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Effects of Olive Leaf (Oleuropein) Supplementation on Quality of Breast Meat in Broilers

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ABSTRACT

In this study, we investigated the effect of dietary olive leaf (oleuropein) supplementation at different levels on breast meat color and pH_{24} in along with TBA values of breast meats which were stored at +4 °C during the 11-day in broilers. For this purpose 320 one-day-old Ross-308 chicks randomly assigned to four groups (5 replicates per group, 16 chicks per replicates). In trial, dietary treatments consist of corn-soybean meal diet without or with 125, 250 and 500 mg kg^{-1} oleuropein supplementation respectively. At the end of trial, two chicks per replicate were slaughtered and meat samples were collected for lipid oxidation, color and pH_{24} measurement. According to the obtained findings, 250 mg kg^{-1} oleuropein supplementation on broiler diets significantly ($P<0.05$) decreased TBA values (mg MDA kg^{-1} meat) of breast meats compared with other groups. TBA values of breast meats significantly ($P<0.05$) increased during storage time. While breast meat brightness (L*) and yellowness (b*) values and pH_{24} were not significantly ($P>0.05$) affected by oleuropein supplementation at different levels, redness (a*) value significantly ($P<0.05$) increased compared with control group. As a result of the study, it is possible to say that oleuropein demonstrated antioxidant activity linked with supplementation level and it can be used at level of 250 mg kg^{-1} as phytobiotic antioxidant in broiler diets.

Keywords: Olive leaf; Oleuropein; Broiler; Antioxidant; Lipid oxidation

1. Introduction

Lipid oxidation and microbial growth are major problems during the storage of chicken meat. Therefore, the use of antimicrobial and antioxidants in broiler is quite common. Nowadays, the use of natural antioxidants instead of synthetic antioxidants (butyl hydroxyanisole, butyl hydroxy toluene, tertiary butyl hydroxyquinonone and propyl gallates) are on the agenda because of consumers' demand for safety food and natural feed additives (Botsoglou et al 2010a). In this demand, the use of vitamins such as E or C vitamins and some phytobiotics as natural antioxidants are emphasized. Phytobiotics can be defined as extracts or essential oils from various parts of plants. The use of some plants and spices such as carnations, rosemary, sage, green tea, thyme and olive leaf, which are antioxidant, in broiler chickens has been discussed in recent years. These products can be used either directly or processed extract form or essential oil form on broiler feed (Loetscher et al 2013).

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Olive and olive oil production are important and traditional agro-industrial activity in the Mediterranean countries since the ancient times and almost all of the world's olive production and consumption is carried out in Mediterranean countries (Basmacıoğlu-Malayoğlu & Aktaş 2011). It is reported that olive leaf, olive oil processing by products, contains more than 30 phenolic compounds which are generally classified as phenolic acids, phenolic alcohols, flavonoids, secoiridoid and lignans. One of these, oleuropein is the major bioactive compound and may reach concentrations of 90 g kg^{-1} in dried olive leaves (Benavente-Garcia et al 2000). Antimicrobial and antioxidant effects of this compound have been demonstrated with some studies (Bisignano et al 1999; Markin et al 2003; Lee & Lee 2010).

In this study, we aimed the investigate effects of oleuropein supplementation at different levels on meat quality and TBA values of breast meat during the storage time in broilers.

2. Material and Methods

2.1. Animals and experimental treatments

In this trial, 320 one-day-old Ross308 chicks were randomly assigned to 4 groups (5 replicates/group, 16 chicks/replicates). At the beginning of study chicks were weighed, wing banded and distributed into 20 floors. Each 1.2×1.1 m^2 floor pen was furnished with wood shavings litter, a round feeder and a round drinker. Water and feed were offered *ad libitum*. Temperature and relative humidity was maintained within the optimum range. Lighting was 23 h light and one hour darkness. The trial lasted for 6 weeks. The basal diet formulated in granule form for starter (1-10 days of age) and pellet forms for grower (11-24 days of age) and finisher (25-42 days of age) periods. The ingredients and composition of basal diets used at different ages is shown in Table 1. The experimental groups consisted of control group fed the basal diet (no oleuropein supplementation) and three groups fed the basal diet supplemented with oleuropein at levels of 125 (OLE125), 250 (OLE250) and 500 (OLE500) mg kg^{-1} of diet, respectively. For this purpose firstly oleuropein content of olive leaf was analyzed. Olive leaf's chemical composition, oleuropein and total phenol contents is shown in Table 2. Then olive leaf was mixed at different levels to soybean meal used in ration and then mixture was added to the compound feed to provided oleuropein levels. The oleuropein contents of diets supplemented with olive leaf were as follows: 125 mg kg^{-1}, 250 mg kg^{-1} and 500 mg kg^{-1} respectively. Dried olive leaf used in trial was obtained from a commercial company operating in the Edremit/Turkey.

