by the mean HDL value.

The caecum content of two birds slaughtered per replicate (10 birds per treatment) was aseptically collected to determine caecal microbial counts. The total counts of *Lactobacillus* spp., *Enterococcus* spp., *Clostridium* spp., *Staphylococcus aureus* (*S. aureus*), *Campylobacter* spp., and *Escherichia coli* (*E. coli*) were measured according to the procedure described by Ozturk et al. (2010). The enumeration of these bacteria was performed using the traditional method of counting the colonies recovered on agar plates. For the *Clostridium* count, TSC Agar (Tryptose Sulfite Cycloserine Agar) Base (Merck 1.11972) was used. The plates were incubated for 24 h at 35 at 37 °C. Slanetz and Bartley Agar (Oxoid CM0377) was used for the enumeration of *Enterococcus*. The plates were incubated aerobically at 37 °C for 24 h, and the number of colonies grown was determined. For the enumeration of *Lactobacilli* spp., MRS Agar (Lactobacillus Agar according to De Man, Rogosa and Sharpe, Merck 1.10660) was used. The plates were incubated for three days at 35 °C under microaerophilic conditions. Baird Parker Agar (Oxoid CM275) was used for the enumeration of *Staphylococcus*. All the plates were aerobically incubated at 37 °C for 24 to 48 h, and the number of colonies grown was determined. For the *Campylobacter* count, Campy CVA Agar (BD BBL 297246) was used, and the plates were incubated for 18 to 24 h at 42 °C. *Salmonella* spp. were placed on Salmonella Shigella Agar (SS Agar, Oxoid CM0099, UK) and incubated aerobically for 18 to 24 h at 37 °C. The enumeration of *E. coli* used Eosin Methylene Blue Agar (Oxoid CM0069). After incubation at 37 °C for 18 to 24 h, all blue-coloured colonies were counted as *E. coli*. All bacteria counts were expressed as $\log_{10}$ cfu g^{-1}.

For performance data (n = 5), pen mean values served as the experimental unit for statistical analysis. For data on carcass weight carcass, dressing percentage, relative weights and lengths of organs (n = 15), caecal microflora count (n = 10), and plasma variables (n = 15), individually slaughtered birds were considered the experimental unit. To verify the homogeneity of variances and normality, data were subjected to Levene's tests and Shapiro-Wilk test, respectively. All proportional data were transformed by taking arcsine square roots prior to analysis. The data were analysed as a completely randomised design using the generalised linear mixed models (GLMM) procedure of the Statistical Package for the Social Sciences (SPSS 21.0, SPSS Inc., Chicago, IL, USA). The distribution type (normal, β or Poisson) of the response variable was included in the GLMM model statement. Moreover, to determine the linear and quadratic effects of increasing concentrations (from 0 to 600 mg kg^{-1}) of supplemental OLE, the data were analysed as orthogonal polynomial contrasts. Tukey's test was used to determine differences between means. The probability level of $P<0.05$ was considered significant.

Results

The result obtained regarding the chemical components of the OLE used in the present study indicated that OLE contained plant secondary metabolites such as oleuropein, rutin, hydroxytyrosol, vanillin, vanillic acid, caffeic acid, and catechin (Table 2). The percentage of oleuropein, which accounted for 112.9 g kg^{-1}, was higher than that of other components.

Mortality during this study was low (4%) and was not associated with any specific treatment. The dietary supplementation of OLE had a linear effect on daily BWG, FI, and FCR ($P<0.01$; Table 3). Daily BWG of the OLE150, OLE300, and OLE600 broilers were higher than those of the control birds. The

OLE600 broilers had the highest FI among the treatments. The OLE75 birds consumed less feed than the OLE150 birds (P<0.05). There were significant improvements in the FCR of OLE300 and OLE600 broilers compared with the control, OLE75, and OLE150 birds. The control and OLE75 birds had similar BWG and FCR values.

The carcass weights of the OLE150, OLE300, and OLE600 birds were higher than those of the control birds (P<0.01; Table 4). The carcass weight of broilers in the OLE600 group was higher (P<0.05) than those in the other groups, while the carcass weights of the OLE150 and OLE300 groups were higher (P<0.05) than for the control and OLE75 groups. The OLE600 diet increased the dressing percentage compared with the OLE75, OLE150, and OLE300 diets (P<0.05). The effects for carcass weight were linear (P<0.01), whereas the effect for the dressing percentage was quadratic (P<0.01). The relative weight of whole gut increased for OLE75 and OLE150 groups compared with the other groups, while the relative length of the gut increased in the OLE75 group compared with the other groups (P<0.05 for quadratic effect). The relative abdominal fat weight of the control birds was lower than in all OLE groups (P<0.01 for linear effect).

