The effects of α-tocopherol acetate and pomegranate peel extract on the raw breast meat of the oxidative stress-induced broiler

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SUMMARY

The present study was conducted to compare the efficacy of the dietary combined supplementation of α-tocopherol acetate (TA) and pomegranate peel extract (PPE) on the total phenolic content (TPC), total aerobic bacteria count (TABC) and quality of the raw breast meat of broilers with the dietary supplementation of TA or PPE alone. A total of 600 Ross 308 newly hatched male broiler chicks were randomly distributed into five treatments with 5 replicates of 24 chicks each. The experimental diets included: CONT: a control diet containing 50 mg/kg α-tocopherol acetate and 4% flaxseed oil; TA200, PPE100, PPE200 and TA100+PPE100: the diets supplemented with 200 mg/kg α-tocopherol acetate, 100 mg/kg PPE, 200 mg/kg PPE or 100 mg/kg α-tocopherol acetate+100 mg/kg PPE, respectively, to the CONT diet. As a result of this study, the TA200, PPE100, PPE200 and TA100+PPE100 diets increased (P <0.01) the TPC and decreased the TABC (P <0.001) and the cooking loss (CL) (P <0.001) of the raw breast meat of broilers compared to the CONT diet. The pH values at the 15th min and the 24th h of the raw breast meat of the broilers were decreased (P <0.001) by the PPE100, PPE200 and TA100+PPE100 diets compared to those of the CONT and TA200 diets. Feeding the PPE100, PPE200 and TA100+PPE diets increased (P <0.01) the water holding capacity (WHC) of the raw breast meat of broilers compared to the CONT diet. The drip loss of the raw breast meat of broilers was not significantly affected by the DTs (P >0.05). The effects of the combined supplementation of TA and PPE on the TPC and the TBAC of the raw breast meat of broilers were higher than when offered alone, but, similarly improved its WHC and the CL compared to those of the supplementation of TA and PPE alone.

KEY WORDS

Breast Meat Quality; Broiler; Lipid Oxidation; Microbial Content; Natural Antioxidants.

INTRODUCTION

An increased awareness of the relationship between human diet and disease inhibition, has promoted interest in developing of healther and functional meat and meat products through dietary nutritional strategies[1,2]. Particular attention has been paid to improve the fatty acids composition in the fat of meat and meat products[1]. In this context, the production and consumption of broiler meat has gained more importance worldwide[3]. Broiler meat is favoured as a healthy product by consumers because of its desirable nutritional characteristics, such as high protein, low intramuscular fat content and relatively high concentrations of polyunsaturated fatty acids (PUFAs) compared to beef or pork meat[4]. However, cereal-based diets commonly used in the broiler nutrition essentially provide omega-6 PUFAs and only a small amount of omega-3 PUFAs[1]. The reported benefits of especially omega-3 PUFAs, mainly eicosapentaenoic and docosahexaenoic acids, on human health have attracted interest to enrich in omega-3 PUFAs of broiler meat[5]. Enrichment of broiler meat with PUFAs, especially omega-3 PUFAs, is achieved by the inclusion of fish oil or vegetable oils (e.g. flaxseed or canola), oil seeds or meals (plant- or fish-derived) in broiler diets. However, increasing the degree of fatty acids unsaturation by dietary manipulation enhances the susceptibility of broiler meat to the oxidative deterioration of lipids during storage and cooking[1,6]. Oxidation of lipids, especially PUFAs, leads to the production of harmful chemicals such as hydroperoxides, which are further decomposed to seconder reaction products such as short-chain aldehydes, ketones and other oxygenated compounds[6]. The consisted compounds adversely influenced lipids, proteins, carbohydrates, vitamins and pigments[5,6]. These changes negatively affect the overall quality by causing loss of flavor, color and nutritive value consumer acceptability and consequently the shelf-life of the broiler meat and meat products[7].