2.2. Determination of breast meat quality

At the end of the trial, 40 broilers (10 chicks in each group) were randomly sampled for determination of breast meat quality. After slaughter carcasses were trimmed for breast meat by removing skin, bones and connective tissue. Following trimming, breast meat from each chick was separated into two sections. Right section was used for lipid oxidation, the other for color and pH_{24} measurements. Samples were placed on the plastic plates and covered with polyethylene film and stored in the refrigerator at +4 °C for lipid oxidation measurement. Thiobarbituric acid value (TBA) was determined on days 1, 5 and 11 as malondialdehyde (MDA) equivalence during 11 days storage. In order to determine the TBA value (mg MDA kg^{-1}), 5 g sample was blended for 2 minute in a homogenizer (AM-7, Nissei Co., Tokyo, Japan) with extracting solution (50 mL 2 M phosphoric acid) which containing 20 percent trichloroacetic acid in. The resulting slurry was diluted to 100 mL with distilled water and homogenized and filtered (Whatman No. 1 filter paper). 5 mL of filtrate was homogenized (5 mL of 2-thiobarbituric acid) and reserved in the dark for 15 h at room temperature. The resulting color was measured with spectrophotometer at 530 nm (Witte et al 1970). For color (L*- lightness, a* - redness and b* - yellowness) and pH at pH_{24} measurement breast meat samples were stored at 4 °C for 24 hours after slaughter. Objective measurement of color was performed at the surface of breast meat using a Minolta CR-300 colorimeter to measure CIE

Table 1- Ingredients and nutrients composition of the basal diets

	Starter (0-10 days)	*Grower (11-24 days)*	*Finisher (25-42 days)*
Ingredients (g kg^{-1})			
Corn	263.60	276.00	224.80
Soybean meal	351.90	305.80	185.00
Wheat	150.00	160.00	200.00
Full fat soybean	64.40	47.30	120.00
Wheat bran	60.00	80.00	140.00
Sunflower meal	20.00	30.00	50.00
Soybean oil	49.80	65.00	47.70
Dicalcium phosphate	15.50	12.50	10.40
Limestone	10.00	9.50	9.50
Common salt	3.50	2.90	2.90
DL-Methionine	3.40	2.80	3.10
L-Lysine	3.00	3.00	2.50
L-Threonine	0.70	0.50	0.50
Sodium bicarbonate	1.10	1.60	1.10
Premix[1,2,3,4]	1.00	1.00	1.00
Enzyme+Phytase	1.00	1.00	1.00
Anticoccidials	0.60	0.60	-
Choline chloride	0.50	0.50	0.50
Analyzed values (g kg^{-1})			
Dry matter	893.50	895.40	896.30
Crude protein	231.07	210.40	190.60
Ether Extract	71.50	92.10	8.40
Crude ash	62.55	50.30	50.40
Crude fiber	34.00	33.70	38.20
Calcium	10.20	9.10	8.40
Total phosphorus	7.37	6.80	7.20
Starch	348.42	372.50	393.70
Sugar	46.47	41.50	40.80
Metabolizable energy*, Kcal kg^{-1}	3025.00	3150.00	3200.00

[1], vitamin premix per kg of starter diet; 11000 IU vitamin A; 5000 IU vitamin D_3; 75 IU vitamin E; 3 mg vitamin K; 3 mg vitamin B_1; 8 mg vitamin B_2; 60 mg niacin; 15 mg Pantothenic acid; 0.15 mg biotin; 2 mg folic acid; 0.16 mg Vit B_{12} [2]vitamin premix per kg of grower diet: 9000 IU vitamin A; 5000 IU vitamin D_3; 50 IU vitamin E; 3 mg vitamin K; 2 mg vitamin B_1; 6 mg vitamin B_2; 60 mg niacin; 15 mg Pantothenic acid; 0.10 mg biotin; 1.75 mg folic acid; 0.16 mg Vit B_{12} [3]Vitamin premix per kg of finisher diet: 9000 IU vitamin A; 4000 IU vitamin D_3; 50 IU vitamin E; 2 mg vitamin K; 2 mg vitamin B_1; 5 mg vitamin B_2; 40 mg niacin; 15 mg Pantothenic acid; 0,10 mg biotin; 1.50 mg folic acid; 0.10 mg Vit B_{12} [4]Mineral premix per kg of diet: 100 mg Zn; 120 mg Mn; 40 mg Fe; 16 mg Cu; 1,25 mg I; 0,30 mg Se. *, Calculated value

