Antioxidant status and thigh meat quality of broiler chickens fed diet supplemented with \( \alpha \)-tocopherolacetate, pomegranate pomace and pomegranate pomace extract

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**ABSTRACT**

The effects of supplementation with \( \alpha \)-tocopherol acetate (\( \alpha \)-Toc), pomegranate pomace extract (PPE) and pomegranate pomace (PP) into chicken feed on antioxidant status, oxidation susceptibility and quality of the thigh meat during refrigeration were investigated. During six weeks broiler chickens were fed eight dietary treatments, which included: control diet, \( \alpha \)-tocopherol diet (200 mg kg\(^{-1}\)), PPE diets (0.1, 0.2 and 0.3 g kg\(^{-1}\)) and PP diets (1, 2 and 3 g kg\(^{-1}\)). Feed efficiency was significantly improved by supplementing chickens fed diet with 0.2 g kg\(^{-1}\) PPE. Long chain polyunsaturated fatty acids (LC PUFA) n-3 level was higher in the thigh of broilers fed with \( \alpha \)-Toc and PPE diets (except 0.1 g kg\(^{-1}\)) than in chickens fed control and PP diets (\( p < .05 \)). Total phenolic content, lipid peroxidation level and 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity in the thigh meat were significantly improved when chickens were fed diets containing \( \alpha \)-Toc and PPE (\( p < .05 \)). Supplementation of different antioxidant preparations into diets had no influence on plasma superoxide dismutase and glutathione peroxidase activities, in contrast, serum lipid peroxidation level was reduced in chickens fed diets supplemented with PPE (except 0.1 g kg\(^{-1}\)) and \( \alpha \)-Toc. In conclusion, the broiler thigh meat may be successfully enriched with LCPUFA n-3 and its antioxidant potential and quality characteristics may be improved by supplementing diets with 0.2 and 0.3 g kg\(^{-1}\) PPE. Moreover, the antioxidant potential of PPE supplementation was equal to that of \( \alpha \)-tocopherol acetate in refrigerated meat.

**Introduction**

Consumer preferences, nutrient profile, availability and low production cost make poultry meat a major source of animal food protein worldwide. Poultry meat enriched with long-chain polyunsaturated fatty acids n-3 (LCPUFA n-3) can be a nutritionally meaningful contribution to western type diet in which content of LCPUFA n-3 is rather low. The enrichment of poultry meat with these fatty acids is usually achieved by fish oil inclusion into broiler diet (Rymer and Givens 2010). However, the meat enriched in such a way is more susceptible to quality deterioration by increased lipid oxidation during storage or cooking, which leads to nutritive value reduction and lipid oxidation products accumulation (Aziza et al. 2010). Oxidation is a very general process affecting lipids, pigments, proteins, DNA, carbohydrates and vitamins (Kanner 1994), however in excess it can be very harmful.

The oxidative stability has been improved by antioxidant supplementation into foods of animal origin. \( \alpha \)-Tocopherols (\( \alpha \)-Toc) or other synthetic antioxidants have been used to control the intensification of lipid oxidation in meat. However, due to consumer concerns about the safety and toxicity of synthetic antioxidants, the recent researches have been focussed on naturally occurring antioxidants. The previous studies have shown that the negative outcome of lipid oxidation in chicken meat was diminished by the use of diets containing antioxidants such as medicinal herb mix, grape pomace and \( \alpha \)-Toc. These are natural antioxidants rich in polyphenols (Sahin and Kucuk 2003; Goñ et al. 2007).