A notable strategy to prevent or decrease the lipid oxidation in omega-3 PUFA-enriched meat is the supplementation of natural or synthetic antioxidants[1,8,9]. Synthetic antioxidants due to their low cost and high effectiveness were previously and widely used to prevent the lipid peroxidation of meat and meat products[8]. However, consumer concern about the safety and toxicity of synthetic antioxidants due to their carcinogenic and mutagenic potential caused to a growing interest in the use of natural antioxidants[7,10]. As a result, much special attention has been focused on natural antioxidants derived from the inexpensive and healthy agrifood waste products rich in phenolic compounds resulting from the processing of fruits and fruit juice industry by-products such as pomegranate peel[6]. Pomegranate peel makes up about 50% of the whole fruit, which is an

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important source of several bioactive compounds including hydrolysable tannins, flavonoids, anthocyanins and other phenolic compounds[11,12]. The ability of the bioactive polyphenolic compounds in the pomegranate peel extract (PPE) to act as a free radical scavenger on the active forms of reactive oxygen species, which has been implicated in the initiation and progressive phases of oxidation, is partly related to their standard one-electron donation breaking the free radical chain reaction or preventing metal ion chelation[13,14,15]. In addition, Kanatt et al.[13] reported that PPE was more effective than butylated hydroxy toluene (BHT) in scavenging hydroxyl and superoxide anion radical. Pomegranate peel powder or PPE as a natural antioxidant[16] resulted in low pH value[3,6,17], low cooking loss[6] and inhibition of discoloration[6,17] of broiler meat.

In the above *in vivo* studies, the effects of the levels of pomegranate peel powder or PPE as an antioxidant source in the oxidative stress-induced broiler were compared with those of α-tocopherol acetate or BHT. However, the objective of the present study was to compare the efficacy of the combined supplementation of α-tocopherol acetate and PPE to diet containing flaxseed oil, which is rich in omega-3 polyunsaturated fatty acids, in the prevention of oxidative stress via their effects on the total phenolic content, the total aerobic bacteria count and the quality of the raw breast meat of broilers.

MATERIALS AND METHODS

Ethics statement

The present study was reviewed and approved by the Animal Experimentation Ethics Committee (Process no. 2009-HADYEK-007) of Tokat Gaziosmanpasa University.

Animals, diets and experimental design

On the day of hatching, 600 Ross 308 male broilers were acquired from a commercial hatchery (Anadolu Ross, Ankara, Turkey). The broiler chicks were weighed, wing-banded and randomly distributed into five treatments with 5 replicates of 24 chicks each. The inital body weights of the broiler chicks were 39.58 g, 39.54 g, 39.41 g, 39.41 g and 39.47 g for the CONT, TA200, PPE100, PPE200 and TA100+PPE100 groups, respectively.

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 32°C for the first week, 28°C 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*.

The feed ingredients were ground through a 1-mm screen in preparation for chemical analysis. Prior to experimental diet formulation, the feed ingredients were analyzed for their crude protein (CP), ether extract (EE), starch and total sugar content according to the methods of the Association of Analytical Chemists[18]. The metabolizable energy (ME) of the feed ingredients was calculated based on the analyzed values of feedstuffs[19]. The diets per treatment were formulated to cover all the fattening period (starter, grower and finisher). The starter diet (23% CP and 3025 Kcal/kg ME) from 0 to 10 d, the grower diet (22% CP and 3150 Kcal/kg ME) from 11 to 28 d and the finisher diet (19% CP and 3200 Kcal/kg ME) from 29 to 42 d were provided for broilers during the experiment. The experimental diets included: CONT: a control diet containing 50 mg/kg α-tocopherol acetate and 4% flaxseed oil; TA200: the diet supplemented with 200 mg/kg α-tocopherol acetate to a control diet; PPE100: the diet supplemented with 100 mg/kg PPE to a control diet; PPE200: the diet supplemented with 200 mg/kg PPE to a control diet; TA100+PPE100: the diet supplemented with 100 mg/kg α-tocopherol acetate+100 mg/kg PPE to a control diet. The α-tocopherol acetate was supplied by Kartal Chemistry (Izmit, Turkey). The ingredients and the nutrient composition of the control diet were presented in Table 1.

