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Effects of dietary α-tocopherol acetate and pomegranate peel extract on growth performance and antioxidative potential and lipid oxidation of meat of broiler

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ABSTRACT: The present study compared the effects of the dietary supplementation of α-tocopherol acetate (TA) and pomegranate peel extract (PPE) alone or in combination in order to prevent oxidative stress created by the enrichment of the diet by 4 % flaxseed oil interms of the growth performance, and fatty acid composition, total phenolic contents, antioxidative profile and lipid oxidation of meat of broilers. A total of 600 day old Ross 308 male broiler chicks were randomly distributed into five treatments with 5 replicates of 24 chicks each.The diets included: CONT: a control diet containing 50 mg/kg TA and 4 % flaxseed oil; TA200: the diet supplemented with 200 mg/kg TA to the CONT diet; PPE100, PPE200 and TA100+PPE100: the diets supplemented with 100 and 200 mg/kg PPE and 100 mg/kg TA+100 mg/kg PPE to the CONT diet.The dietary treatments had no significant effect on growth performance. The TA200, PPE100, PPE200 and TA100+PPE100 diets significantly increased the contents of total polyunsaturated fatty acids (PUFAs), omega-6 fatty acids (FAs)and omega-3 FAs, total phenolic contents (TPCs), the 2,2-dipheny l-1-picry l hydrazy l radical scavenging activity and ferric reducing antioxidant power, and significantly decreased the concentrations of total saturated FAs, total monounsaturated FAs, the omega-6/omega-3 ratio and the malondialdehyde (MDA) values of the thigh meat and breast meat compared to the CONT diet. In conclusion, the PPE100, PPE200 and TA100+PPE100 diets were as effective as TA200 diet alone in terms of enriching broiler thigh meat and breast meat with the omega-3 PUFAs. The dietary combination of TA and PPE was a more effective antioxidant in terms of the lowest omega-6/omega-3 ratio of broiler breast meat, and the highest TPCs and the lowest MDA values of broiler thigh meat and breast meat enriched with omega-3 PUFAs compared to TA200 alone.

Keywords: Pomegranate peel extract, meat, antioxidative potential, lipid oxidation,broiler

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INTRODUCTION

Consumer preferences, nutrient profile, availability and low production cost make poultry meat a major source of animal food protein worldwide (Kim and Voy, 2021).Poultry meat has many desirable characteristics such as high protein and low fat contents and relatively high concentrations of polyunsaturated fatty acids (PUFAs) that can be further increased through dietary manipulations compared to beef- or pork-meat (Moyo*et al.,*2021). Enrichment of poultry meat with PUFAs, especially omega-3 PUFAs, due to their benefits for human health is achieved by the inclusion of fish or vegetable oils (e.g. flaxseed or canola), oil seeds or meals (plant- or fish-derived) in broiler diets (Saleh *et al.,* 2018). The increase in the degree of lipid unsaturation by dietary manipulation increases the susceptibility of poultry meat to oxidative deterioration of lipids, especially PUFAs, during storage and cooking (Akuru *et al.,* 2020). Lipid oxidation also impairs the nutritional quality and organoleptic characteristics, shortens the shelflife and reduces consumer acceptability of meat and meat products (Kishawy *et al.,* 2019). Lipid peroxidation in meat and meat products has been prevented by the dietary supplementation ofnatural antioxidantssuch as α-tocopherol acetate, ascorbic acid and β-carotene or synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene and tertiary butyl hydroquinone (Gümüş *et al.,* 2021). Synthetic antioxidants because of their low cost and high effectiveness have previously been widely used to prevent the lipid peroxidation of meat and meat products (Gümüş *et al.,* 2021). However, recent concerns over their use due to the carcinogenic and mutagenic potential have led to a growing interest in the use of natural antioxidants instead of synthetic antioxidants (Kishawy *et al.*, 2019; Mitterer-Daltoé *et al.,* 2021). However, natural antioxidants are more expensive and less effective than synthetic antioxidants (Gümüş *et al.,* 2021). As a result, much special attention has been focused on natural antioxidants derived from inexpensive and phenolic-rich waste products resulting from the processing of fruits and fruit juice industry by-products such as pomegranate peel (PP) (Kishawy *et al.,* 2019; Akuru *et al.,* 2020, 2021; Ahmadipour *et al.,* 2021).

Pomegranate (*Punica granatum* L.) is a major commercial fruit widely grown in many tropical and subtropical countries (de Oliveira *et al.,* 2020). Turkey is an important pomegranate producer and exporter in the World and its total production amounted to559 000tons in 2019 (Gungor *et al.,* 2021).In Turkey, pomegranate fruit is generally processed into juice and juice concentrate, and its peel is an inedible by-product obtained during processing pomegranate into juice (Akuru *et al.,* 2020; Ahmadipour *et al.,* 2021). PPmakes up about50 % of the whole fruit, which is an important source of several bioactive compounds including hydrolyzable tannins, flavonoids, anthocyanins and other phenolic compounds (Gullón *et al.,* 2020).The ability of these bioactive polyphenolic compounds in PP to act as a free radical scavenger on the active forms of reactive oxygen species, which has been implicated in the initiation and progression stagesof oxidation, is partly related to their standard one-electron donation, breaking the free radical chain reaction or preventing metal ion chelation (Sumathy *et al.,* 2013; Kishawy *et al.,* 2019; Akuru *et al.,* 2020, 2021).

Although the antioxidant activity of PP has been demonstrated by many scientific *in vivo* studies (Saleh *et al.,* 2017; Al-Shammari *et al.,* 2019; Kishawy *et al.,* 2019;Sharifian *et al.,* 2019; Akuru *et al.,* 2020; Baset *et al.,* 2020; Ahmadipour *et al.,* 2021; Akuru *et al.,* 2021; Eid *et al.,* 2021)in broilers, there is currently no published *in vivo* research regarding the effects of the combined supplementation of α-tocopherol acetate (TA) and pomegranate peel extract (PPE) to the broiler diets enriched with omega-3 PUFAs.

