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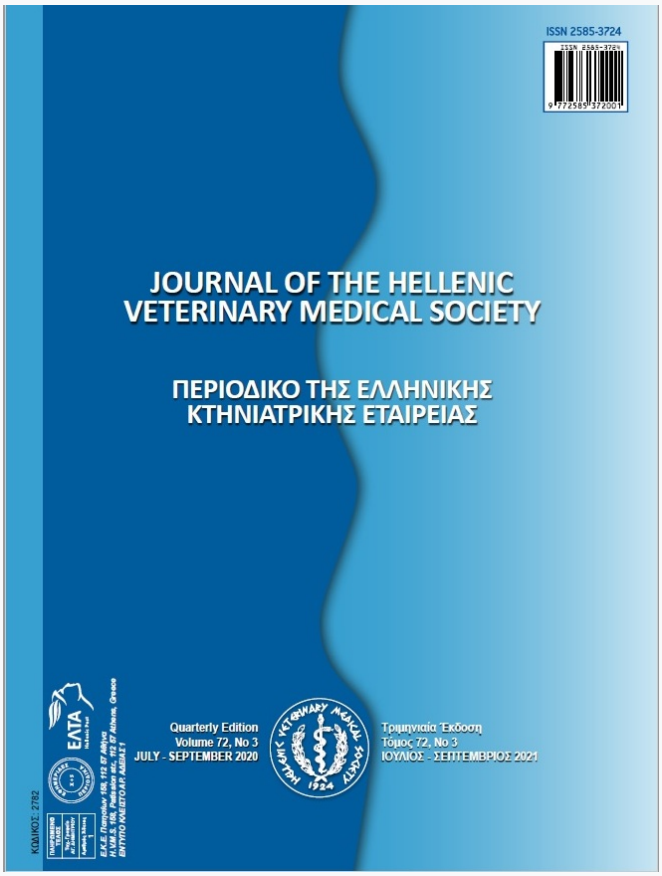

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Research article
Ερευνητικό άρθρο

Influence of dietary olive paste flour on the performance and oxidative stress in chickens raised in field conditions

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ABSTRACT: Olive oil contains a variety of antioxidants, including vitamin E. Its consumption protects against oxidative stress, which is involved in many pathological conditions, affecting animals' development and their general welfare. The present study aimed to investigate the effect of olive paste flour (OPF) on the antioxidant status and performance of broiler chickens raised in field conditions. Total of 18.000 broilers was randomly allocated in equal numbers in two poultry houses. The chickens were grouped according to their diet as follows: **Control group**: chickens fed commercial poultry feed, and **OPF group**: chickens fed control dietsin the starter period, but they got a supplement of OPF in grower, and finisher 1 and 2periods, respectively. The birds were raised under identical field conditions (ventilation, vaccination, lighting, etc.). Antioxidant status was assessed by measuring the concentration of vitamin E in plasma, as well as the total antioxidant capacity (TAC) in plasma and muscle tissue. According to the statistical analysis of the results, the addition of OPF to chickens' diet significantly enhanced plasma α-tocopherol concentration ($p \leq 0.05$). TAC did not show any significant differences in chicken plasma nor muscle tissue($p > 0.05$).Feed intake (FI) was higher in OPF group, while Body weight (BW) was lower. Liveability was similar for the two groups. The feed conversion ratio (FCR) was higher, and the European production efficiency factor (EPEF) was lower in broilers of the OPF group compared to those of the control group.Samples of roasted breast from chickens of both groups were used for organoleptic characteristics evaluation. Results showed that samples of the OPF group smelled more intensely and were more tasteful than those of the control group.However, other organoleptic characteristics did not differ. In conclusion, the results demonstrated that although the addition of OPF to chickens' diet can cause growth retardation, it can significantly increase the plasma α-tocopherol concentration. Further studies are needed to optimize the concentration of OPF in poultry feed in order to avoid growth retardation or even to promote growth in broiler chicks.

***Keywords*:** Olive paste flour, α-tocopherol, total antioxidant capacity, performance, field conditions

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INTRODUCTION

Olive oil is obtained from olive tree fruit (*Olea europaea* L.) and is an essential constituent of Mediterranean diet, which is widely accepted as a healthy diet. Many of the beneficial effects of olive oil on human and animal health are attributed to the presence of several bioactive compounds such as mono/polyunsaturated fatty acids, phenols, phytosterols, carotenoids and tocopherols (Cárdeno et al., 2013; Scoditti et al., 2014). Olive oil production generates a significant amount of wastes, whose disposal causes severe environmental problems (Rinaldi et al., 2003; Venieri et al., 2010). The exploitation of such wastes could be an ideal solution in avoiding environmental pollution and toxicity and meets the targets of a circular economy to eliminate waste and the continual use of resources.