The pomegranate fruits (Hicaz Pomegranate) 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. The modified method of Sarica and Urkmez[20] was applied to obtain the PPE. 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 -50°C and 0.028 mbar, and the crude extracts were kept in vacuum bags at -80°C until use.

Data and sample collection

On day 42, the diets were withdrawed 6 hours ago prior to slaughter. After 6 h feed withdrawal, 15 broiler chickens 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 total phenolic content, the total aerobic bacteria count and the quality parameters (pH, water holding capacity, drip loss, cooking loss) of the raw breast meat. After slaughtering, 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 and packaged in a vacuum bag and stored in a deep freezer at -80°C until required for analysis.

Right section of the frozen breast meat was used for analysis of its total phenolic content and total aerobic bacteria count. The breast meats were placed on the plastic plates and covered with polyethylene film and stored in the refrigerator at +4°C until the 9th day to determine their total phenolic contents and total aerobic bacteria counts[21].

Chemical analysis

The raw breast meat (3 g) were homogenized in 15 ml of distilled water at 1130 × *g* for 1 min. 10 ml of chloroform was added to the homogenates. The mixture was then shaken strongly 2-3 times and centrifugated at 2090 × *g* for 15 min. The supernatant was used for the measurement of the total phenolic content of the raw breast meat on days 0th and 9th [22]. Total phenolic content (TPC) of the breast meat was estimated by the Folin–Ciocalteu method[23]. The Folin–Ciocalteu reagent (0.2 ml) and 3 ml sodium carbonate solution (5%) were added to a 0.1 mL aliquot. The reaction mixture was vortexed and incubated for 1 h at 23°C. After incubation, the absorbance was measured with a spectrophotometer (Perkin Elmer, 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[22].

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
P available, %	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.

The total aerobic bacteria count (TABC) of the raw breast meat on days 0th and 9th was determined according to the methods of Lee et al.[24]. Media for the enumeration of the total aerobic bacteria were prepared by tryptic soy agar.

For this analysis, breast meat samples (10 g) were homogenized with 90 ml of physiological saline water (0.85% NaCl) using a homogenizer for 2 min and the homogenate was serially diluted 10-fold with saline solution.

Each diluent (100 µl) was spread in triplicate on each agar plate and the plate was incubated at 37°C for 24 h. Colony forming units (CFU) per gram were counted and expressed as log cfu/g.

Left section of the frozen breast meat was used for analysis of

the quality parameters (pH, water holding capacity, drip loss, cooking loss) of the breast meat.

The upper one-third from the left section of the raw breast meat was used for pH measurement. The pH values were determined for 15 min post slaughter (initial pH, pHi) and after chilling for 24 h at +4°C in self-sealed plastic bags, using a protable meat pH meter (Testo 205) equipped with a stainless electrode (pH57-SS) [25].

The water holding capacity (WHC) of the raw breast meat was estimated[26] by centrifuging 1 g of the meat, placed on tissue paper inside a tube for 4 min at 1500 *x g*. The water remaining after centrifugation was quantified by drying the samples at 70°C overnight. WHC was calculated as: (weight after centrifugation-weight after drying)/initial weight x 100.

The drip loss (DL) of each breast meat was measured using the suspension method[27,28]. Each meat sample was trimmed to an approximately equal size (12 cm x 10 cm), weighed, placed in a polyethylene bag and hung at 4°C for 24 h. The meat sample was removed from the bag, blotted dry and reweighed to determine drip loss. DL was calculated as: (initial weight-final weight)/initial weight x 100.

The raw breast meat was weighed before and after cooking to determine percentage of cooking loss (CL). The meat samples (20-25 g) were put in a plastic bag and then cooked for 40 min in a water bath with constant temperature of 70°C. After the meat samples were cooled to room temperature (25°C), removed from the bag and blotted dry. All samples from a given replicate were cooked and chilled as one batch. The cooked breast meat was reweighed to determine the cooking loss[29]. CL was calculated as: (initial weight-final weight)/initial weight x 100.