Iran is an important pomegranate (\( Punicagranatum \) \( L \)) producer and exporter in the world and its total production accounted to 990,000 tons in 2016 (Iran Statistical Year Book 2016). Pomegranate pomace (PP) is the residue left after pomegranate juice extraction...
by pressing pomegranates in the juice production industry. The recent investigations have stressed the importance of by-products from plant materials particularly rich in a wide range of polyphenols. The studies have shown that polyphenols have the capacity to act as powerful antioxidants by scavenging free radicals and terminating oxidative reactions (Yilmazand Toledo 2004). The peel and rind of pomegranate are rich sources of tannins, anthocyanins and flavonoids (Naveena et al. 2008). Hydrolysable tannins are the most abundant polyphenols and antioxidant compounds in pomegranates and include gallotannins, ellagitannins and gallagyl esters such as punicalagin and punicalin in condensed tannins (Madrigal-Carballo et al. 2009). The hydrolysable tannins are usually more soluble in water and methanol solvents than condensed tannins (Reed 1995). Methanol extract of PP shows the highest antioxidant activity among all the extracts (Singh et al. 2002). However, no research has been conducted to assess the effect of processing method on the efficacy of pomegranate as a feeding additive. The objective of this study was to evaluate the effects of dietary α-Toc, pomegranate pomace extract (PPE) and PP on antioxidant status, the susceptibility to oxidation and quality of the broiler thigh meat during refrigeration.

**Material and methods**

**Preparation of pomegranate pomace and its extract**

Peels of pomegranates were obtained from pomegranate trees (Ardestani variety) in Khorasan Razavi province (north eastern Iran) in October 2011. The peels were manually removed and air dried under ambient conditions and then powdered in a grinder to pass 40-mesh. The peels were stored at –20°C until extraction. The proximate analysis of PP is shown in Table 1. Dried powder of pomace (2.5 g) was extracted with 40 ml of methanol solvent at room temperature for 6 h. The extract was filtered through Whatman No. 42 filter paper to remove fine particles. After extraction, the solvent was evaporated using a rotary evaporator (under vacuum at 30°C) and the concentrated extracts were stored in a freezer (Çam and Hisıl 2010). Total polyphenol content (TPP) was determined using the Folin–Ciocalteu colorimetric method as described by Çam and Hisıl (2010). A gallic acid aqueous solution (80 g ml⁻¹) was applied for calibration. TPP content was converted into mg of gallic acid equivalents (GAE) per g of dry matter (DM) (mg GAE g⁻¹ DM).

Hydrolysable tannins (HTs) content was determined in three replicates using KIIO₃ according to Çam and Hisıl (2010). The results were expressed as mg of tannic acid equivalent per g of DM (mg TAE g⁻¹ DM). Methanol extraction treated with 5 ml l⁻¹ of HCl-butanol for 3 h at 100°C was used in order to determine condensed tannins (CT). Condensed tannins were calculated from the absorbance at 550 nm of the cyanidin solutions.

**Birds, feeding and management**

The experimental protocol was approved by the Animal Care Committee of the Ferdowsi university of Mashhad (Mashhad, Khorasan Razavi, Iran). 384 1-day-old male broiler chickens (Ross® 308 Broiler) were obtained from a commercial hatchery (Larin Amol, Mazandaran, Iran). Broilers were randomly allotted to eight groups with four replicates pen per treatment and 12 chicks per pen birds and reared for 42 days in a standard condition of temperature (24–32°C), humidity (70%), ventilation and 23 h of constant overhead fluorescent lighting. Eight dietary treatments (Table 2) included control diet without feed additives, control diet mixed with α-Toc (200 mg kg⁻¹), control diet mixed with PPE (0.1, 0.2 and 0.3 g kg⁻¹) and control diet mixed with PP (1, 2 and 3 g kg⁻¹). The source of dietary fibre for control and PPE diets was cellulose. Pomace pomegranate replaced cellulose in PP diets. Water and mash diets were offered ad libitum. In all diets, 2% of fish oil was added to enhance the enrichment with PUFA n–3 fatty acid. All diets were isocaloric and isonitrogenous according to ROSS 308 recommendation. Broilers were weighed at the beginning and at the end of the experiment and feed efficiency were calculated at the end of the experimental period.