Toward this direction, some wastes of olive oil production (olive paste and olive oil mill wastewater, OMWW) could be incorporated into animal feed due to their significant antioxidant content (Anniva and Tsimidou, 2009; Cardinali et al., 2010; Frankel et al., 2013; Gerasopoulos et al., 2015; Padalino et al., 2018; De Bruno et al., 2021).To be more specific, olive paste contains vitamin E (Anniva and Tsimidou, 2009; Padalino et al., 2018; De Bruno et al., 2021), among other essential antioxidants such as polyphenols.

Vitamin Eis the most active antioxidant ingredient of the olive oil, in terms of antioxidant and anti-stroke activity and can protect against lipid peroxidative damages (De Luca, 1978; Gimeno et al., 2002). It is known that the term vitamin E denotes two groups of related compounds, having similar biological activity: α, β, γ and δ-tocopherols and α, β, γ, and δ-tocotrienols. The greatest physiological interest is represented by tocopherols (Hansen and Warwick, 1966), especially α-tocopherol, one of the most often encountered in nature. It is the only one present in animal bodies.

The incorporation of olive-based byproducts into animals' diets has shown contradictory results concerning the animals'performance. For instance, the supplementation of broiler diet with5% of olive cake (OC) showed the best performance compared to the broilers fed with 10% OC, and the control. Particularly, the body weight gain (BWG), FI,and FCR were higher for the broilers fed with 5% OC, except for EPEF which was higher in broilers fed with 10% OC (Al-Harthi, 2016). Pappas et al. (2019) reported that broilers fed with olive pulp up to 5% performed well and maintained good health, final body weight, and carcass yield without impacting to feed:gain ratio. Remarkably, broilers fed 8% of olive pulp showed meaningfully poorer FCR and numerically higher feed intake (FI). Also, the addition of 2.5% or 5% of olive pulp in the starter period and5% in the finisher periodpresented the best performance results. Furthermore, Shafeyet al. (2013) showed that the replacement of15g or 30 g of wheat bran with olive leavesdid not significantly influence the growth performance and carcass characteristics of the chickens fed the olive leaves. However,the replacement of 50 g/kg of wheat bran with olive leaves reduced theB-WG but increased FI and significantly increased FCR.

The effect of olive byproducts on animals' redox status is also debatable.Gerasopoulos et al. (2015) proposed that incorporation of OMWW retentate into broiler diet may improve the redox status of chickens and reduce the oxidative stress by scavenging free radicals, as well as improve meat nutritional value. This study assessed the antioxidant effects of supplementation with OMWW by measuring oxidative stress biomarkers (protein carbonyl levels, TBARS; TAC; GSH;catalase, CAT) in broiler blood and different tissues (i.e. muscle, heart and liver). This research concluded that OMWW is a supplement that can enhance the polyphenolic content of animal feed and thus protect animals against various pathologies.

By contrast, Pappas et al. (2019) reported that the addition of 5% olive pulp into chicken diet did not significantly affect the total antioxidant activity measured in blood by several biochemical markers (GPx, GST, SOD, GR, CAT, ABTS, FRAP).Furthermore, it was reported that differences in antioxidant activity between studies could be attributed to the experimental conditions, but also to the different content of polyphenols between the examined olive-based products. The latter is further justified by the fact that indeed there are differences in phenolic content during olive processing, oil extraction and storage (Frankel et al., 2013). Finally, Pappas et al. (2019) stated that their study was applied in small-scale production and suggested that commercial conditions in large-scale production may affect the analyzed biochemical markers.

In this work, the diet of commercial broiler chickens was supplemented on top with an olive paste-based product, rich in antioxidant substances, coming from wastes of olive oil production. Particularly, we aimed to estimate the influence of olive paste flour on the redox status and performance of broiler chick-

ens raised in field conditions in large-scale production systems. The chosen dosage of 2.5% and 5% OPF in grower, and finisher 1 and 2 periods was based on previous studies (Shafeyet al., 2013; Al-Harthi 2016; Pappas et al., 2019),which showed that olive byproducts up to 5% affected positively the performance in broilers raised in small-scale production.The total antioxidant capacity was studied in chicken plasma and muscle extract, while α-tocopherol levels were determined in broiler plasma.Organoleptic attributes were evaluated by tasters. BW and liveability were recorded daily, FI was calculated at the end of each period, whereas FCR and EPEF were calculated at the end of the experiment for estimating growth performance.