Statistical analysis

Univariate General Linear Model using the SPSS (17.0)® statistic package[30] was applied to data related to the TPC and TABC of the raw breast meat with a model including dietary treatments (DTs) and storage days (SDs) and interaction between DTs and SDs. In addition, one-way ANOVA using the SPSS (17.0)® statistic package[30] was applied to evaluate the effect of DTs on data related to the raw breast meat quality of broiler. Significant differences between treatment means were separated using Duncan's multiple range test[31]. All statements of significance were based on $P < 0.05$.

Table 2 - The effects of the DTs and the SDs on the TPC of the raw breast meat of broilers on days 0th and 9th of refrigerated storage (μg gallic acid equivalent/g fresh weight).

DTs	Days	TPC
CONT	0	16.115
	9	8.185
TA200	0	19.095
	9	9.419
PPE100	0	18.722
	9	9.644
PPE200	0	19.078
	9	10.020
TA100+PPE100	0	19.529
	9	10.925
SEM[1]		6.646
SDs	0	18.508[a]
	9	9.639[b]
SEM[1]		5.049
DTs	CONT	12.150[c]
	TA200	14.257[b]
	PPE100	14.183[b]
	PPE200	14.549[b]
	TA100+PPE100	15.227[a]
SEM[1]		7.981
P		
DTs		**
SDs		***
DTs x SDs Interaction		ns

[a-c] Values in the same column not sharing a common superscript differ significantly. ** $P < 0.01$, *** $P < 0.001$.

[1] SEM: Standard Error of the Means; DTs: Dietary Treatments; SDs: Storage Days; TPC: Total Phenolic Content.

CONT: a control diet containing 50 mg/kg α-tocopherol acetate and 4% flaxseed oil; TA200: the diet supplemented with 200 mg/kg α-tocopherol acetate to a control diet; PPE100: the diet supplemented with 100 mg/kg PPE to a control diet; PPE200: the diet supplemented with 200 mg/kg PPE to a control diet; TA100+PPE100: the diet supplemented with 100 mg/kg α-tocopherol acetate+100 mg/kg PPE to a control diet.

RESULTS AND DISCUSSION

The effects of the DTs and the SDs on the TPC of the raw breast meat of broilers on days 0th and 9th of refrigerated storage were given in Table 2.

As shown in Table 2, the TPC of the raw breast meat ($P < 0.01$) was increased by the TA200, PPE100, PPE200 and TA100+PPE100 diets compared to that of broilers fed the CONT diet. The highest TPC of the raw breast meat was obtained by feeding the TA100+PPE100 diet. The finding related to the total phenolic content of the raw breast meat of broilers is in agreement with the results published by Saleh et al.[32] who reported that the breast meat of the broilers fed diets supplemented with α-tocopherol acetate at 200 mg/kg level and PPE at the levels of 100 and 200 mg/kg had significantly higher levels of the total phenolic contents compared to a control diet.

Likewise, Kishawy et al.[7] stated that the addition of PPE at levels of 0.05% or 0.1% to the broiler diets containing linseed oil resulted in a significant increase in the total phenolic content of breast meat. In addition, Sharma and Yadav[2] showed that the dietary supplementation of PPE significantly increased the total phenolic content of chicken meat compared to the control meat. In the present study, the high TPC of the raw breast meat may show that breast meat is fortified with the supplementation of natural antioxidant such as PPE. Moreover, 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[32]. In a previous study, it was reported that PPE is a polyphenol natural product[7] and may directly combine with free radicals and lead to their inactivation, which in turn may reduce the intracellular concentration of free radicals such as superoxide, peroxyl and hydroxyl radicals. As a result of this, the polyphenolic compounds in PPE without oxidation are ab-

Table 3 - The effects of the DTs and the SDs on the TABC (log CFU/g) of the raw breast meat of broilers on days 0th and 9th of refrigerated storage.