**Chemical analysis in blood plasma**

One broiler chicken was randomly selected from each pen at 42 day of life. Blood was collected via wing vein puncture using a syringe and about 2 ml of blood was immediately placed in a heparinised tube and kept on ice to obtain the plasma samples and then
centrifuged. Thiobarbituric acid reactive substances (TBARs) and the activity of antioxidant enzymes including superoxide dismutase (SOD) and glutathione peroxidase (GPx) were determined in plasma (Yagi 1984). The GPx and SOD activity were measured using RANSEL and RANSOD kits (Randox Laboratories Limited, Crumlin, UK). The TBARS concentration in homogenates was measured according to Jo and Ahn (1998) method. The plasma TBARS concentration was expressed as nmol mL$^{-1}$.

**Chemical analysis of thigh broiler meat**

Chickens were scarified by cervical dislocation at 42 day of life. The thigh muscles were trimmed and immediately stored at $-20^\circ$C for further analysis of fatty acid composition. Sample of raw thigh meat was minced twice (4 mm plate) using a grinder and was stored at 4 and $-20^\circ$C for further analyses of antioxidative potential and various meat quality characteristics, which were performed on 0, 7 and 11 day of refrigerated storage ($4^\circ$C).

Each thigh meat sample (5 g) was placed in a distilled water (15 ml) and homogenised (T 10 basic ULTRA-TURRAX, Ika Works Inc., Breisgau, Germany) at 1130 g for 2 min. Chloroform (9 ml) was added to the homogenates and the mixture was vigorously shaken for 2–3 times to separate the lipids. Total phenols content in the aqueous supernatant was estimated by the Folin–Ciocalteu method (Çam and Hısil 2010). 1 mL of diluted sample (1:4, vol/vol) was added to the Folin–Ciocalteu reagent (500 µL) followed by addition of 1 mL of sodium carbonate solution (10%). The reaction mixture was vortexed and the absorbance was measured with a spectrophotometer (UV 1600PC, Shimadzu, Kyoto, Japan) at 700 nm after 1h of incubation at room temperature. The quantification of phenolics was done based on the standard curve generated with gallic acid and expressed as gallic acid equivalent.

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was estimated with the aqueous supernatant obtained from thigh meat according to Blois (1958) method with slight modifications. Briefly, 200 µL of diluted aqueous supernatant was added to 800 µL of water and 1 mL of methanolic DPPH solution (0.2 mM). The mixture was vortexed and left to stand at room temperature for 30 min. A tube containing 1 mL of distilled water and 1 mL of methanolic DPPH solution (0.2 mM) served as the control. The

| Table 2. Ingredients and nutrient composition of experimental diets. |
|------------------------|---------------|------------------|------------------|------------------|
|                       | α-Toc, mg kg$^{-1}$ | PPE, g kg$^{-1}$ | PP, g kg$^{-1}$ |
| Indices | Control | 0.1 | 0.2 | 0.3 | 1 | 2 | 3 |
| Ingredients, g kg$^{-1}$ fed | | | | | |
| Maize Corn (8.1% CP) | 544.00 | 544.00 | 544.00 | 544.00 | 544.00 | 544.00 | 544.00 |
| Soya bean | 315.60 | 315.60 | 315.60 | 315.60 | 315.60 | 315.60 | 315.60 |
| Gluten | 40.00 | 40.00 | 40.00 | 40.00 | 40.00 | 40.00 | 40.00 |
| Animal fat | 35.00 | 35.00 | 35.00 | 35.00 | 35.00 | 35.00 | 35.00 |
| Fish oil | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 | 20.0 |
| Cellulose | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| PP | | | | | | | |
| Limestone | 10.7 | 10.7 | 10.7 | 10.7 | 10.7 | 10.7 | 10.7 |
| Salt | 4.70 | 4.70 | 4.70 | 4.70 | 4.70 | 4.70 | 4.70 |
| Vitamin-mineral premix kg$^{-1}$ | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| DL-methionine | 2.60 | 2.60 | 2.60 | 2.60 | 2.60 | 2.60 | 2.60 |
| L-lysine | 2.80 | 2.80 | 2.80 | 2.80 | 2.80 | 2.80 | 2.80 |
| DL-threonine | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 | 0.60 |
| Calculated composition, % DM | | | | | | | |
| AME, MJ kg$^{-1}$ | 13.12 | 13.12 | 13.12 | 13.12 | 13.12 | 13.12 | 13.12 |
| Crude protein, % | 21.43 | 21.43 | 21.43 | 21.43 | 21.43 | 21.43 | 21.43 |
| Crude fibre, % | 5.51 | 5.51 | 5.51 | 5.51 | 5.51 | 5.51 | 5.51 |
| Calcium | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 |
| Available P | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 | 0.55 |
| Calculated polyphenol composition, g kg$^{-1}$ DM | 1.03 | 1.04 | 1.11 | 1.19 | 1.27 | 1.22 | 1.41 |
| TPC | 0.74 | 0.75 | 0.80 | 0.86 | 0.92 | 0.87 | 1.00 |
| CT | 0.00 | 0.00 | 0.01 | 0.02 | 0.03 | 0.07 | 0.13 |