MATERIALS AND METHODS

All the procedures were performed in accordance with PINDOS welfare guidelines.

Materials

Olive paste flour (Sparta INNOLIVE) used on top of the commercial chicken diet was purchased from Sparta Life S.A (Greece). Sodium Phosphate used in muscle tissue homogenisation was purchased from Fluka (Switzerland). Hexane, Ethanol and Methanol used in evaluation of α-tocopherol and TAC respectively, were obtained from Fisher Scientific (USA). α-tocopherol, used to prepare the Standard solution of vitamin E, as well as DPPH used in TAC evaluation, were purchased from Sigma Aldrich (Germany).

Animals and their diet

Eighteen thousand broiler chicks were randomly allocated equally in two poultry houses, as described below: house 1that hosted the control group, which served as the negative control, and house 2that hosted the OPF group, whose diet was supplemented with 5% olive paste flour. The birds were as hatched and originated from the same parent stock and hatchery. They were raised under the same field conditions (equipment, ventilation, vaccination program, lighting program, etc.). Two production cycles were performed for each treatment. Chickens were offered from Agricultural Poultry Cooperative of Ioannina "PINDOS" (Greece).

To meet the nutrient requirements of the broiler chicks during the experimental period, four complete basal diets (Table 1) were formulated each one for the starting (0-10d), growing (11-24d), finishing 1 (25-39d) and finishing 2 (40-slaughter) periods, respectively. Feed formulation and chemical analysis of rations are presented in Table 2. The addition of 2.5 and 5% OPF was done on top in grower and finisher rations. Chemical analysis of OPF is illustrated in Table 3. No antibiotic growth promoters, organic acids and phytobiotics were used. Feed and drinking water were offered to all birds ad libitum throughout the experiment.

Table 1. Diet formulation of the tested groups

Ingredient (kg/ton)	Starter 0-10 days		Grower 11-24 days		Finisher 1 25-39 days		Finisher 2 40 day-slaughter	
Corn	200 [a]	200 [b]	200 [a]	200 [b]	0 [a]	0 [b]	0 [a]	0 [b]
Wheat	385 [a]	385 [b]	391 [a]	401 [b]	660 [a]	700 [b]	689 [a]	719 [b]
Soya-meal	340 [a]	340 [b]	312 [a]	327 [b]	220 [a]	230 [b]	195 [a]	215 [b]
Sparta INNOLIVE	0 [a]	- [b]	25 [a]	- [b]	50 [a]	- [b]	50 [a]	- [b]
Palm oil	5 [a]	5 [b]	12 [a]	12 [b]	22	22 [b]	25 [a]	25 [b]
Soya oil	30 [a]	30 [b]	27 [a]	27 [b]	17 [a]	17 [b]	13 [a]	13 [b]
Limestone	14 [a]	14 [b]	13 [a]	13 [b]	12 [a]	12 [b]	12 [a]	12 [b]
Premix	26 [a]	26 [b]	20 [a]	20 [b]	19 [a]	19 [b]	16 [a]	16 [b]

[a] OPF group; [b] conventional group.

Table 2. Dietary ingredients and chemical composition[1] of broiler feed

		Starter		Grower		Finisher 1		Finisher 2	
Age	days	0-10		11-24		25-39		40-slaugther	
Metabolizable Energy	kcal/kg	3000		3100		3200		3200	
AMINO ACIDS		**Total**	**Digest**	**Total**	**Digest**	**Total**	**Digest**	**Total**	**Digest**
Lysine	%	1.44	1.28	1.29	1.15	1.15	1.02	1.08	0.96
Methionine+Cystine	%	1.08	0.95	0.99	0.87	0.90	0.80	0.85	0.75
Methionine	%	0.56	0.51	0.51	0.47	0.47	0.43	0.44	0.40
Threonine	%	0.97	0.86	0.88	0.77	0.78	0.68	0.73	0.64
Valine	%	1.10	0.96	1.00	0.87	0.89	0.78	0.84	0.73
Isoleucine	%	0.97	0.86	0.89	0.78	0.80	0.70	0.75	0.66
Arginine	%	1.52	1.37	1.37	1.37	1.21	1.09	1.14	1.03
Tryptophan	%	0.23	0.20	0.21	0.18	0.18	0.16	0.17	0.15
Leucine	%	1.58	1.41	1.42	1.27	1.26	1.12	1.19	1.06
Crude Protein	%	23.0 [a] 23.0 [b]		21.5 [a] 21.5 [b]		19.5 [a] 19.1 [b]		18.3 18.2	
Crude Fat	%	5.1 [a] 5.1 [b]		6.5 [a] 5.8 [b]		6.4 [a] 5.6 [b]		6.4 [a] 5.2 [b]	
Crude Fiber	%	2.4 [a] 2.4 [b]		3.2 [a] 2.4 [b]		3.5 [a] 2.3 [b]		3.4 [a] 2.2 [b]	
Moisture	%	11.5 [a] 11.5 [b]		11.5 [a] 11.5 [b]		11.3 [a] 11.3 [b]		12.0 [a] 11.5 [b]	
Ash	%	5.5 [a] 5.5 [b]		5.1 [a] 5.0 [b]		4.9 [a] 4.6 [b]		4.8 [a] 4.4 [b]	
MINIMUM PECIFICATION									
Choline per kg	mg	1700		1600		1500		1450	
Linoleic Acid	%	1.25		1.20		1.00		1.00	