In the above mentioned *in vivo* studies, the effects of levels of pomegranate peel powder or PPE aloneas an antioxidant source in the oxidative stress-induced broiler and laying hen diets have been compared with those of TA. Therefore, the objective of the present study was to compare the effects of the dietary supplementation of α-tocopherol acetate (TA) and pomegranate peel extract (PPE) alone or in combination in order to prevent oxidative stress created by the enrichment of the diet by 4 % flaxseed oil in terms of the growth performance, and fatty acid composition, total phenolic contents, antioxidative profile and lipid oxidation of meat of broilers.

MATERIALS AND METHODS

Animal care, experimental design and diets

The complete protocol was reviewed and approved by the Animal Care and Use Committee (2009-HADYEK-007) of Tokat Gaziosmanpasa University. A total of 600 day-old Ross 308 male broiler chicks were weighed, wing-banded and randomly distributed into five treatments with 5 replicates of 24 chicks each. From hatching until 6 weeks of age, the chicks were kept on floor pens bedded with fresh

wood shavings as litter. Temperature was kept at 32ºC for the first week, 28ºC for the second week and 21˚C thereafter. A fluorescent lighting schedule of 23 h light and 1 h dark was used during the experiment with an average light intensity of 20 lux. The diets in mash form and drinking water were provided *ad libitum*.

All diets were formulated according to phase feeding practices as the broiler chickens advanced in age and weight, as recommended by the breeder (Ross 308, 2007); the starter phase lasted from day 0 to 10, the grower phase was from day 11 to 28 and the finisher phase was from day 29 to 42. The ingredients and the nutrient composition of the control diet are presented in Table 1. The experimental diets included: CONT: a control diet containing 50 mg/kg TA

Table 1. Ingredients and chemical compositions of the control diet, g/kg

Ingredients	Days		
	0-10	11-28	29-42
Corn	498.53	577.59	621.51
Soybean Meal	361.02	303.95	241.93
Flaxseed Oil	40.00	40.00	40.00
Fish Meal	20.00	-	-
Corn Gluten Feed	42.79	-	-
Corn Gluten Meal	-	39.85	59.22
Dicalcium Phosphate	16.30	18.04	18.37
Limestone	9.16	9.45	9.64
Salt	2.41	3.33	3.34
Vitamin Premix[1]	2.50	2.50	2.50
Trace Mineral Premix[2]	1.00	1.00	1.00
DL-Methionine	3.85	2.68	1.42
L-Lysine	1.67	1.61	1.07
L-Threonine	0.77	-	-
Chemical Composition (Calculated)			
Dry Matter, %	87.83	87.45	87.26
Crude Protein, %	23.00	22.00	19.00
Crude Fiber, %	3.33	2.88	2.68
Crude Ash, %	5.75	5.38	5.09
Crude Fat, %	6.35	6.25	6.28
ME, Kcal/kg	3025	3150	3200
Ca, %	1.00	0.90	0.90
Pavailable, %	0.50	0.45	0.45
Methionine, %	0.74	0.60	0.47
Methionine+Cystine, %	1.09	0.94	0.80
Lysine, %	1.44	1.20	1.00
Na, %	0.17	0.16	0.16
Tryptophan, %	0.26	0.22	0.20
Arginine, %	1.54	1.33	1.17
Chemical Composition (Analyzed)			
Dry Matter, %	87.78	87.15	87.00
Crude Protein, %	22.87	21.96	18.83
Crude Fat, %	6.21	6.19	6.09
Crude Ash, %	5.60	5.21	4.97
Crude Fiber, %	3.42	2.91	2.71
Ca, %	0.95	0.86	0.84
P total, %	0.75	0.67	0.61
ME, Kcal/kg	3022	3143	3195

[1] Vitamin premix/kg diet: 12 000 IU vitamin A; 1 500 IU vitamin D_3; 50 mg vitamin E; 5 mg vitamin K_3; 3 mg vitamin B_1; 6 mg vitamin B_2; 5 mg vitamin B_6; 0.03 mg vitamin B_{12}; 25 mg niacin; 12 mg Ca-D-pantothenate; 1 mg folic acid; 0.05 mg D-biotin; 2.5 mg apo-carotenoic acid ester; 400 mg choline chloride

[2] Trace Mineral Premix/kg diet: 80 mg Mn; 60 mg Fe; 60 mg Zn; 5 mg Cu; 0.2 mg Co; 1 mg I; 0.15 mg Se

and 4 % flaxseed oil; TA200: the diet supplemented with 200 mg/kg TA to a CONT diet; PPE100: the diet supplemented with 100 mg/kg PPE to a CONT diet; PPE200: the diet supplemented with 200 mg/kg PPE to a CONT diet; TA100+PPE100: the diet supplemented with 100 mg/kg TA+100 mg/kg PPE to a CONT diet. TA was supplied by Kartal Chemistry (Izmit, Turkey). The flaxseed oil obtained froma local market inTokat was antioxidant free. The fatty acid compositions of the flaxseed oil and the control diet are given in Table 2.

The pomegranate fruits (*Punica granatum*Hicaz) were supplied by the West Mediterranean Research Institute (Antalya, Turkey). The peels of the pomegranate fruits were immediately removed and freeze-dried, ground to pass through a 2-mm screen, and then stored in a dry and dark place. Ten grams of pomegranate peel powder was extracted over 4 h with 100 ml of 50 % (v/v) aqueous ethanol at room temperature using a shaking incubator fixed at 200 rpm. The filtrates were placed in a rotary evaporator to remove ethanol under reduced pressure at 38°C and 120 rpm. The remaining aqueous solutions were lyophilized at -50ºC and 0.028 mbar, and the crude extracts were kept in vacuum bags at -80ºC until use.