PPE: pomegranate peel extract; PP: pomegranate peel; AME: Apparent Metabolism Energy; TPC: total polyphenol content; HTs: hydrolysable tannins; CT: condensed tannin; α-Toc: α-tocopherol; Vitamin-mineral mix suppling per kg of diet: mg:α-tocopherol 30, cholecalciferol 62.5, menadione sodium bisulphite 3, thiamine hydrochloride 1, riboflavin 5, pyridoxine hydrochloride 3, cyanocobalamin 0.02, niacin 30, pantothenic acid 10, folic acid 0.8, biotin 0.05 ascorbic acid 10, choline chloride 480, Mn 55, Zn 50, Fe 85, Cu 5, Se 0.1 and I 0.18. 
Table 3. Effect of diet supplementation with α-tocopherol (α-Toc), pomegranate pomace extract (PPE) and pomegranate pomace (PP) on feed intake, daily weight gain and feed conversion ratio of broilers (0–42 days).

| Indices                          | Control | 200 | 0.1 | 0.2 | 0.3 | 1 | 2 | 3 | p value | SEM |
|---------------------------------|---------|-----|-----|-----|-----|---|---|---|---------|-----|
| Body weight gain, g per bird per day | 55.48 a | 55.02 b  | 52.83 b  | 56.08 a  | 54.14 d | 50.94 b  | 48.67 c | 46.02 a | .01    | 4.80 |
| Feed intake, g per bird per day  | 106.21 a | 104.18 a | 103.71 a  | 105.12 a  | 106.31 a | 103.47 ab | 100.57 b | 98.56 b | .01  | 8.42 |
| Feed efficiency                 | 1.79     | 1.78     | 1.83 b  | 1.74 d  | 1.80     | 1.80     | 1.80     | 1.80     | .01  | 0.73 |

α-Toc, mg kg⁻¹  PPE, g kg⁻¹  PP, g kg⁻¹

Mean values with different superscripts within a column are significantly different at p < .05; SEM: standard error of means.

absorbance of the solution was measured at 517 nm using a spectrophotometer (UV 1600 PC, Shimadzu, Kyoto, Japan). The percentage of DPPH radical scavenging was obtained from the following equation:

Radical–scavenging activity
= [1 – (absorbence value of testing solution / absorbence value of control solution)] × 100

Each meat sample stored at −20 °C (5 g) from various storage periods was placed in 15 mL distilled water and homogenised at 1130 g for 1 min (T 10 basic ULTRA-TURRAX, Ika WorksInc., Breisgau, Germany). Sample homogenate (5 mL) was transferred to a test tube and lipid oxidation was determined by the TBARS value using the method described by Ahn et al. (1999). Briefly, 50 μL of butylatedhydroxyanisol (7.2%) and 5 mL of TBA-trichloroacetic acid solution (20 mM TBA in 15% trichloroacetic acid) were added to the test tube. Tubes were heated in a boiling water bath for 15 min, cooled and then centrifuged at 966 g for 15 min. Absorbance of the supernatant was measured at 532 nm with a spectrophotometer (UV 1600 PC, Shimadzu, Kyoto, Japan). Lipid oxidation was reported as mg of malondialdehyde (MDA) per kg of meat.