[1]Ingredients and chemical composition was noted according to feed formulation and NIR tests for verification; a: OPF group; b: conventional group; MINERALS % (Minerals are those used on diet): Calcium: starter: 0.96; grower: 0.87; finisher 1: 0.78; finisher 2: 0.75; Available Phosphorus: starter: 0.480; grower: 0.435; finisher 1: 0.390; finisher 2: 0.375; Magnesium: starter; grower; finisher 1; finisher 2: 0.05-0.50; Sodium: starter; grower: 0.16-0.23; finisher 1; finisher 2: 0.16-0.20; Chloride: starter; grower; finisher 1; finisher 2: 0.16-0.23; Potassium: starter: 0.40-1.00; ADDED TRACE MINERALS PER KG (mg): Copper: starter; grower; finisher 1; finisher 2: 16; Iodine: starter; grower; finisher 1; finisher 2: 1.25; Iron: starter; grower; finisher 1; finisher 2: 20; Manganese: starter; grower; finisher 1; finisher 2: 120; Selenium: starter; grower; finisher 1; finisher 2: 0.30; Zinc: starter; grower; finisher 1; finisher 2: 110;ADDED VITAMINS PER KG (IU): Vitamin A: starter: Wheat based feed: 13,000; Maize based feed: 12,000; grower: Wheat based feed: 11,000; Maize based feed: 10,000; finisher 1: Wheat based feed: 10,000; Maize based feed: 9000; finisher 2: Maize based feed: 10,000; Maize based feed: 9000; Vitamin D3: starter: Wheat based feed; Maize based feed: 5000; grower: Wheat based feed; Maize based feed: 4500; finisher 1: Wheat based feed; Maize based feed: 4000; finisher 2: Maize based feed; Maize based feed: 4000; Vitamin E: starter: Wheat based feed; Maize based feed: 80; grower: Wheat based feed; Maize based feed: 65; finisher 1: Wheat based feed; Maize based feed: 55; finisher 2: Maize based feed; Maize based feed: 55; Vitamin K (Menadione) (mg): starter: Wheat based feed; Maize based feed: 3.2; grower: Wheat based feed; Maize based feed: 3.0; finisher 1: Wheat based feed; Maize based feed: 2.2; finisher 2: Maize based feed; Maize based feed: 2.2; Thiamin (B1) (mg): starter: Wheat based feed; Maize based feed: 3.2; grower: Wheat based feed; Maize based feed: 2.5; finisher 1: Wheat based feed; Maize based feed: 2.2; finisher 2: Maize based feed; Maize based feed: 2.2; Riboflavin (B2) (mg): starter: Wheat based feed; Maize based feed: 8.6; grower: Wheat based feed; Maize based feed: 6.5; finisher 1: Wheat based feed; Maize based feed: 5.4; finisher 2: Maize based feed; Maize based feed: 5.4; Niacin (mg): starter: Wheat based feed: 60; Maize based feed: 65; grower: Wheat based feed: 55; Maize based feed: 60; finisher 1: Wheat based feed: 40; Maize based feed: 45; finisher 2: Maize based feed: 40; Maize based feed: 45; Pantotheninic Acid (mg): starter: Wheat based feed: 17; Maize based feed: 20; grower: Wheat based feed: 15; Maize based feed: 18; finisher 1: Wheat based feed: 13; Maize based feed: 15; finisher 2: Maize based feed: 13; Maize based feed: 15; Pyridoxine (B6) (mg): starter: Wheat based feed: 5.4; Maize based feed: 4.3; grower: Wheat based feed: 4.3; Maize based feed: 3.2; finisher 1: Wheat based feed: 3.2; Maize based feed: 2.2; finisher 2: Maize based feed: 3.2; Maize based feed: 2.2; Biotin (mg): starter: Wheat based feed: 0.30; Maize based feed: 0.22; grower: Wheat based feed: 0.25; Maize based feed: 0.18; finisher 1: Wheat based feed: 0.20; Maize based feed: 0.15; finisher 2: Maize based feed: 0.20; Maize based feed: 0.15; Folic Acid (mg): starter: Wheat based feed; Maize based feed: 2.20; grower: Wheat based feed; Maize based feed: 1.90; finisher 1: Wheat based feed; Maize based feed: 1.60; finisher 2: Maize based feed; Maize based feed: 1.60; Vitamin B12 (mg): starter: Wheat based feed; Maize based feed: 0.017; grower: Wheat based feed; Maize based feed: 0.017; finisher 1: Wheat based feed; Maize based feed: 0.011; finisher 2: Maize based feed; Maize based feed: 0.011.