The contents of punicalagin and ellagic acid in PPE were determined using ultra-HPLC-tandem mass spectrometer (MS/MS) on a Shimadzu system (Kyoto, Japan). The punicalagin and ellagic acid contents in PPE were 9.17 mg/g and 8.78 mg/g dry weight, respectively.

The total phenol content of PPE was determined using the Folin-Ciocalteu method, with a modification described by Jayaprakasha *et al.* (2001) using UV spectrophotometer (PerkinElmer, Waltham, MA, USA) at 760 nm. The results were expressed as milligrams of gallic acid equivalents (GAEs) per fresh weight. The phenol content of PPE was calculated as 159.89±2.515 mg/GAEq/g extract.

The total flavonoid content of PPE was determined by the aluminum chloride method (Chang *et al.,* 2002)using UV spectrophotometer (PerkinElmer, Waltham, MA, USA) at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg of rutin equivalent per g of dry weight. The total flavonoid content of PPE was 37.89 ± 4.8 mg/rutin/g extract.

The proanthocyanidin content of PPE was determined according to the method proposed by Sun*et al.* (1998)using UV spectrophotometer (PerkinElmer, Waltham, MA, USA) at 500 nm. The result was expressed as milligrams of (+)-catechin equivalents.

Table 2. The fatty acid compositions of the flaxseed oil and the control diet, %

Fatty Acids (FAs)	Flaxseed Oil	Control Diets		
		Starter (0-10 days)	Grower (11-28 days)	Finisher (29-42 days)
C12:0	0.01	-	-	-
C14:0	0.04	5.19	0.54	0.63
C14:1	-	5.47	0.63	1.88
C15:0	-	1.53	1.30	2.27
C16:0	4.46	10.69	9.38	8.07
C16:1	0.07	3.46	4.17	2.26
C17:0	-	0.08	0.47	0.04
C17:1	-	0.56	1.25	0.38
C18:0	3.98	13.57	7.70	6.58
C18:1n-9	17.37	19.43	28.92	26.26
C18:2n-6	16.74	31.07	35.98	42.45
C18:3n-6	0.10	0.05	0.64	0.14
C18:3n-3	57.23	8.90	8.02	8.04
SFAs	8.49	31.06	19.39	9.52
MUFAs	17.44	28.92	34.97	30.78
PUFAs	74.07	46.02	50.64	56.63
Omega-3 FAs	57.23	8.90	9.02	9.04
Omega-6 FAs	16.84	31.12	36.62	42.59
Omega-6/Omega-3 Ratio	0.29	3.50	4.07	4.71

The proanthocyanidin content of PPE was found to be 8.1878 ±2.525 mg/ ((+)-catechin)/g extract.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of PPE was determined as described by Jung *et al.* (2010) with some modificationusing UV spectrophotometer (PerkinElmer, Waltham, MA, USA) at 517 nm. The DPPH radical scavenging activity of PPE was EC50 4.455 µl/ml.

The vitamin E (α-tocopherol acetate) concentration in PPE was determined using an HPLC system, after the saponification of the sample with ethanolic KOH in the presence of pyrogallol (Surai *et al.,* 1996). The α-tocopherol acetate concentration of PPE was 0.96 mg/g ± 0.01.

Growth performance and sample collection

During the 42-day experimental period, the growth performance of broilers was evaluated by recording their body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR). The body weight (BW) of broilers was recorded at the beginning of the experiment and on a weekly basis thereafter. FCR was calculated weekly as the amount of feed consumed per unit of BWG. Throughout the experiment, broilers were handled according to the principles for the care of animals in experimentation (Ross 308, 2007). Mortality was recorded daily.

Analysis of the fatty acids compositions of meat

At the end of the experiment, 15 broilers, whose BWs were similar to the group average, were selected from each of the treatment groups and a total of 75 broilers were slaughtered by severing the jugular vein to determine the fatty acids (FAs) compositions of meat. And then, the thigh meat and breast meat were trimmed by removing the skin, bones and connective tissue. Following trimming, the thigh meat and breast meat samples were vacuum packaged and stored in a freezer at -80ºC until analysis of theirFAs compositions. Fat extraction was conducted by the method described by Folch *et al.* (1957) to prepare the sample for FA analysis using chloroform/methanol (2:1; vol/vol). The FA methyl esters were prepared from lipid samples according to Anonymous (1987), and subsequent FA profiles were obtained by gas chromatography (GC) (Shimadzu GC-2010, Shimadzu Corporation, Kyoto, Japan) with a flame detector. A Supelco wax-fused silica capillary column (30 m x 0.32 mm i.d.) with a film thickness of 0.25 l (JW Scientific, Milford, CT, USA) was used. The injection, detector and oven temperatures were 230ºC, 195ºC and 240ºC respectively. The carrier gas was helium at a flow rate of 0.3 ml/min and the split ratio was 1:80. The peaks were identified by comparing their retention times using a Supelco 37 Component FAME mix and an unsaturated FA mix. The FAs compositions of the flaxseed oil, the control diet and the thigh meat and breast meat were determined according to the methoddescribed above.

Analysis of the total phenolic contents and antioxidant potentials of meat

The meats were stored in the refrigerator at +4ºC until day 9 to determine their total phenolic contents (TPCs) and antioxidant potentials. The TPCs and antioxidant potentials of the thigh meat and the breast meat on days 0^{th} and 9^{th} of storage were measured. The thigh meat and the breast meat (3 g) were homogenized in 15 ml of distilled water at 1130 *x g* for 1 min. Next, 10 ml of chloroform was added to the homogenate. The mixture was then shaken strongly 2-3 times and centrifuged at 2090 × g for 15 min. The supernatant was used for the measurement of TPC, DPPH radical scavenging activity and ferric reducing antioxidant power (FRAP) of the thigh meat and breast meat (Jung *et al.,* 2010).