The sample total lipids were extracted using chloroform:methanol (2:1, v/v) mixture according to Folch et al. (1957) procedure. The fatty acid (FA) methyl esters were prepared from the extracted lipids with BF₃-methanol (Sigma-Aldrich, St. Louis, MO). The fatty acid methyl esters were then separated with the use of a UNICAM gas chromatograph (UNICAM, Wisford, UK) equipped with a flame ionisation detector (FID). A split inlet (split ratio, 50:1) was used to inject samples into a sun grid engine (SGE) capillary column (30 m × 0.22 mm × 0.25 μm) and a ramped oven temperature was used (160 °C for 3 min, increased to 180 °C at 2.5 °C min⁻¹ and maintained for 5 min and then increased to 220 °C at 2.5 °C min⁻¹ and maintained for 25 min). The injector temperature was programmed at 240 °C and the detector temperature was tuned to 280 °C. Helium was the carrier gas at constant flow of 0.7 mL min⁻¹. Fatty acids (g of weight per 100 g of total fatty acids) were identified by comparison of retention times to known standards. Relative quantities of fatty acids were expressed in each sample.

Statistical analysis
The obtained data were subjected to analysis of variances (ANOVA) using the GLM procedure of SAS (Version 9.1, SAS Institute, 2003) and single degree of freedom linear contrast was used to separate treatments. Linear and quadratic effects were also analysed. Significant differences among treatments were determined at p < .05 by Duncan’s multiple range test.

Results
Broiler performance
The addition of PP into diets impaired chicken growth performance indices such as body weight (BW) gain, feed intake and feed efficiency (Table 3) as compared to those fed with the un-supplemented diet or diets supplemented with PPE and α-Toc (p < .05). Feeding diet with 0.2 g kg⁻¹ PPE significantly improved the chickens daily body weight gain and decreased the feed efficiency with no effect on daily feed intake.

Blood indices
The supplementation of diet with PP, PPE and α-Toc did not influence the activity of SOD and GPx (Table 4). Nevertheless, the birds given the antioxidant diet showed a tendency towards lower TBARS concentrations in plasma in comparison to the control group suggesting a decrease in lipid peroxidation. The plasma lipid peroxidation level was decreased in all birds fed diets with additional antioxidants (p < .5) in comparison to the control ones. The more pronounced effect was observed in group fed the α-Toc and PPE (except 0.1 g kg⁻¹) diets.

Meat quality
The FA composition in thigh meat was modified by examined antioxidants supplementation (Table 5). The concentrations of myristic acid (C14:0), palmitic...
Table 4. Effect of diet supplementation with α-tocopherol (α-Toc), pomegranate pomace extract (PPE) and pomegranate pomace (PP) on antioxidant enzymes activity and lipid peroxidation (thiobarbituric acid reactive substances (TBARS) content) in broilers blood at 42 day of age.

| Indices                      | Control | 200  | 0.1   | 0.2   | 0.3   | 1     | 2     | 3     | p value | SEM |
|------------------------------|---------|------|-------|-------|-------|-------|-------|-------|---------|-----|
| GPx activity, U l⁻¹          | 176     | 181  | 180   | 193   | 192   | 174   | 181   | 178   | .12     | 30.3|
| SOD activity, U l⁻¹          | 154     | 162  | 169   | 179   | 178   | 166   | 169   | 157   | .09     | 26.6|
| TBARS content, nmol ml⁻¹     | 8.95a   | 6.76b| 6.69b | 6.66b | 6.76b | 7.3a  | 7.2a  | 7.4a  | .05     | 3.22|

Statistical significance (p value of contrast)

- control vs. α-Toc
- control vs. PPE
- control vs. PP
- α-Toc vs. PPE
- α-Toc vs. PP
- PPE vs. PP

| Type of response due to percentage of PPE in diet | Linear | Quadratic |
|--------------------------------------------------|--------|-----------|
| Linner                                           | .05    | .05       |
| Quadratic                                        | .23    | .39       |

| Type of response due to percentage of PP in diet |
|--------------------------------------------------|
| Linner                                           | .28    |
| Quadratic                                        | .36    |

GPx: glutathione peroxidase; SOD: superoxide dismutase; SEM: standard error of means. All data points represent values of four analyses (one bird per replicate).