Table 3. Chemical analysis of the complementary OPF

Chemical Analysis	%
Proteins	9.5
Total Fat	16.0
Total Carbohydrates	62.9
Sugars	0.7
Total Ash	6.4
Total Fibers	22.5
Salt	0.46
Moisture	5.23
Lysine	0.105
Threonine	0.468

Performance

The body weight (BW) was measured at the end of the experiment on 45th day of age, while the feed conversion ratio (FCR) was calculated cumulatively for the total production period as feed intake divided by body weight gain. Mortality was recorded daily, while European Production Efficiency Factor (EPEF) was calculated at the end of the experiment, according to the type: (BW (kg) x Liveability (%) x 100)/ (FCR x slaughter age (d)). Feed intake was calculated per growing phase.

Antioxidant status

Blood and muscle tissue samples were collected in the slaughterhouse at the 45th day and stored at -80 °C, until needed for processing. In particular, blood samples from 20 birds per group were collected by jugular vein-puncture in ethylene diamine tetra-acetic acid (EDTA) tubes, centrifuged in 1.500 rpm for 10 minutes in 4 °C, while plasma was collected in eppendorf tubes. Homogenized muscle tissue samples were received after treating them with buffer, containing 1 M NaCl, 2 mM EDTA, 50 mM Tris-HCl (pH 7.3), 0,5 v/v Triton X-100, sonicated in ice and centrifuged in 20.000 x g for 30 min at 4 °C. The supernatant was collected for further processing, while the pellet was discarded.

Determination of Total Antioxidant Capacity

The estimation of total antioxidant capacity was based on the measurement of DPPH in 520 nm. The DPPH reduction assay was performed by adding 20 μl of blood plasma or 40 μl homogenate muscle extract to 480 or 460 μl of 10 mM sodium phosphate buffer (pH 7.4) respectively. Then the total volume of the samples (500 μl) was added to 500 μl of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanol solution. Then the samples were incubated for 30 minutes at 21 °C. Absorbance was measured at 520 nm according to standard protocol (Janaszewska and Bartosz, 2002). Total Antioxidant Capacity (TAC) represents mmol of DPPH reduced to 2,2-diphenyl-1-picrylhydrazine (DPPH:H) by the existing antioxidants in plasma or muscle tissue. Evaluation of antioxidant capacity of the samples, either plasma or muscle extract, was achieved with calculation of % Radical Scavenging Activity which is derived from the type: $[(Absorbance_{Control}-Absorbance_{Sample})/Absorbance_{Control})] *100$.

Determination of α-tocopherol

The evaluation of α-tocopherol levels was based on the measurement of the fluorescence of the extracted α-tocopherol, into hexane, at specific excitation and emission wavelengths. Initially, a Standard and a Working solution of vitamin E were made: Standard solution of vitamin E, 2000 μg/ml: 500 mg of α-tocopherol were dissolved in 100 ml of filtered ethanol and then to 250 ml. Working solution of vitamin E, 20 μg/ml: 1 ml of vitamin E standard solution was dissolved in 100 ml of filtered ethanol.

Then a variety of solutions were prepared in 15 ml centrifuge tubes (with stopper): 1. Blank: in 1.2 ml water, 2 ml of filtered ethanol was added under stirring. 2. Pattern: in 0.2 ml Working solution, 1.2 ml of water was added and mixed for 30 sec. Then 1.8ml of filtered ethanol was added under stirring. 3. Unknown: in 0.2ml plasma of each sample, 1ml of water was added and mixed for 30 sec. Then 2 ml of filtered ethanol were added under stirring.