Table 2 - Plant secondary metabolites of olive leaf extract

Secondary metabolite	g kg^{-1}
Oleuropein	112.9
Rutin	34.5
Hydroxytyrosol	16.7
Vanillin	7.3
Vanillic acid	4.7
Caffeic acid	4.4
Catechin	1.6

Table 3 - Body weight gain, feed intake, and feed conversion ratio of broilers fed diets supplemented with olive leaf extract (OLE)

Item[1]	Control	OLE75	OLE150	OLE300	OLE600	SEM	P-value	L	Q
Daily weight gain (g bird^{-1})	53.7d	53.7d	55.3c	57.6b	59.4a	0.33	0.010	**	NS
Daily feed intake (g bird^{-1})	100.2bc	99.9c	100.3bc	102.1b	105.2a	0.55	<0.001	**	NS
Feed conversion ratio (g feed:g gain)	1.87a	1.86a	1.81b	1.77c	1.77c	0.013	<0.001	**	NS

Control: basal diet with no oleuropein; OLE75: basal diet with 75 mg kg^{-1} oleuropein; OLE150: basal diet with 150 mg kg^{-1} oleuropein; OLE300: basal diet with 300 mg kg^{-1} oleuropein; OLE600: basal diet with 600 mg kg^{-1} oleuropein.
SEM - standard error of the mean; L and Q - linear and quadratic response, respectively, for the oleuropein level; NS - non-significant.
Means in the same row not sharing a common letter are significantly different (P<0.05).
** P<0.01.
[1] Data represent the mean value of five replicate pens of 15 birds.

Table 4 - Carcass weight (g), dressing percentage, weights of whole gut, edible inner organs, and abdominal fat (g 100 g BW^{-1}), and length of whole gut of broilers fed diets supplemented with olive leaf extract (OLE)

Item[1]	Control	OLE75	OLE150	OLE300	OLE600	SEM	P-value	L	Q
Carcass weight	1637c	1637c	1686b	1745b	1834a	9.9	<0.001	**	NS
Dressing percentage	73.1ab	72.0b	72.3b	72.1b	74.1a	0.08	<0.001	NS	**
Relative weight of									
Whole gut	7.83b	8.96a	9.32a	7.81b	7.80b	0.09	<0.001	NS	*
Edible inner organs	6.19	5.76	6.35	5.43	5.87	0.11	0.060	NS	NS
Abdominal fat	1.09d	1.23c	1.38b	1.46ab	1.51a	0.03	<0.001	**	NS
Relative length of whole gut	10.53d	11.28ab	10.74cd	10.42d	10.36d	0.09	0.001	NS	*

Control: basal diet with no oleuropein; OLE75: basal diet with 75 mg kg^{-1} oleuropein; OLE150: basal diet with 150 mg kg^{-1} oleuropein; OLE300: basal diet with 300 mg kg^{-1} oleuropein; OLE600: basal diet with 600 mg kg^{-1} oleuropein.
SEM - standard error of the mean; L and Q - linear and quadratic response, respectively, for the oleuropein level.
Means in the same row not sharing a common letter are significantly different (P<0.05).
NS - non-significant, P>0.05; * P<0.05; ** P<0.01.
[1] Data represent the mean value of 15 birds (five replicate pens × three birds per pen).

The OLE75, OLE150, and OLE600 broilers had higher triglycerides than the control group (P<0.05 for quadratic effect) (Table 5). The HDL level increased with OLE at the tested doses compared with the control treatment, while the LDL level decreased in the OLE150, OLE300, and OLE600 groups compared with the control and OLE75 groups (P<0.05 for quadratic effect). As a result, the LDL:HDL cholesterol ratio was at its lowest and highest in the OLE600 (1.18) and control (1.59) groups, respectively (P<0.05 for quadratic effect). The total cholesterol concentrations of birds in the control, OLE150, and OLE600 groups remained lower compared with the value of the OLE75 group. Broilers in the OLE150, OLE300, and OLE600 groups had higher LDL cholesterol values than those in the control and OLE75 groups, whereas the control birds had a lower value than all OLE-fed birds in terms of HDL cholesterol.