Table 5. Fatty acids composition of thigh (mg kg⁻¹) of broiler chickens fed diets supplemented with α-tocopherol (α-Toc), pomegranate pomace extract (PPE) and pomegranate pomace (PP).

| Fatty acids | α-Toc, mg kg⁻¹ | PPE, g kg⁻¹ | PPE, g kg⁻¹ |
|-------------|----------------|-------------|-------------|
|             | Control 200    | 0.1 0.2 0.3 | 1 2 3       |
| C14:0       | 109.72         | 107.64      | 108.77      |
| C16:0       | 3088.90        | 3088.90     | 3088.75     |
| C16:1       | 513.52         | 460.56      | 483.35      |
| C18:0       | 1238.48        | 1370.08     | 1342.15     |
| C18:1       | 3490.34        | 3513.35     | 3503.72     |
| C18:2       | 2931.09        | 3119.53     | 2971.79     |
| C18:3       | 161.39         | 194.40      | 172.26      |
| C20:3       | 160.85         | 171.37      | 163.11      |
| C20:4       | 186.05         | 197.43      | 184.70      |
| EPA         | 160.66         | 197.12      | 164.26      |
| DPA         | 461.82         | 529.67      | 488.59      |
| DHA         | 195.46         | 239.94      | 207.76      |
| SFA         | 4537.10        | 4533.62     | 4539.49     |
| MUFA        | 3968.86        | 3960.19     | 3997.50     |
| PUFA        | 4257.31        | 4443.46     | 4422.49     |
| n-3         | 979.33         | 1155.41     | 1022.88     |
| LC-n-3      | 817.94         | 960.73      | 860.62      |
| n-6         | 3277.98        | 3488.32     | 3389.60     |
| n-6+n-3     | 3.35           | 3.02d       | 3.28ab      |

| p value | SEM |
|---------|-----|
| 10.05   | 30.3|
| .09     | 26.6|
| .05     | 3.22|
| .05     | 3.22|
| .05     | 3.22|
| .05     | 3.22|
| .05     | 3.22|
| .05     | 3.22|

Table 6. Indices of control diet.

- PPE, g kg⁻¹/C0
- α-Toc, mg kg⁻¹/C0
- -Toc, mg kg⁻¹/C0

EPA: eicosapentaenoic acid; C20:5; DHA: Docosahexaenoic acid, C22:6; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; n-3 and n-6; C18:2, C18:3, C18:1, C16:0, C16:1, C18:1, C18:2, C20:3, C20:4; SEM: standard error of means. All data points represent mean fatty acid content from four analyses (one bird per replicate).

α-Toc, α-tocopherol; PPE, Pomegranate Pomace Extract; -Toc, -tocopherol; PPE, pomegranate pomace extract; PP, pomegranate pomace; α-Toc, α-tocopherol.
C18:3; except 1 and 3 g PP kg\(^{-1}\) diet), eicosapentaenoic acid (EPA, C22:5; except 0.1 and 1 g PP kg\(^{-1}\) diet), docosapentaenoic acid (DPA, C20:6) and docosahexaenoic acid (DHA, C22:6; only 0.3 g PPE kg\(^{-1}\) diet) in the thigh meat of broilers fed diet containing PP, PPE and \(\alpha\)-Toc was higher than that of the control birds \((p < .05)\). So, the sum of n-3 PUFA as well as long chain PUFA (LC PUFA) n-3 was increased in all examined groups but the increase was pronounced mostly in chickens fed diet supplemented with PPE at a dose of 0.2 and 0.3 g kg\(^{-1}\). The n-6/n-3 ratio in the thigh meat from broilers was decreased by dietary supplementation with PP, PPE and \(\alpha\)-Toc \((p < .05)\).