Table 2- Chemical composition, oleuropein and total phenol contents of olive leaf

Analyzed values	*(g kg^{-1})*
Dry matter	952.60
Crude protein	91.90
Ether extract	72.80
Crude ash	52.40
Crude fiber	170.21
Oleuropein	25.10
Total phenol (mg GAE g^{-1})	46.47

L*, a*, b* values (Kim et al 2007). The instrument was calibrated with standard white measuring plate before measurements. The pH_{24} value of the meat samples at 3 different locations was determined using a pH meter (Hanna Instruments HI 8314) and measured using a direct electrode by thrusting the probe into the incised breast and probe was cleaned in each measurement.

2.3. Statistical analysis

All experimental data analyzed using general linear model procedure in SPSS 16.0 package program. In addition, the effect of treatment (oleuropein levels) the MDA contents of samples was determined by regression analysis by defining orthogonal polynomial contrast as linear and quadratic. Differences between the treatment groups were assessed according to the Duncan's Multiple Range test and differences were considered significant at P<0.05 (SPSS 2007).

3. Results and Discussion

At the completion of the study, performance parameters did not differ significantly (P>0.05) among groups (data presented in thesis), indicating that the incorporation of olive leaves to the diets had no adverse influence on the growth rate of broilers (Yavaş 2013).

3.1. Lipid oxidation on breast meat

The effect of dietary oleuropein supplementation and storage time on breast meat lipid oxidation was presented in Table 3 and lipid oxidation alteration depending on the storage time was presented in Figure 1. As shown in Table 3, extension of the storage time significantly increased the levels of TBA of breast meat (P<0.05). Moreover, results showed that dietary oleuropein supplementation at level of 250 mg kg^{-1} was significantly decreased lipid oxidation on breast meat compared with other groups (P<0.05).

Our results supported by the findings of studies on Japanese quails (Sarıca & Toptas 2014) mentioned that 150 and 200 mg kg^{-1} oleuropein supplementation on quail diets were effective in delaying lipid oxidation. Similarly, olive leaf supplementation studies on turkeys (Govaris et al

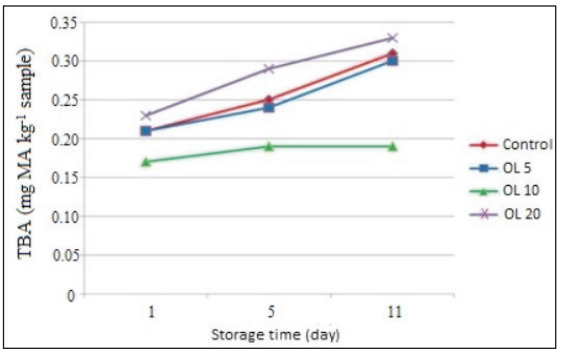

Figure 1- The alteration of TBA value on breast meat samples in experimental groups depends on storage time

Table 3- Effect of treatment and storage time on breast meat lipid oxidation (TBA, mg MDA kg^{-1} sample)

Sources of variation		*TBA (mg MDA kg^{-1} sample)*
Treatments	Control	0.26^{b}
	OLE125	0.25^{b}
	OLE250	0.19^{a}
	OLE500	0.28^{b}
	SEM	0.01
	P	<0.001
	Linear	0.830
	Quadratic	<0.001
Storage time, day	1.	0.21^{a}
	5.	0.24^{b}
	11.	0.28^{c}
	SEM	0.01
	P	<0.001
P values		
Treatment (T)		< 0.001
Storage Time (ST)		< 0.001
T x ST		0.364

$^{a-c}$, means within a column with different superscripts are significantly different (P<0.05); SEM, standard error of means