The TPCs of the thigh meat and the breast meat were estimated by the Folin-Ciocalteu method (Subramanian *et al.,* 1965) with a spectrophotometer (PerkinElmer, Waltham, MA, USA) at 765 nm. The quantification of phenolics was based on the standard curve generated with the use of gallic acid, and expressed as gallic acid equivalent (Jung *et al.,* 2010).

The DPPH radical scavenging activities of the thigh meat and the breast meat were determined according to the modified method of Blois (1958) with a spectrophotometer (PerkinElmer, Waltham, MA, USA) at 517 nm. The percentage of DPPH radical scavenging activity was calculated using the following equation (Jung *et al.* 2010):

DPPH radical scavenging activity= [1−(absorbance of sample/absorbance of control)] × 100

The FRAP of the thigh meat and the breast meat was estimated according to the method described by Oyaizu (1986) using a spectrophotometer (PerkinElmer, Waltham, MA, USA) at 700 nm: higher absorbance indicates higher FRAP values (Jung *et al.,* 2010).

Analysis of the lipid oxidation of meat

On day 42, an additional 15 broilers, whose BWs were similar to the group average, were selected from each treatment group. A total of 75 broilers were slaughtered by severing the jugular vein to determine the lipid oxidation of the thigh meat and the breast meat. The meats were stored in the refrigerator at +4ºC until day 9. Lipid oxidation was determined according to the method described by Botsoglou *et al.* (2003a) on days 0th and 9th of refrigerated storage using UV spectrophotometer (PerkinElmer, Waltham, MA, USA) at 530 nm. Third-order derivative spectra were produced by digital differentiation of the normal spectra using a derivative wavelength difference setting of21 nm.The concentration of malondialdehyde (MDA) (ng/g wet tissue) in the analyzed extracts was calculated on the basis of the height of the third-order derivative peak at 521.5 nm by referring to the slope and intercept data of the computed least-squares fit of standard calibration curve prepared using 1,1,3,3-tetraethoxypropane.

Statistical analysis

All statistical analyses were performed using the SPSSusing SPSS (17.0)® statistic package (SPSS-WIN, 2007).The data related to growth performance and the FAs contents of the thigh meat and the breast meat were analyzed with one-way ANOVA. The univariate general linear model was applied to data related to the total phenolic contents, DPPH radical scavenging activity, FRAP and MDA values of the thigh meat and the breast meat with a model including dietary treatments (DTs) and storage days (SDs) and the interaction between DTs and SDs. Significant differences between treatment means were separated using Duncan's multiple range test (Duncan, 1955). All statements of significance were based on *P* value <0.05.

RESULTS AND DISCUSSION

Growth performance

The effects of the DTs on the initial and final BW and BWG, FI and FCR of broilers from hatching to 42 days are shown in Table 3.

As shown in Table 3, there is no significant difference (*P*>0.05) among the DTs in terms of the initial BW of broiler chicks. The final BW and BWG, FI and FCR ofbroilers from 0 to 42 days of age were not affected by the DTs. The results related to the BWG and FIof broilers are in agreement with the findings reported by Saleh *et al.* (2017) who stated that BWG and FI of broilers were not influenced by the dietary supplementation of PPE at 100 and 200 mg/kg levels when compared to the diet with α-TA at 200 mg/kg level. Similarly, Kishawy *et al.* (2019) reported that PPE supplementation at 0.1 % level to a diet containing linseed oil did not significantly affect the BWG, FI and FCR of broilers compared to the control group without any supplementation.In contrast, the finding related to FCR of the present study did not concur with the results published by Sharifian *et al.* (2019). They reported that the supplementation of PPE at the increasing levels to the diet of broiler chickens reared under heat stress significantly improved the FCR of broilers throughout overall experimental periods.

The inconsistent results related to growth performance may be attributed to the differences in stress condition, the length and severity of imposed stress, the extraction method and the solvent used during the extraction process, the total phenolic concentration in the diet and the polyphenolic profile of the PPE used in different studies.

The fatty acids contents of meat

The effects of the DTs on the fatty acids (FAs) contents of the thigh meat and the breast meat are sum-

Table 3. The effects of the DTs on the initial and final BW and BWG, FI and FCR ofbroilers from hatching to 42 days

Parameters	DTs					SEM	*P-value*
	CONT	TA200	PPE100	PPE200	TA100+PPE100		
Initial BW, g	39.58	39.54	39.41	39.41	39.47	0.080	0.956
Final BW, g	2416.80	2460.40	2448.00	2440.20	2415.60	21.317	0.963
BWG, g	2377.26	2420.93	2408.59	2400.79	2376.02	21.332	0.963
FI, g	4494.43	4533.09	4557.06	4515.40	4438.75	33.655	0.855
FCR, g:g	1.89	1.87	1.89	1.88	1.87	0.012	0.167

SEM: Standard Error of Mean
DTs: Dietary Treatments

marized in Table 4 and 5, respectively.

As indicated in Table 4, feeding the TA200, PPE100, PPE200 and TA100+PPE100 diets decreased the total saturated fatty acids (SFAs) (P <0.01) and monounsaturated fatty acids (MUFAs) (P <0.001) contents and omega-6/omega-3 ratio (P<0.001), however, increased the total polyunsaturated fatty acids

Table 4. The effects of the DTs on the fatty acid contents of the thigh meat of broilers, %