Significant differences were observed in caecal microflora populations between treatments, except for *Lactobacillus* spp. For the *Campylobacter* spp. count, the effects were linear (P<0.01), whereas the effects for the *Enterococcus* spp. (P<0.05), *Clostridium* spp. (P<0.01), *S. aureus* (P<0.05), and *E. coli* (P<0.05) counts were quadratic (Table 6). *Salmonella* spp. could not be detected in any treatments. The OLE75 treatment decreased the *Enterococcus* spp. count compared with the control and OLE150 treatments. The other OLE treatments did not affect the *Enterococcus* spp. count compared with the control group. The *Clostridium* spp. counts of the control birds were higher than those of the OLE75, OLE150, and OLE300 birds. The OLE150 and OLE600 treatments both resulted in higher *Clostridium* spp. counts than the OLE75 treatment. The OLE75, OLE150, and OLE600 treatments did not affect the *S. aureus* count compared with the control group. The *S. aureus* count of the OLE300 birds was lower (P<0.05) than that of the control and OLE75 birds. Although all OLE treatments tended to increase the *Campylobacter* spp. count, the birds in the OLE600 group had a higher *Campylobacter* spp. count compared with the control group. The OLE at the tested doses, excluding the OLE300 group, decreased the *E. coli* count compared with the control group. The *E. coli* count of the OLE150 birds was lower than that of the OLE300 birds.

Table 5 - Plasma metabolites of blood from broilers receiving diets supplemented with olive leaf extract (OLE)

Item[1] (mg dL^{-1})	Control	OLE75	OLE150	OLE300	OLE600	SEM	P-value	L	Q
Triglyceride	141.3c	146.9ab	147.0ab	143.6bc	147.2ab	1.13	0.046	NS	*
Total cholesterol	175.2c	183.5ab	176.4c	178.8bc	175.6c	1.21	0.007	NS	*
High-density lipoprotein (HDL)	60.9b	69.0a	70.2a	68.6a	70.4a	0.94	0.044	NS	*
Low-density lipoprotein (LDL)	96.8a	100.0a	85.8b	88.1b	83.2b	1.74	<0.001	NS	*
LDL:HDL ratio	1.59a	1.45b	1.22c	1.28c	1.18c	0.077	0.048	NS	*

Control: basal diet with no oleuropein; OLE75: basal diet with 75 mg kg^{-1} oleuropein; OLE150: basal diet with 150 mg kg^{-1} oleuropein; OLE300: basal diet with 300 mg kg^{-1} oleuropein; OLE600: basal diet with 600 mg kg^{-1} oleuropein.
SEM - standard error of the mean; L and Q - linear and quadratic response, respectively, for the oleuropein level.
Means in the same row not sharing a common letter are significantly different (P<0.05).
NS - non-significant, P>0.05; * P<0.05.
[1] Data represent the mean value of 15 birds (five replicate pens × three birds per pen).

Table 6 - Bacterial counts from the cecal content of broilers fed diets supplemented with olive leaf extract (OLE)

Item[1] (log_{10} cfu g^{-1})	Control	OLE75	OLE150	OLE300	OLE600	SEM	P-value	L	Q
Lactobacillus spp.	7.07	6.79	6.88	6.77	6.76	0.046	0.253	NS	NS
Enterococcus spp.	5.07ab	4.77c	5.45a	5.00bc	4.86bc	0.062	0.036	NS	*
Clostridium spp.	2.00a	0.56c	1.40b	0.85bc	1.68ab	0.128	0.001	NS	**
Staphylococcus aureus	4.63ab	4.67a	4.27bc	4.13c	4.23bc	0.073	<0.001	NS	*
Campylobacter spp.	1.39b	1.41b	1.71ab	1.60ab	1.93a	0.078	0.032	**	NS
Escherichia coli	6.73a	6.06bc	5.95c	6.21ab	6.05bc	0.068	0.002	NS	*

Control: basal diet with no oleuropein; OLE75: basal diet with 75 mg kg^{-1} oleuropein; OLE150: basal diet with 150 mg kg^{-1} oleuropein; OLE300: basal diet with 300 mg kg^{-1} oleuropein; OLE600: basal diet with 600 mg kg^{-1} oleuropein.
SEM - standard error of the mean; L and Q - linear and quadratic response, respectively, for the oleuropein level.
Means in the same row not sharing a common letter are significantly different (P<0.05).
NS - non-significant, P>0.05; * P<0.05; ** P<0.01.
[1] Data represent the mean value of 10 birds (five replicate pens × two birds per pen).