In thigh meat of broilers fed diets containing \(\alpha\)-Toc and PPE (except diet containing 0.1 g PPE kg\(^{-1}\) diet) significantly higher total phenolic content was estimated when compared to groups fed with control or PP-supplemented diets \((p < .05\); Figure 1\).

Two indicators (DPPH scavenging ability and MDA content) were used to evaluate the antioxidant effect of used antioxidants in meat. The extent of lipid oxidation, as measured by malondialdehyde (MDA) formation, differed \((p < .01)\) between treatments after 1, 7 and 11 day of refrigeration (Figure 2). The development of lipid oxidation in the thigh meat from broilers was delayed by the addition of all three antioxidants (PP, PPE and \(\alpha\)-Toc). The MDA value of all meat samples gradually increased with shelf life. The thigh meat of broilers fed PPE, \(\alpha\)-Toc and PP supplemented diets contained lower content of MDA in spite of the higher content of PUFA LCN-3 in diets \((p < .05)\). The dietary treatment had a major impact on the DPPH radical scavenging activity of the meat during the refrigerated storage (Figure 3). The DPPH radical scavenging activity of the thigh meat from the broilers fed diets supplemented with \(\alpha\)-Toc and 0.2 and 0.3 g kg\(^{-1}\) PPE was significantly higher than that of the control birds during the entire storage period, whereas lower significant difference was found in broilers fed diets with PP in comparison to control birds \((p < .05)\). In this experiment, the inclusion of \(\alpha\)-Toc and PPE was more effective than PP in lipid oxidation reduction. However, diets with addition of PPE (0.2 and 0.3 g kg\(^{-1}\)) roughly exhibited similar performance of lipid oxidation reduction when compared to \(\alpha\)-Toc diet.

**Discussion**

The inclusion of PP into diets impaired growth performance of chickens. The addition of 15 g kg\(^{-1}\) PP into diets caused decreased BW and feed intake in broilers (Rajani et al. 2011). In the current study, PP contained 19% of total extracted by the Folin method compound polyphenols and HTs and CT constituted 13.7% and 0.6% of total phenols, respectively (Table 2). Similarly, the effect of polyphenols has also been studied in chickens using ingredients such as grape seed extract, grape pomace, sorghum and faba bean. In general, relatively high dietary concentrations of polyphenols caused by the addition of these ingredients, reduced performance in chickens as well as in other livestock animals (Gori et al. 2007). Nevertheless, in our study, reduced chickens performance was only observed in birds fed diets with PP. The low concentration of condensed tannins present in the PPE diet may be relatively low to produce a growth depression.

Feed intake, growth rate and feed efficiency of the poultry are impaired by environmental stress, mainly heat stress (Bartlett and Smith 2003). Improved feed efficiency of quail was reported by
supplementation of vitamin C as an antioxidant that affected the utilisation of dietary nutrients (Sahin and Kucuk 2003). Previous studies reported that dietary antioxidants, such as vitamin C, E, flavonoids and phenolics, could reduce oxidative damage in animals, which is generated by different stress sources (Brisibe et al. 2009). Therefore, the dietary supplementation of PPE, which contained natural antioxidant HTs, may improve feed efficiency by reducing oxidative damage in broilers fed diet supplemented with fish oil.

Figure 2. Effect of diet supplementation with pomegranate pomace extract (PPE), pomegranate pomace (PP) and \( \alpha \)-tocopheryl acetate (\( \alpha \)-Toc) and refrigerated storage duration on lipid oxidation in raw chicken thigh meat MDA -malondialdehyde, a–e bars with different superscripts are significantly different at \( p < .05 \). All data points represent mean MDA content from four analyses ± SEM (one bird per replicate).