Fatty acids (FAs)	DTs					SEM	*P-value*
	CONT	TA200	PPE100	PPE200	TA100+PPE100		
C14:0	0.52^{a}	0.34^{b}	0.48^{a}	0.51^{a}	0.49^{a}	0.012	0.000
C14:1	0.40^{b}	0.17^{c}	0.13^{c}	0.52^{a}	0.08^{d}	0.021	0.000
C15:0	0.06^{c}	0.08^{ab}	0.06^{bc}	0.04^{d}	0.08^{a}	0.002	0.000
C16:0	25.04^{a}	23.85^{b}	23.76^{b}	24.69^{a}	24.81^{a}	0.104	0.000
C16:1	6.31^{a}	4.95^{c}	5.60^{b}	5.47^{b}	5.09^{b}	0.085	0.000
C17:0	0.09^{a}	0.07^{b}	0.02^{d}	0.02^{d}	0.04^{c}	0.004	0.000
C17:1	0.10^{a}	0.06^{b}	0.12^{a}	0.11^{a}	0.04^{c}	0.005	0.000
C18:0	7.95^{b}	8.36^{a}	7.83^{b}	7.18^{b}	6.90^{c}	0.109	0.000
C18:1n-9	39.59^{a}	37.42^{c}	38.37^{b}	38.33^{b}	37.58^{c}	0.188	0.000
C18:2n-6	16.39^{d}	17.91^{ab}	17.67^{ab}	17.04^{bc}	18.27^{a}	0.124	0.000
C18:3n-3	5.44^{b}	6.74^{a}	6.44^{a}	6.25^{a}	6.88^{a}	0.070	0.000
C18:3n-6	0.21^{a}	0.14^{b}	0.17^{b}	0.22^{a}	0.04^{c}	0.010	0.000
SFAs	33.66^{a}	32.70^{b}	32.15^{b}	32.44^{b}	32.32^{b}	0.211	0.002
MUFAs	46.40^{a}	42.60^{c}	44.22^{b}	44.43^{b}	42.79^{c}	0.247	0.000
PUFAs	22.04^{d}	24.79^{b}	24.28^{b}	23.51^{c}	25.19^{a}	0.139	0.003
Omega-6 FAs	16.60^{c}	18.05^{a}	17.84^{b}	17.26^{b}	18.31^{a}	0.070	0.000
Omega-3 FAs	5.44^{b}	6.74^{a}	6.44^{a}	6.25^{a}	6.88^{a}	0.120	0.000
Omega-6/Omega-3 ratio	3.05^{c}	2.68^{a}	2.77^{b}	2.76^{b}	2.66^{a}	0.044	0.000

$^{a-d}$ Values in the same row not sharing a common superscript differ significantly (**P<0.01; ***P<0.001)
SEM: Standard Error of Mean
DTs: Dietary Treatments

Table 5. The effects of the DTs on the fatty acid contents of the breast meat ofbroilers, %

FAs	DTs					SEM	*P-value*
	CONT	TA200	PPE100	PPE200	TA100+PPE100		
C14:0	0.49^{a}	0.43^{bc}	0.46^{ab}	0.49^{a}	0.41^{d}	0.007	0.000
C14:1	0.34^{a}	0.15^{c}	0.14^{c}	0.15^{c}	0.27^{b}	0.012	0.000
C15:0	0.19^{a}	0.07^{c}	0.07^{c}	0.05^{c}	0.13^{b}	0.007	0.000
C16:0	25.56^{a}	24.11^{b}	25.46^{a}	25.04^{ab}	24.56^{ab}	0.179	0.048
C16:1	6.64^{a}	4.92^{c}	4.75^{c}	5.53^{b}	5.57^{b}	0.109	0.000
C17:0	0.09^{a}	0.06^{b}	0.05^{b}	0.05^{b}	0.05^{b}	0.004	0.001
C17:1	0.11^{a}	0.05^{b}	0.11^{a}	0.04^{b}	0.09^{a}	0.005	0.000
C18:0	8.19^{a}	7.52^{ab}	6.95^{b}	7.89^{a}	7.70^{a}	0.113	0.005
C18:1n-9	39.30^{a}	37.93^{bc}	37.22^{c}	38.37^{ab}	37.67^{bc}	0.174	0.001
C18:2n-6	16.17^{c}	17.90^{a}	18.07^{a}	16.77^{bc}	17.22^{b}	0.133	0.000
C18:3n-3	5.50^{c}	6.77^{a}	6.61^{a}	6.08^{b}	6.84^{a}	0.072	0.000
C18:3n-6	0.27^{a}	0.23^{b}	0.18^{c}	0.19^{c}	0.18^{c}	0.008	0.000
SFAs	34.52^{a}	32.19^{c}	32.99^{bc}	33.52^{b}	32.85^{bc}	0.279	0.001
MUFAs	46.39^{a}	43.05^{c}	42.22^{d}	44.09^{b}	43.60^{bc}	0.204	0.001
PUFAs	21.94^{c}	24.90^{a}	24.86^{a}	23.04^{b}	24.24^{a}	0.167	0.000
Omega-6 FAs	16.44^{c}	18.13^{a}	18.25^{a}	16.96^{b}	17.40^{b}	0.146	0.000
Omega-3 FAs	5.50^{c}	6.77^{a}	6.61^{a}	6.08^{a}	6.84^{a}	0.072	0.000
Omega-6/Omega-3 ratio	2.99^{a}	2.68^{c}	2.76^{b}	2.79^{b}	2.54^{d}	0.042	0.000

$^{a-d}$ Values in the same row not sharing a common superscript differ significantly (*P<0.05; **P<0.01; ***P<0.001)
SEM: Standard Error of Mean
DTs: Dietary Treatments

(PUFAs) (P <0.01), omega-6 FAs (P <0.001) and omega-3 FAs(P <0.001) contents in the broiler thigh meat compared to those of broilers fed the CONT diet. Further, the omega-6/omega-3 ratio in the thigh meat of broilers fed the TA100+PPE100 diet was equal to that of broilers fed the TA200 diet (Table 4). In agreement with the results of the present study, Ahmed *et al.* (2015) reported that feeding diet supplemented with 2 % pomegranate by-products significantly reduced the total SFAs content and omega-6/omega-3 ratio, and increased the total omega-3 FAs content of the broiler thigh meat when compared to those of broilers fed the non-supplemented diet. In partial consistent, Saleh *et al.* (2018) stated that the supplementation of α-TA and pomegranate pomace extract at 0.1 and 0.2 g/kg levels into the diet contained 2% fish oil significantly increased the total PUFAs, omega-3 FAs and omega-6 FAs contents and decreased the omega-6/omega-3 ratio, however, did not significantly influence the total SFAs content of the broiler thigh meat compared to the control diet.