2010; Botsoglou et al 2010a; Botsoglou et al 2010b) observing that 10 g kg^{-1} olive leaf supplementation on turkey diets was decreased lipid oxidation on breast meat which was stored for 12 days at +4 °C compared to control and summarized that 10 g kg^{-1} olive leaf supplementation more effective to prevent lipid oxidation than 10 g kg^{-1} rosemary and 150 mg kg^{-1} α-tocopherol acetate (Govaris et al 2010) and 10 g kg^{-1} thyme (Botsoglou et al 2010b) supplementation respectively. Study on broilers (Marangoni et al 2017) also reported that 5 g kg^{-1} olive leaf supplementation has antioxidant effect on chicken meat during the frozen storage. But these studies not mentioned olive leaf's oleuropein levels. Other *in vivo* study on laying hens (Aktaş 2012) also reported antioxidant effect of olive leaf extract. In addition to *in vivo* studies, studies conducted on *in vitro* also reported antioxidant effect of olive leaf or its phenolic substances (Briante et al 2002; Lee et al 2009; Kiritsakis et al 2010). The antioxidant effect of oleuropein has been reported in studies that it inhibits the formation of free radicals by binding with metal ions such as iron and copper and under favour of this bond suppresses the activities of many inflammatory enzymes such as lipoxygenase (Andrikopoulos et al 2002; Visioli et al 2002; Botsoglou et al 2010a). Studies also have shown that olive leaf has 15 antioxidant substances besides oleuropein such as hydroxytriosol, luteolin-7-glucoside, verbascoside, tirosol, vanillic acid (Benavente-Garcia et al 2000; Silva et al 2006). Our result of antioxidant effect of oleuropein is consistent with the reported in the literature. However, OLE500 dietary group in our study did not decrease the TBA value in breast meat sample compared to the control group, on the contrary increased as a numerally, in other words its preventive effect on lipid oxidation was not detected. In a study conducted in *in vitro* conditions with turmeric extract (Saefudin et al 2014), reported that the use of turmeric extract in high levels causes prooxidant properties instead of antioxidant activity. It is known that polyphenol compounds can display both antioxidant and prooxidant effects (Decker 1997) depending on several factors (chelating potential, solubility, bioavailability and stability in tissues). In this respect 250 mg kg^{-1} oleuropein leaf supplementation to diet showed the expected antioxidant effect but highest level of supplementation showed prooxidant effect related to usage level.

3.2. Breast meat color and pH_{24}

The effect of dietary oleuropein supplementation on 24 hours stored at 4 °C breast meat color and pH_{24} were presented in Table 4. As shown in Table 4, while there was no difference in breast meat lightness, yellowness and pH_{24} among the treatments ($P>0.05$) dietary oleuropein supplementation at different level significantly increased redness value compared to the control group ($P<0.05$).

Table 4- Effect of treatment on breast meat color and pH_{24} value

Treatments	*L**	*a**	*b**	*pH_{24}*
Control	48.85	3.19[a]	5.16	6.15
OLE125	50.67	5.67[b]	5.47	6.11
OLE250	51.43	6.52[b]	5.62	6.10
OLE500	49.33	7.83[b]	5.66	6.19
SEM	0.874	0.774	0.619	0.054
P	0.156	0.001	0.937	0.600
Linear	0.575	0.000	0.550	0.601
Quadratic	0.031	0.459	0.829	0.232

L*, lightness; a*, redness; b*, yellowness; [a-b], means within a column with different superscripts are significantly different ($P<0.05$); SEM, standard error of means

Our results supported by the findings of study (Marangoni et al 2017) observing that olive leaf supplementation to broiler feed has no effect on L* and b* values of breast meat but were contrast to findings of a* value of breast meat and pH. In that study, while olive leaf supplementation has no effect on a* value, it decreased pH of the meat during long storage condition (60 days). Studies with different phytobiotics on broiler breast meat color and pH (Hong et al 2012; Kırkpınar et al 2014; Cho et al 2014; Li et al 2015) reported that increase or decrease in meat color might be related to type and rations of carotenoids found in phytobiotic structures, management, breeding type, carcass weight and pH. Olive leaf's carotenoid amount was reported by

Cayan & Erener (2015) as 2 mg β-carotene and 8 mg lutein 100 g^{-1} olive leaf in literature and olive leaf supplementation at any level on layer diet increased yolk yellowness in that study. In our study, amount of carotenoid in olive leaf was not determined and increases in a* value may be related to amount and type of carotenoid in olive leaf.

4. Conclusions

As a result, 250 mg kg^{-1} oleuropein supplementation to broiler diets, decreased lipid oxidation by decreasing TBA value of breast meat sample and it is possible to say that olive leaf demonstrated antioxidant activity linked with oleuropein supplementation level and it can be used at level of 250 mg kg^{-1} as phytobiotic antioxidant in broiler diets.

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