DTs	Days	TABC
CONT	0	$^{A}3.64^{b}$
	9	$^{A}5.64^{a}$
TA200	0	$^{C}3.15^{b}$
	9	$^{D}5.23^{a}$
PPE100	0	$^{B}3.29^{b}$
	9	$^{B}5.41^{a}$
PPE200	0	$^{B}3.26^{b}$
	9	$^{C}5.35^{a}$
TA100+PPE100	0	$^{C}3.10^{b}$
	9	$^{E}5.17^{a}$
SEM[1]		0.150
SDs	0	3.29^{b}
	9	5.36^{a}
SEM[1]		0.008
DTs	CONT	4.64^{a}
	TA200	4.19^{d}
	PPE100	4.35^{b}
	PPE200	4.31^{c}
	TA100+PPE100	4.13^{e}
SEM[1]		0.013
P		
DTs		***
SDs		***
DTs x SDs Interaction		*

[a-e] Values in the same column not sharing a common superscript differ significantly. *P <0.05, ***P <0.001.
[a-b] Different letters on the right show the interaction between DTs and SDs.
[A-E] Different letters on the left show the interaction between SDs and DTs.
[1] SEM: Standard Error of the Means; DTs: Dietary Treatments; SDs: Storage Days; TABC: Total Aerobic Bacteria Count.
CONT: a control diet containing 50 mg/kg α-tocopherol acetate and 4 % flaxseed oil; TA200: the diet supplemented with 200 mg/kg α-tocopherol acetate to a control diet; PPE100: the diet supplemented with 100 mg/kg PPE to a control diet; PPE200: the diet supplemented with 200 mg/kg PPE to a control diet; TA100+PPE100: the diet supplemented with 100 mg/kg α-tocopherol acetate+100 mg/kg PPE to a control diet.

sorbed from the digestive tract and then distributed, retained and remained functional in broiler meat[32].

The SDs influenced (P <0.001) the TPC of the raw breast meat (Table 2). Prolongation of the refrigerated storage period from 0th to 9th days decreased (P <0.001) the TPC of the raw breast meat (Table 2).

On the other hand, there was no significant interaction between the DTs and the SDs in terms of the TPC of the raw breast meat (P >0.05) (Table 2).

The effects of the DTs and the SDs on the TABC of the raw breast meat of broilers on days 0th and 9th of refrigerated storage were given in Table 3.

As indicated in Table 3, the TABC of the raw breast meat (P <0.001) was reduced by the TA200, PPE100, PPE200 and TA100+PPE100 diets compared to that of broilers fed the CONT diet. This result concurs with the findings published by Dakheli[33] who reported that the total aerobic bacteria count of poultry carcass was decreased by the treatment with pomegranate pomace extract at the levels of 20, 40 and 60 ml/l compared to the control. The low TABC of the raw breast meat of broilers fed diets containing PPE in the current study might be related to the high phenolic compounds of the breast meat[17]. The mechanism of antibacterial activity of the phenolic compounds in PPE is likely to be due to the disorder in the bacteria cell membrane[33]. The SDs influenced (P <0.001) the TABC of the raw breast meat (Table 2). Prolongation of the refrigerated storage period from 0th to 9th days increased (P <0.001) the TABC of the raw breast meat (Table 3).