Discussion

The main active components of olive leaf are oleuropein and its derivatives, such as hydroxytyrosol, caffeic acid, vanillic acid, vanillin, and rutin (Ryan et al., 2002; Farag et al., 2003; Altiok et al., 2008). The OLE added to broiler diet was similar in composition to that found in the literature (e.g. Bianco and Uccella, 2000; Ryan et al., 2002; Farag et al., 2003). A slightly higher amount of oleuropein was observed; however, it must be underlined that the content of this isomer is usually a good OLE quality indicator (Ryan et al., 2002; Farag et al., 2003; Altiok et al., 2008).

The results of the present study show that growth performance improved with OLE supplementation. However, it was also evident that the standards expected for male broilers under controlled environment conditions were not reached. In some studies, dietary oleuropein supplementation improved the performance of broilers (El-Damrawy et al., 2013; Jabri et al., 2017), laying hens (Cayan and Erener, 2015; Ahmed et al., 2017), and quail (Bahsi et al., 2016). In contrast to these reports, oleuropein supplementation at increasing levels exerted no growth-promoting effect when incorporated into quail diets (Sarica and Toptas, 2014).

The linear effects observed for daily BWG, FI, and FCR indicate that the concentrations of OLE used in this experiment were still below the critical level for yielding appreciable additive effects on the performance of broiler chickens. Accordingly, at least 600 mg kg^{-1} OLE can be recommended in the diet. In addition, diets with OLE caused a measurable variation in the studied plasma variables and caecal microflora populations, as well as the performance and carcass and digestive tract traits of broilers. Moreover, the results with respect to daily BWG and FCR confirmed that the experiment was not performed under ideal conditions, because the growth rate of chicks fed the control diet was below the standard expected for male chicks under good management practices (53.7 vs. 68.5 g d^{-1}). Thus, these results indicate that broilers in the present study were kept in conditions that possibly led to the enhancement of the growth-promoting effect or efficacy, if any, of the level of OLE or oleuropein in the treatment. These effects can result in higher economic efficiency in broiler-meat production.

Higher BWG in broilers fed with OLE in the present study could be related to higher FI (Richards, 2003). Indeed, BWG and FI increased with OLE supplementation in the diet, leading to an improved FCR. This may be related to an improvement in the flavour and palatability of feed or the enhancement of the activities of digestive enzymes and nutrient absorption by phytogenic feed additives (Hernández et al., 2004; Cross et al., 2007; Zeng et al., 2015). An improvement in productive performance may also be associated with changes in caecal microflora (Toghyani et al., 2011; Zeng et al., 2015) and in studied blood metabolites (Jemai et al., 2008) when feeding OLE. Accordingly, our results with respect to production performance indicate that OLE has antimicrobial properties as herbal additive, and this product is a feasible alternative to feed additives used as growth promoters (Ocak et al., 2008; Amad et al., 2011). Unfortunately, in the present study, the birds were not fed a diet supplemented with an antibiotic. The results of previous studies and the present study indicate that the effects of plant extracts on BWG, FI, and FCR are inconsistent. This may be attributable to differences in the composition of the various phytogenic additives, the concentrations of the active substances and their biological activity, harvest time, extraction method, and level of extract used (Ryan et al., 2003; Ranalli et al., 2006; Ocak et al., 2008; Amad et al., 2011).

It was observed that the BWG of broilers resulted in different carcass weights and dressing percentages. However, the effect of OLE on the BWG of broiler chickens was not reflected in the dressing percentage, although low BW has been associated with low carcass yield (Fanatico et al., 2005; Choo et al., 2014). Carcass yield, expressed as dressing percentage, is affected by a number of factors such as genetics, feed, slaughtering conditions, and BW and sex of birds (Brickett et al., 2007; Choo et al., 2014). As such, as a result of the discrepancies in FI, the amount of metabolizable energy and protein ingested by the birds could explain the differences observed in dressing percentage (Richards, 2003; Brickett et al., 2007). Richards (2003) reported that the level of FI is a basic and important factor that determines the growth rate and body composition achieved by animals throughout their life cycles. Zubair and Leeson (1996)

stated that higher abdominal fat may be due to the fast growth rate until slaughtering age, because a fast growth rate is accompanied by increased body fat deposition. Consequently, it may be stated that diets supplemented with high levels of OLE have a disadvantage with regard to high body fat.