Then all tubes were mixed for 30 sec, 5 ml hexane was added, and they were shaken well for 5 min by hand and Vortex. Next the tubes were centrifuged for 5min at 2000 rpm. The hexane layer of each tube was transferred to fluorescence cuvettes. The absorbance was measured at 330 nm. The results were processed by the equation: μg of free vitamin E/ml = $[(F_x-F_b)/(Fs-Fb)] *20$. Where F_x = absorption of unknown, F_s = standard absorbance, F_b = blank absorption (Hansen and Warwick, 1966).

Evaluation of organoleptic attributes

At the end of the experiment (45th day), 30 birds per group were slaughtered at PINDOS slaughterhouse for evaluating the organoleptic characteristics. Chicken breasts of each group were roasted at 200 °C for 30 min and served to different people to investigate the possible organoleptic differences. The sam-

ples were presented one pair at a time and in random order to each taster. Tasters were asked to judge about "more", "less", or "no difference" for taste, odour, colour, consistency, and texture of the meat. None of the animals was destined to be marketed/consumed.

Statistical Analysis

Assays were performed in a series of samples equally for both groups in duplicate. Results are shown as mean, while standard deviation (SD), standard error of the mean (SEM) and statistical significance (*p* value) were determined according to normality and calculated with independent t test, using the software IBM® SPSS® Statistics 26.

RESULTS

Performance

The BWat slaughter age was significantly lower (p≤0.05) in the OPF groupcompared to the control group (Table 4). FI, liveability,FCR, and EPEFwere calculated on a farm level and statistical analysis and evaluation was performed. However, the high number of birds per farm as well as the application of identical farm management practices and micro-environmental conditions could allow to draw out some conclusions. The FI and FCRwere higher and the EPEF was lower in the OPF group compared to the control group, while liveability was similar between experimental groups (Tables 2,4).

Antioxidant status

As it is shown in Table 5 (Figure 1), α-tocopherol could pass from the diet in chicken plasma. The amount of α-tocopherolin plasma of broilers fed with OPF was significantly higher (p≤0.05) than α-tocopherol content of plasma of broilers fed with the control diet (control group).TAC of chicken plasma and muscle tissue was not significantly different as it is conducted from % RSA (p>0.05) (Table5, Figs. 2, 3).

Table 4. Performance of broilers during the experimental period

	Control group	**OPF group**
Final BW (kg)	2.73	2.50
SD	0.29	0.13
SEM	0.09	0.04
P value	≤0.05	
Liveability	94.88	95.70
SD	2.12	1.77
SEM	0.67	0.56
FCR	1.73	1.83
SD	0.06	0.03
SEM	0.02	0.01
EPEF	332.90	285.50
SD	22.59	20.18
SEM	7.14	7.38
Feed Intake kg/broiler		
Starter	0.40 [b]	0.41 [a]
Grower	1.14 [b]	1.21 [a]
Finisher 1	1.56 [b]	1.73 [a]
Finisher 2	0.88 [b]	1.02 [a]

BW: Body Weight; FCR: Feed Conversion Ratio; EPEF: European Production Efficiency Factor; SD: Standard Deviation; SEM: Standard Error of Means.

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Table 5. Effect of OPF on a-tocopherol in broiler plasma and ontotal antioxidant capacity in broiler plasma and muscle tissue

	α-tocopherol				total antioxidant capacity							
	plasma				plasma				muscle tissue			
	Vitamin E (μg/ml)	SD	SEM	P value	% RSA	SD	SEM	P value	% RSA	SD	SEM	P value
Control	9.0	4	0.8	≤0.05	70.6	4.8	2	>0.05	81.6	1.67	0.53	>0.05
OPF	13.2	6.7	1.5		64	11.9	3		81.8	2.05	0.65	

RSA: Radical Scavenging Activity; SD: Standard Deviation; SEM: Standard Error of Means.

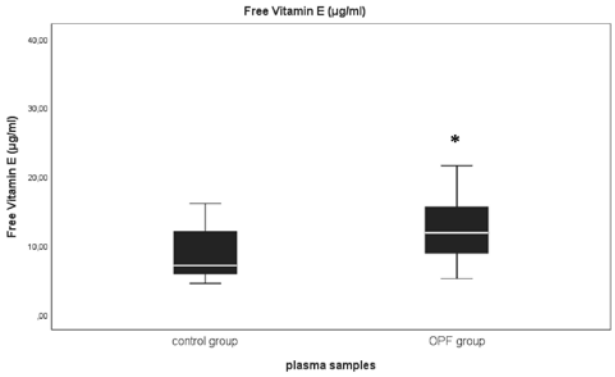

Figure 1. Free Vitamin E (µg/ml) in chicken plasma fed with OPF compared to control. *Significantly different from the values of the control group at the same sampling time (p≤0.05).