There was a significant interaction (P <0.05) between the DTs and the SDs in terms of the TABC of the raw breast meat (Table 3). As shown in Table 3, the TABC of the raw breast meat was increased by prolongation of the refrigerated storage period from 0th to 9th days regardless of the DTs (P <0.05). Moreover, there was a significant interaction (P <0.05) between the SDs and the DTs in terms of the TABC of the raw breast meat (Table 3). The TABC of the raw breast meat on both the 0th day and the 9th day of refrigerated storage was decreased by the TA200, PPE100, PPE200 and TA100+PPE100 diets compared to that of broilers fed the CONT diet (P <0.05). The lowest TABC of the raw breast meat on both the 0th day and the 9th day of refrigerated storage was obtained by feeding the TA100+PPE100 diet. This finding is in agreement with the results published by Lee et al.[24] who reported that dietary supplementation of the mixture of gallic and linoleic acid at the levels of 0.5% and 1.0% significantly reduced the total aerobic bacteria count of the meat on the 7th day of refrigerated storage compared to that of the control diet. Likewise, Kamboh et al.[34] pointed out that the total aerobic bacteria count of the breast meat on days 0 and 15 of storage was significantly decreased by the dietary supplementation of the mixture of soy genistein and/or hesperidin at the levels of 5, 10 and 20 mg/kg compared to that of the control diet. In the current study, the observed highest efficiency of dietary supplementation of PPE in combination with α-tocopherol acetate in decreasing TABC of the raw breast meat on both the 0th day and the 9th day of refrigerated storage might be attributed to its higher total phenolic content compared to those of broilers fed the other diets. The lowest TABC of the raw breast meat of broilers fed the TA100+PPE100 diet in the present study may explain the synergistic effect between TA and PPE[35]. The synergistic interactions between TA and PPE may be explained as follows: 1. Regeneration of α-TA through the donation of a hydrogen atom 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[35,36]. The higher phenolic compounds in the breast meat due to the synergistic interactions between TA and PPE interact with pathogen bacterial cell walls, leading to distruption of the cell wall, releasing of cellular contents and leading to cell death. Furthermore, the phenolic compounds also influence protein biosynthesis, change metabolic processes and biofilm formation in bacterial cells and inhibit ATP andd DNA synthesis (suppressing DNA gyrase). This is probably caused by the ability of phenolic compounds to bind proteins associated with the bacteria cell membrane[37].

The effects of the DTs on the pH values at the 15th min and the 24th h, water holding capacity (WHC), drip loss (DL) and cook-

Table 4 - The effects of the DTs on the pH values at the 15th min and the 24th h, WHC, DL and CL of the raw breast meat of broilers of refrigerated storage.

DTs	pH		WHC (%)	DL (%)	CL (%)
	15th min	24th hour			
CONT	6.51[a]	5.97[a]	53.64[b]	2.51	16.93[a]
TA200	6.50[a]	5.91[a]	54.06[ab]	2.44	10.32[b]
PPE100	6.38[b]	5.83[b]	54.25[a]	2.41	10.07[b]
PPE200	6.31[b]	5.84[b]	54.50[a]	2.47	10.86[b]
TA100+PPE100	6.30[b]	5.83[b]	54.87[a]	2.41	10.03[b]
SEM[1]	0.021	0.012	0.351	0.046	0.657
P	***	***	**	ns	***

[a-b] Values in the same column not sharing a common superscript differ significantly. ** P <0.01, *** P <0.001.
[1] SEM: Standard Error of the Means.
DTs: Dietary Treatments.
CONT: a control diet containing 50 mg/kg α-tocopherol acetate and 4 % flaxseed oil; TA200: the diet supplemented with 200 mg/kg α-tocopherol acetate to a control diet; PPE100: the diet supplemented with 100 mg/kg PPE to a control diet; PPE200: the diet supplemented with 200 mg/kg PPE to a control diet; TA100+PPE100: the diet supplemented with 100 mg/kg α-tocopherol acetate+100 mg/kg PPE to a control diet.
WHC: Water Holding Capacity; DL: Drip Loss; CL: Cooking Loss.

ing loss (CL) of the raw breast meat of broilers of refrigerated storage were given in Table 4.

As shown in Table 4, the pH values at the 15th min and the 24th h of the raw breast meat were decreased (P <0.001) by the PPE100, PPE200 and TA100+PPE100 diets compared to those of the CONT and TA200 diets.