Some blood metabolites such as HDL, LDL, total cholesterol, and triglycerides are metabolites of hepatic lipid metabolism and are not simple markers of avian health. However, the alteration of blood parameters, as in the present study, is a frequently recorded variable in research, as it reflects changes in the physiological state, and is very important in evaluating and interpreting the results of investigations related directly to animal health (Toghyani et al., 2011; Ozturk et al., 2012).

The results with respect to blood parameters are in agreement with the results of previous studies (Coni et al., 2000; Andreadou et al., 2006; Sarica and Toptas, 2014). Indeed, it has been reported that the addition of oleuropein to the diet reduced the plasmatic levels of total (Coni et al., 2000; Andreadou et al., 2006), free, and ester cholesterols (Andreadou et al., 2006), as well as triglycerides (Coni et al., 2000; Andreadou et al., 2006) in rabbits. In addition, Sarica and Toptas (2014) noted that the diet supplemented with 200 mg kg^{-1} oleuropein decreased serum total and LDL cholesterol concentrations in quail. A reduction in circulating total cholesterol and LDL levels may be related to the hypocholesterolaemic effect of oleuropein, oleuropein aglycone, and hydroxytyrosol-rich extracts in rats (Fki et al., 2007; Jemai et al., 2008; Oi-Kano et al., 2008), rabbits (Coni et al., 2000; Andreadou et al., 2006), and quail (Sarica and Toptas, 2014). Our results and the results of previous studies indicate that OLE has the ability to lower total plasma cholesterol and LDL cholesterol levels. However, OLE as a feed additive had a detractive impact on the total cholesterol level in terms of avian health, although it had a beneficial effect on HDL content. In fact, the lowering of cholesterol in chickens is not an objective, because cholesterol is needed for proper fat digestion and cell membrane stability.

The antimicrobial effects of herbs and phytogenic products result from controlling and limiting the growth and colonization of numerous pathogenic and non-pathogenic species of bacteria in the gut of poultry (Toghyani et al., 2011). It is known that antibiotics as a growth promoter may positively change the growth and productivity of birds, increasing the uptake and absorption of nutrients. The improved FCR and enhanced growth, in spite of the higher FI of broilers fed the diet supplemented with 600 mg kg^{-1} oleuropein, may be attributed to the antimicrobial effects of OLE, as reported by Bedford (2000). Indeed, *Lactobacillus* spp., which are beneficial microorganisms, were not affected by the treatments, although all levels of OLE had a considerable antibacterial effect on *Clostridium* spp. and *S. aureus* populations compared with the control group. Based on the results of previous *in vitro* (Markin et al., 2003; Sudjana et al., 2009) and *in ovo* (Ahmed et al., 2014; Jabri et al., 2017) studies and the present study, OLE may be added to the set of non-antibiotic growth promoters.

In vivo studies on the antimicrobial action of OLE in poultry are rare, and at the same time, the results obtained from phytogenic feed additives are difficult to compare due to the use of different methods. Therefore, these observations support the hypothesis that phytogenic feed additives, including oleuropein, may favourably affect gut functions (Furneri et al., 2002; Markin et al., 2003; Jamroz et al., 2005; Pereira et al., 2006).

Conclusions

When feeding olive leaf extract, especially at the level of 600 mg kg^{-1}, the improvement in growth and feed efficiency is associated with changes in the caecal microflora. Therefore, the alcoholic liquid olive leaf extract can be used as an alternative to antibiotics to maintain growth performance and plasma variables such as total cholesterol, HDL and LDL cholesterols, and triglycerides associated with the feed additive supply of broilers under the present trial conditions. Although the mode of action of oleuropein contained in olive leaf extract seems to be related to more efficient nutrient digestion, growth promotion, cholesterol-lowering, and antimicrobial effects, the underlying mechanisms require more in-depth characterization. Therefore, present results emphasize that broiler chickens should be given olive leaf extract at maximum dose to produce satisfactory antibiotic effects.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: G. Erener, E. Ozturk and S. Cankaya. Formal analysis: S. Cankaya and A. Altop. Investigation: G. Erener, N. Ocak, E. Ozturk, S. Cankaya and A. Altop. Methodology: N. Ocak, R. Ozkanca and A. Altop. Project administration: G. Erener, N. Ocak and A. Altop. Software: S. Cankaya. Writing-original draft: G. Erener and N. Ocak. Writing-review & editing: N. Ocak.

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