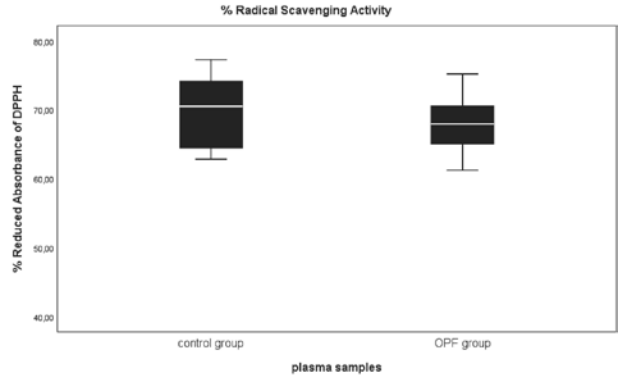

Figure 2. Total antioxidant capacity of broiler plasma against DPPH. (p>0.05).

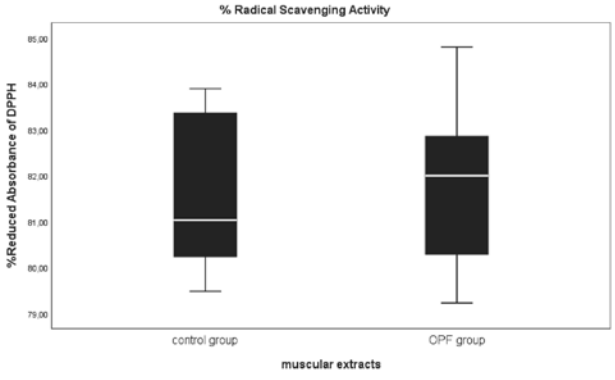

Figure 3. Total antioxidant capacity of broiler muscle tissue against DPPH. (p>0.05).

Evaluation of organoleptic characteristics

In the context of organoleptic control, chicken meat from both groups was roasted and tested by 10 people to determine organoleptic differences. Concerning the taste sensory, the two different groups of chickens presented differences. Broilers fed with OPF were tastier and had increased smell attributes than the conventional ones. However, no other organoleptic characteristic like meat appearance, namely colour, consistency and texture were differentiated in the two different groups.

DISCUSSION

Vitamin E is the most important lipophilic antioxidant in mammals. It can provide protection against lipid peroxidation (Pompeu et al., 2018) and thus improve meat quality (Nam and Ahn, 2003; Arshad et al., 2013). Also, vitamin E can increase the antioxidant status thus results in protection from pathological problems or mitigation of their symptoms (Deaton et al., 2004; Lykkesfeldt and Svendsen, 2007; Abuelo et al., 2015).Natural antioxidants are safer than the synthetic ones, and they have been used widely in broiler feed for reducing lipid oxidation (Arshad et al., 2013; Starčević et al., 2015).Olive paste and OMWW are sources of natural antioxidants as it has been shown previously (Anniva and Tsimidou, 2009; Cardinali et al., 2010; Frankel et al., 2013; Gerasopoulos et al., 2015; Padalino et al., 2018; De Bruno et al., 2021). Furthermore, olive paste contains vitamin E (Anniva and Tsimidou, 2009; Padalino et al., 2018; De Bruno et al., 2021), which is one of the dominant antioxidants presented in olive oil (Gimeno et al., 2002). So, it is conducted that products stemming from olive oil production like olive paste could be an excellent supplement for animal feed.

The findings of the current study propose that the incorporation of olive paste into the feed increases α-tocopherol levels in poultry plasma.The results suggest that α-tocopherol may be transferred from olive to the processed feed (olive paste flour) and finally to broiler plasma.Results of the present work are in accordance with a study conducted by Paiva-Martins et al., (2009) who found that incorporation of 5% or 10% of olive leaves into pig diet increases α-tocopherol content of the meat. Furthermore, similarly to our results, various studies (Guo et al., 2001; Surai and Kochish, 2019) reported that supplement of vitamin E to broiler diets increases α-tocopherol concentration either in chicken plasma or in tissues.

Total antioxidant capacity did not exhibit significant differences between the two groups in broiler plasma (p>0.05). These findings agree with a study (Pappas et al., 2019)conducted under conditions of small-scale production which showed that total antioxidant activity did not alter in animal blood after the addition of olive pulp in broiler diet in various proportions. Vossen et al. (2011) also reported that the plas-

ma redox status and lipid oxidation in broilers have not changed by incorporating natural antioxidants into broiler diet. Contrary to our results, it has been suggested that olive oil mill wastewater (OMWW) retentate with 1% olive oil might improve the redox status of chickens (Gerasopoulos et al., 2015). This could indicate that the supplemented diet used in the above study maybe contain more antioxidant compounds and vitamin E than the OPF used in this work.