As summarized in Table 5, the TA200, PPE100, PPE200 and TA100+PPE100 diets reduced the total SFAs and MUFAs contents (P <0.01), the omega-6/omega-3 ratio, and increased the total PUFA, omega-6 FAs and omega-3 FA contents(P <0.001) in the broiler breast meat compared to the CONT diet. Moreover, the omega-6/omega-3 ratio of the breast meat of broilers fed the TA100+PPE100 diet was lower than that of broilers fed the TA200 diet (P<0.001) (Table 5).In agreement with our results, Saleh *et al.* (2017) demonstrated that the supplementation of α-TA(200 mg/kg level) and PPE (100 and 200 mg/kg levels) into the diet contained 2 % fish oil resulted in a significant increase in the concentrations of the total PUFAs, omega-3 FAs and omega-6 FAs, however, in a significant reduction in the total MUFAs content and omega-6/omega-3 ratio ofthe broiler breast meat compared to the CONT diet. Likewise, Kishawy *et al.* (2019) showed that the supplementation of PPE at the levels of 0.05 % and 0.1 % to a diet containing linseed oil (2 %) significantly enhanced the omega-3 FA contents of the breast meat of broilers when compared to those of broilers fed a non-supplemented diet. In contrast, Ahmed *et al.* (2015) reported that the omega-6/omega-3 ratio of the broiler breast meat was not significantly influenced by dietary supplementation of pomegranate by-products at increasing levels (0.5 %, 1 % and 2 %) compared to the non-supplemented diet. This discrepancy between studies regarding the omega-6/omega-3 ratio of broiler meat may be explained by the differences in pomegranate by-products, the total phenolic concentration in the diet and the polyphenolic profile of the pomegranate by-products.

In summary, the increase in the total PUFAs contents and the decrease in the total MUFAs contents of the broiler thigh meat and breastmeat in the present studycan be explained by the preventive effect of TA and phenolic compounds in PPE on the oxidation of PUFAs (Saleh *et al.,*2017). Consequently, an increase in the PUFA contents leads to decreased MUFA synthesis through inhibiting the activity of9-desaturase enzyme complex, which is the key enzyme needed for the conversion of SFAs to MUFAs (Pinchasov and Nir, 1992). Moreover, the current study demonstrated that the supplementation of TA or PPE alone or in combination to the linseed oil-containing diets retained the omega-3 PUFAs in the thigh meat and the breast meat (Kishawy *et al.,* 2019). This effect may be attributed to the high antioxidant activity of the phenolic compounds in PPE towards removing and decreasing the free radicals in meats and their protective effect that prevents the oxidation of omega-3 PUFAs in linseed oil (Saleh *et al.,* 2017; Kishawy *et al.,* 2019).

The total phenolic contents of meat

The effects of the DTs and the SDs on the total phenolic contents (TPCs) of the thigh meat and the breast meat at 0^{th} and 9^{th} days of refrigerated storage are given in Table 6.

As presented in Table 6, the TPCs of the thigh meat (P<0.05) and the breast meat (P <0.01) were increased by the TA200, PPE100, PPE200 and TA100+PPE100 diets compared to those of broilers fed the CONT diet. The highest TPCof the thigh meat and the breast meat was obtained by the TA100+PPE100 diet. The findings related to the TPCsof the breast meat of broilers are in agreement with the results published by Saleh *et al.* (2017) who reported that the breast meat of broilers fed diets supplemented with α-TA at 200 mg/kg level and PPE at the levels of 100 and 200 mg/kg had significantly higher levels of the TPCs compared to a control diet. Likewise, Kishawy *et al.* (2019) stated that the addition of PPE at the levels of 0.05 % or 0.1 % to broiler diets containing linseed oil (2 %) resulted in a significant increase in the TPCof the breast meat. The results related to the TPCs of the broiler thigh meat concur with the findings published by Saleh *et al.* (2018).They reported that the thigh meat of broilers fed diets containing α-TA and pomegranate pomace extract at the levels of 100 and 200

mg/kg had significantly higher TPC when compared to groups fed the control diet. In the present study, the high levels of the total phenolic compounds in meat can be explained by the protective effect of PPE as a powerful systemic antioxidant against oxidation of the polyphenolic compounds in tissues (Saleh *et al.,* 2017, 2018). As a result of this, the polyphenolic compounds in PPE without oxidation are absorbed from the digestive tract and then distributed, retained and remained functional in the broiler meat (Saleh *et al.,* 2017, 2018). Furthermore, prolongation of the refrigerated storage time from 0th to 9th days decreased(P <0.001) the TPCsof the thigh meat and the breast meat of broilers (Table 6). On the other hand, there was no significant interaction (P>0.05) between the DTs and the SDs in terms of the TPCs of the thigh meat and the breast meat of broilers (Table 6).

The antioxidant potentials of meat

The effects of the DTs and the SDs on the DPPH radical scavenging activity of the thigh meat and the breast meat of broilers on days 0th and 9th of refrigerated storage are given in Table 7.