This finding is in agreement with the result published by Ahmed et al.[3] who reported that the diets supplemented with three levels (0.5%, 1.0% and 2.0%) of pomegranate by-product significantly decreased the pH of breast meat compared to the control diet. In addition, Chandralekha et al.[38] pointed out that the overall mean pH values of chicken meat balls incorporated with 2.5 and 5.0 percent PPE had significantly lower values than the treatment with BHT at 0.005 and 0.01 percent. Likewise, this result concurs with the findings published by Al-Qazzez et al.[39]. They reported that PPE significantly reduced the pH of minced frozen chicken meat. In contrast, Akuru et al.[6] reported that dietary supplementation of pomegranate peel powder meal at four levels (2, 4, 6 and 8 g/kg) did not influence the pH of the broiler breast meat at the 15th min and 24th hour compared to the positive control diet with 200 mg α-tocopherol acetate per kg. Likewise, Sharma and Yadav[2] pointed out that there are no significant differences among the pH values of control and BHT and PPE treated chicken patties on day 0. In the present study, the pH increase of the control breast samples might be due to the accumulation of metabolites such as ammonia etc. by the utilization of amino acids by bacteria, which are released during protein degradation, because the stored glucose has been depleted. Accumulation of ammonia and the products of amino acid decomposition result in an increase in the pH of the breast meat[2,40]. The lower pH value in the raw breast meat of broilers fed the PPE100, PPE200 and TA100+PPE100 diets in the present study may be attributed to the inhibitory effect of the antibacterial bioactive phenolic compounds such as tannins and phenolic acid in PPE on the proliferation and the growth of spoilage microorganisms that metabolize nitrogen compounds[3,6,40].

As shown in Table 4, the PPE100, PPE200 and TA100+PPE100 diets increased (P <0.01) the WHC of the raw breast meat on the 42th day compared to that of broilers fed the CONT diet. This result is in agreement with the finding published by Sharifian et al.[41] who reported that the supplementation of PPE linearly (250, 450 and 650 mg/kg) to diet of broilers under heat stress increased the WHC values of the breast meat on day 0 compared to the control diet. In contrast, Sharma and Yadav[2] pointed out that there are no significant differences among the WHC values of control and PPE treated chicken patties. The high WHC values in the raw breast meat of broilers fed the PPE100, PPE200 and TA100+PPE100 diets in the present study may be due to their high total phenolic compounds. As a result of this, the phenolic compounds may be absorbed on the protein surface and interact with protein in reversible and irreversible ways that caused to variation in charge distribution. The alteration of charge distribution due to a polyphenol-protein complex may have lead to the increase of the WHC by the supplementation of PPE[42].

Table 4 indicated that there are no significant differences among the DTs in terms of the DL of the raw breast meat of broilers (P >0.05).

This result concurs with the finding published by Mazur-Kuśnirek et al.[43] who reported that there are no significant differences among the diets supplemented with a product of polyphenols (200 mg/kg) (PP200), α-tocopherol acetate (200 mg/kg) (TA200) and PP100+TA100 in terms of the drip loss of the breast meat of broilers exposed to high temperature. However, the same authors also reported that the PP200, TA200 and PP100+TA100 diets significantly decreased the drip loss of the breast meat of broilers under heat stress compared to the negative and positive control diets.

As shown in Table 4, the TA200, PPE100, PPE200 and TA100+PPE100 diets decreased (P <0.001) the CL of the raw breast meat of broilers compared to the CONT diet. Likewise, Al-Qazzez et al.[39] reported that the PPE supplementation significantly decreased the cooking loss of minced frozen chicken meat. It may be attributed to the increase of the WHC of PPE that enhanced the ability of meat to retain and reduced the cooking loss during cooking[44]. Contrary to results of the present study, Sharma and Yadav[2] pointed out there are no significant differences among the cooking losses of the control and BHT and PPE treated chicken patties.

CONCLUSIONS

In conclusion, the effects of the combined supplementation of TA and PPE on the total phenolic contents and the total aerobic bacteria count of the raw breast meat of broilers were higher than when offered alone, revealing a synergistic action. However, the dietary supplementation of TA and PPE in combination similarly improved the water holding capacity and the cooking loss of the raw breast meat of broiler chickens compared to those of the supplementation of TA and PPE alone.

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AUTHOR'S CONTRIBUTION

SS conducted the research and the analyses, collected the data, statistically analyzed the collected data and described them in the manuscript and provided insights for interpretation of results and wrote the manuscript. The author read and approved the final manuscript.

CONFLICT OF INTEREST STATEMENT

The author declare that she has no conflicts of interest.

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