Moreover, our data did not show significant differences in TAC of muscle tissue extracts. An explanation could be that the role of vitamin E on the tissue depends on the age of the birds (Jankowski et al., 2016). To investigate the specific issue, studies at earlier ages of birds would have to be conducted.

The effect of the supplemental vitamin E in TAC values varies among studiesmaybe due to the variance of amounts and duration of the supplemental olive-based productor vitamin E, as well as other poultry management factors such as food composition, scale production, living conditions, polyphenolic content, etc.(Pappas et al., 2019; Surai and Kochish, 2019). The most important issue which affects TAC, may bethe differences in phenolic content between the examined olive-based products due to olive processing, oil extraction, and storage (Frankel et al., 2013). The addition of vitamin E (>100 mg/kg) from the beginning growing period until slaughter, or just for a greater period than this study, might improve poultry total antioxidant defense (Surai and Kochish, 2019). In the present work, there was a gradual decrease in the standard commercial supplementation of vitamin E from the starter to the finisher. This might indicate that even though plasma α-tocopherol concentration was elevated by the addition of OPF, still it was not such efficient in increasing TAC.

In addition, the quality evaluation of chicken meat of both groups showed that OPF enhanced ones had higher taste and smell attributes. Maybe this is due to increase in fatty acids, which is known to affect nutritional value of poultry meat (Wood et al., 2004). Furthermore, both groups presented the same meat colour as well as consistency and texture. Maybe the similarity of appearance characteristics is attributed to the production system used in rearing of the chicks (Arreza, 2019) at the poultry farm, but also could be attributed to theirgenotype, processing procedure of the meats, slaughter conditions, and the overall health and nutrition of the poultry animals (Mir et al., 2017).

The results of the study illustrate that introduction of olivepaste flour, into broiler diet, increases the FCR, which could be attributed to the higher FI as well as to the relative final body weight of the OPF group (Masouri et al., 2015; Al-Harthi, 2016; Yi et al., 2018; Pappas et al., 2019). Moreover, EPEF value was decreased in OPF group compared to Control group. This difference could be due to the higher FCR or the significantly lower BW of the OPF group. Decreased EPEF, however, could not be ascribed to the liveability as this parameter was not significantly different in two groups. Higher FI, FCR and lower EPEF values in this studyare in agreement with other reports (Masouri et al., 2015; Pappas et al., 2019). The inclusion of 5% of olive byproducts into broilers' diet has been tested several times and it was reported as one of the best portions to be added into broilers' feed regarding the performance (Shafey et al., 2013; Al-Harthi, 2016; Pappas et al., 2019). Similarly to our results, Pappas et al. (2019) and Shafey et al., (2013) reported higher FI, FCR, and lower BW in broilers fed with 5% of the corresponding byproduct. Also, Al-Harthi (2016) stated that broilers fed with 5% of OC as olive byproduct presented increased FI and FCR, however,in contrast to our results, the EPEF was lower and BW was higher. The differences in FCR and EPEF,that olive byproducts have displayed, among studies, may be due to the variations on α-tocopherol concentration in broiler feed, but also due to the different chicken raising conditions (Rama Rao et al., 2011; Masouri et al., 2015).Also, the differences in performance could be ascribed to the different content of the crude fiber among different olive byproducts, given that certain fiber content may be related to better digestibility and other performance parameters (Al-Harthi, 2016; Pappas et al., 2019).Hence, the high content of the crude fiberin the OPF supplement that was used in the present study, could justify theincreased FI and consequently the higherFCR in the OPF group.

CONCLUSION

In conclusion, the findings of the present study show a significant increase in α-tocopherol in broiler plasma fed with OPF compared to the control group. This indicates that α-tocopherol could pass from olive and olive paste to broiler plasma through diet. TAC did not exhibit significant differences between the two groupsneither inplasma norin muscle tissue. Furthermore, the organoleptic characteristics presented a difference in taste and smell attributes, but color, consistency and texture remained the same for both

groups. In addition, the OPF group showed higher FI and FCR,but decreased BW and EPEF compared to the control group. Finally, given that this study took place in a poultry farm unit under field conditions, these findings are considerable because of their application in large-scale poultry production. Further studies are needed to optimize the concentration of olive paste flour in poultry feed in order to avoid growth retardation or even to promote growth in broiler chicks.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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