The TA200, PPE100, PPE200 and TA100+PPE100 diets showed (P <0.001) high DPPH radical scavenging activityof the thigh meat and the breast meat of broilers when compared to that of the CONT diet (Table 7). This finding is in agreement with the results reported by Saleh *et al.* (2017) who pointed out that the DPPH radical scavenging activity of the breast meat in broilers fed diets supplemented with α-TA at the 200 mg/kg level and PPE at the levels of 100 or 200 mg/kg were significantly higher than that of the control birds. In the current study, the observed efficiency of dietary supplementation of PPE alone or in combination with α-TAin increasing DPPH activities of meats might be attributed to the increase in the TPCs, as a powerful radical-scavenging antioxidant,of the thigh meat and the breast meat(Saleh *et al.,* 2018;

Table 6. The effects of the DTs and the SDs on the total phenolic contents of the thigh meat and the breast meat on days 0th and 9th of refrigerated storage (μg gallic acid equivalent/g fresh weight)

DTs	Days	Thigh Meat	Breast Meat
CONT	0	15.941	16.115
	9	7.019	8.185
TA200	0	18.380	19.095
	9	9.255	9.419
PPE100	0	18.489	18.722
	9	8.714	9.644
PPE200	0	18.544	19.078
	9	8.921	10.020
TA100+PPE100	0	18.798	19.529
	9	9.873	10.925
SEM		6.896	6.646
SDs	0	18.030[a]	18.508[a]
	9	8.756[b]	9.639[b]
SEM		5.521	5.049
DTs	CONT	11.480[c]	12.150[c]
	TA200	13.818[b]	14.257[b]
	PPE100	13.601[b]	14.183[b]
	PPE200	13.733[b]	14.549[b]
	TA100+PPE100	14.335[a]	15.227[a]
SEM		8.723	7.981
P-value			
DTs		0.016	0.008
SDs		0.000	0.000
DTs x SDs Interaction		0.995	0.953

[a-c] Values in the same column not sharing a common superscript differ significantly (*P<0.05; **P<0.01; ***P<0.001)
SEM: Standard Error of Mean
DTs: Dietary Treatments
SDs: Storage Days

Al-Shammari *et al.,* 2019). Moreover, prolongation of the refrigerated storage time from 0th to 9th days decreased the DPPH radical scavenging activity of the thigh meat and the breast meat (P <0.001) (Table 7).

The interaction between the DTs and the SDs did not have a significant effect (P >0.05) on the DPPH radical scavenging activity of the thigh meat (Table 7). On the other hand, there was a significant interaction between the SDs and DTs and DTs and the SDs in terms of the DPPH radical scavenging activity of the breast meat (P <0.05) (Table 7). Prolongation of storage time reduced (P<0.05) the DPPH radical scavenging activity of the breast meat of broilers fed the TA200, PPE100, PPE200 and TA100+PPE100 diets except the CONT diet. Moreover, the DPPH radical scavenging activity of the breast meat on days0th and9th of refrigerated storage was increased (P <0.05) by the TA200, PPE100, PPE200 and TA100+PPE100 diets compared to the CONT diet (Table 7).

The effects of the DTs and the SDs on the FRAP of the thigh meat and the breast meat on days 0th and 9th of refrigerated storage are summarized in Table 8.

The FRAP of the thigh meat (P <0.01) and breast meat (P <0.05) was increased by the TA200, PPE100, PPE200 and TA100+PPE100 diets compared to the CONT diet (Table 8). The higher FRAP in meat containing PPE can be attributed to its increasing phenolic compounds accumulation. The high levels of phenolic compounds in meat may increase the ability of the diets containing PPE to reduce Fe^{3+} to Fe^{2+} form in meat due to their transition-metal-chelating activity (Sultana *et al.,* 2018)

Prolongation of the refrigerated storage time from 0th to 9th days decreased (P <0.001) the FRAP of the thigh meat and the breast meat (Table 8). However, there was no significant interaction (P>0.05) between the DTs and the SDs in terms of the FRAP of the thigh meat and the breast meat (Table 8).

Table 7. The effects of the DTs and the SDs on the DPPH radical scavenging activityof the thigh meat and the breast meat on days 0th and 9th of refrigerated storage (%)

DTs	Days	Thigh Meat	Breast Meat
CONT	0	61.74	$^{B}54.96^{a}$
	9	54.14	$^{C}54.12^{a}$
TA200	0	75.73	$^{A}82.44^{a}$
	9	67.45	$^{A}72.83^{b}$
PPE100	0	79.13	$^{A}80.82^{a}$
	9	70.53	$^{B}66.77^{b}$
PPE200	0	71.35	$^{A}77.92^{a}$
	9	65.71	$^{B}62.48^{b}$
TA100+PPE100	0	73.77	$^{A}79.19^{a}$
	9	67.42	$^{B}64.59^{b}$
SEM		1.122	1.476
DTs	CONT	57.94^{d}	54.54^{d}
	TA200	71.59^{ab}	77.63^{a}
	PPE100	74.83^{a}	73.79^{ab}
SEM	PPE200	68.53^{c}	70.20^{c}
	TA100+PPE100	70.60^{ab}	71.89^{ab}
		1.735	1.525
SDs	0	72.34^{a}	75.07^{a}
	9	65.05^{b}	64.16^{b}
SEM		1.104	0.951
P-value			
DTs		0.000	0.000
SDs		0.000	0.000
DTs x SDs Interaction		0.975	0.023

$^{a-d}$ Values in the same column not sharing a common superscript differ significantly (*P<0.05; ***P<0.001)
$^{a-b}$Small letters in right show the interaction between DTs and SDs
$^{A-C}$ Capital letters in left show the interaction between SDs and DTs
SEM: Standard Error of Mean
DTs: Dietary Treatments
SDs: Storage Days

Table 8. The effects of the DTs and the SDs on the ferric reducing power of the thigh meat and the breast meat on days 0th and 9th of refrigerated storage

DTs	Days	Thigh Meat	Breast Meat
CONT	0	0.506	0.520
	9	0.079	0.076
TA200	0	0.572	0.614
	9	0.107	0.097
PPE100	0	0.548	0.556
	9	0.090	0.104
PPE200	0	0.542	0.542
	9	0.099	0.101
TA100+PPE100	0	0.544	0.585
	9	0.107	0.102
SEM		0.027	0.029
SDs	0	0.542[a]	0.563[a]
	9	0.096[b]	0.096[b]
SEM		0.006	0.010
DTs	CONT	0.292[b]	0.298[b]
	TA200	0.340[a]	0.356[a]
	PPE100	0.319[a]	0.330[a]
	PPE200	0.321[a]	0.322[a]
	TA100+PPE100	0.325[a]	0.344[a]
SEM		0.008	0.016
P-value			
DTs		0.003	0.017
SDs		0.000	0.000
DTs x SDs Interaction		0.499	0.402

[a-b] Values in the same column not sharing a common superscript differ significantly (*P<0.05; **P<0.01; ***P<0.001)
DTs: Dietary Treatments
SDs: Storage Days

Lipid oxidation of meat

The effects of the DTs and the SDs on the malondialdehyde (MDA) values of the thigh meat and the breast meat on days 0th and 9th of refrigerated storage are given in Table 9.

As indicated in Table 9, the MDA values of the thigh meat and the breast meat were reduced (P<0.001) by feeding the TA200, PPE100, PPE200 and TA100+PPE100 diets compared to those of broilers fed the CONT diet. These results are in agreement with the findings published by Saleh *et al.* (2017) who reported that the PPE diet (200 mg/kg) had potential similar to that of α-TA diet in reducing the lipid oxidation of the broiler breast meat on days 0, 7 and 11 of refrigerated storage. Likewise, Sharifian *et al.* (2019) pointed out that dietary PPE supplementation linearly significantly reduced the MDA concentration in the breast meat of broilers reared under heat stress on days 0, 30 and 60 of refrigerated storage. The low MDA values of the thigh meat and the breast meat of broilers fed diets containing PPE in the current study might be related to the high levels of phenolic compounds with the strong antioxidant activity in meat (Baset *et al.,* 2020) that extend the shelf life of meat (Akuru *et al.,* 2020). Moreover, these phenolic compounds can act in a way similar to that of α-TA in the prevention of lipid oxidation in the breast meat and the thigh meat (Saleh *et al.,* 2018). In addition, significantly lowest MDA values of the thigh meat and the breast meat were obtained with the TA100+PPE100 diet (P <0.001) (Table 9). This finding in the present study concurs with the results published by Papageorgiou *et al.* (2003) who reported that a synergistic action between oregano oil and TA (200 mg/kg) resulted in higher oxidative stability in meatcompared to when 200 mg/kg TA alone was fed to turkeys. The lowest MDA values of the meats of broilers fed the TA100+PPE100 diet in the present study may explain the synergistic effect between TA and PPE (Altunkaya, 2014). The synergistic interactions between TA and PPE may be explained as follows: 1. Regeneration of α-TA through the donation of a hydrogen atom

Table 9. The effects of the DTs and the SDs on the malondialdehyde value of the thigh meat and the breast meat on days 0th and 9th of refrigerated storage (mg malondialdehyde per kg meat)

DTs	Days	Thigh Meat	Breast Meat
CONT	0	0.367	0.126
	9	1.048	0.510
TA200	0	0.317	0.093
	9	0.963	0.487
PPE100	0	0.311	0.099
	9	0.984	0.488
PPE200	0	0.313	0.093
	9	0.976	0.477
TA100+PPE100	0	0.290	0.082
	9	0.908	0.461
SEM		0.030	0.017
SDs	0	0.319^{b}	0.098^{b}
	9	0.976^{a}	0.485^{a}
SEM		0.011	0.005
DTs	CONT	0.707^{a}	0.318^{a}
	TA200	0.640^{b}	0.290^{b}
	PPE100	0.648^{b}	0.293^{b}
	PPE200	0.644^{b}	0.285^{bc}
	TA100+PPE100	0.599^{c}	0.272^{d}
SEM		0.017	0.007
P-value			
DTs		0.000	0.000
SDs		0.000	0.000
DTs x SDs Interaction		0.685	0.965

$^{a-d}$ Values in the same column not sharing a common superscript differ significantly(***P<0.001)
SEM: Standard Error of Mean
DTs: Dietary Treatments
SDs: Storage Days

by phenolic compounds in PPE to the tocopheroxyl radical, 2. Metal chelation by one antioxidant sparing a chain breaking antioxidant, 3. Protection of α-TA and PPE by each other against reactive oxygen species (Altunkaya, 2014).

Prolongation of the SDs from 0th to 9th days increased (P<0.001) the MDA value of the thigh meat and the breast meat of broilers (Table 9).This finding concurs with the results reported by Saleh *et al.* (2017, 2018) who stated that the thiobarbituric acid reactive substances values of raw chicken breast meat were significantly increased with increasing storage time. On the other hand, there was no significant interaction (P>0.05) between the DTs and the SDs on the MDA values of the thigh meat and the breast meat (Table 9).

CONCLUSIONS

In our experiment, the dietary inclusion of TA or PPE alone or in combination did not have a significant effect on the growth performance of broilers. Moreover, the supplementation of TA (200 mg/kg) or PPE (100 and 200 mg/kg) alone or in combination (TA100+PPE100) into the diet contained 4% flaxseed oil significantly increased the omega-3 PUFAs and total phenolic contents, and decreased the omega-6/omega-3 ratio and the MDA values of the thigh meat and the breast meat of broilers. In conclusion, the PPE100, PPE200 and TA100+PPE100 diets were as effective as TA200 diet alone in terms of enriching broiler thigh meat and breast meat with the omega-3 PUFAs. The dietary combination of TA and PPE (TA100+PPE100) was a more effective antioxidant in terms of the lowest omega-6/omega-3 ratio of broiler breast meat, and the highest total phenolic contents and the lowest MDA values of broiler thigh meatand breast meat enriched with omega-3 PUFAs compared to TA200 alone. There is a need for detailed nutrigenomic studies to ascertain the effects of dietary supplementation of TA or PPE alone or in combination on the antioxidative potential and lipid oxidation of

broiler meat enriched with omega-3 PUFAs.

CONFLICT OF INTEREST

None declared by the authors.

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