Figure 3. Effect of diet supplementation with pomegranate pomace extract (PPE), pomegranate pomace (PP) and \( \alpha \)-tocopheryl acetate (\( \alpha \)-Toc) on DPPH radical scavenging activity in raw chicken thigh meat, %DPPH - 1,1-diphenyl-2-picrylhydrazyl; a–e bars with different superscripts are significantly different at \( p < .05 \). All data points represent mean DPPH concentration from four analyses ± SEM (one bird per replicate).
Several enzymes such as SOD and GPx can be scavenging formed reactive oxygen species (ROS) which act as antioxidants. Endogenous protection against oxidative stress is achieved by enzymes that catalytically remove free radicals and other reactive species. Mice fed with pomegranate juice revealed decreased protein and DNA damage by declining Glutathione (GSH) and Glutathione disulphide (GSSG) levels without changing the GSH/GSSG ratio and by decreasing hepatic antioxidant endogenous enzymes (GPx, catalase, SOD and GST), most probably in relation with less ROS production (Faria et al. 2007). The antioxidant defence systems include antioxidants (natural or synthetic) and the antioxidant enzymes present in the biological system. Increasing the activity of SOD and GPx would subsequently enhance the clearance capacity of oxygen free radicals in broilers. Together with the increased activity of SOD and GPx, TBARS concentration in the serum was reduced by inclusion of ginger into broiler diets (Zhang et al. 2009; Akbarian et al. 2015). Malondialdehyde is formed as an end-product of lipid peroxidation and therefore, the extent of lipid peroxidation ROS can be monitored by TBARS level. Vitamin E, a major chain-breaking antioxidant of membranes, can scavenge the hydroxyl, alkoxyl, peroxyl and superoxide anion radicals and increase membrane stability. The reduced serum TBARS level in antioxidant-supplemented birds in comparison to those in control group indicated that lipid peroxidation was reduced by PPE and α-Toc via enhancing antioxidant action.

The FA composition of the diets, particularly LCPUFA n-3, was modified and was increased by the inclusion of fish oil into diet. The concentration of SFAs in the thigh meat was not influenced in broilers fed antioxidant diets in comparison to those fed with control diet. The results indicated that the broiler tissue have a limited capacity for SFA alteration. The SFA in poultry are mainly derived from the diet and later from the synthesis in the liver. According to Ayerza et al. (2002), the increase storage of PUFA decreased the synthesis of MUFA by inhibiting via the activity of 9-desaturase complex that is the key enzyme needed to convert SFAs to MUFAs. This was also confirmed in our study.

Content of n-6 fatty acid in the thigh meat was significantly increased by the diet containing antioxidant. Crespo and Esteve-Garcia (2002) has shown that the fatty acid profiles in broiler meat reflect the composition of diet fed to animals. Feeding pigs and broilers diets with the addition of sunflower oil (as a source of LA) resulted in the increase of LA and AA levels in the meat. In our study, the n-6 fatty acid storage amount in thigh tissue of chickens fed diets containing antioxidants (PPE, PP and α-Toc) were the highest and almost the same in all diets. Probably, it was connected with the ability of antioxidants to decrease the free radicals in the diet, tissue and in the storage time and they consequently reduce oxidation. Some authors have also found no variation in FA composition of broiler thighs when animals were fed diets supplemented with α-Toc (Bou et al. 2004).

One of the major results of our study was that the supplementation of antioxidant in diets appeared to improve storage of the LCPUFA n-3 in thigh meat. The antioxidative effects of phenolics compound added to diets can explain this. Diet containing antioxidants may inhibit the oxidation of PUFA. Docosahexaenoic acid levels in the breast meat of broilers fed dietary mixture of gallic acid and linoleic acid were increased. This was explained by the antioxidative effect of gallic acid (Jung et al. 2010). Dietary α-TOC, pomegranate by-products and sorghum have been shown to increase long-chain n-3 fatty acids such as eicosapentaenoic acid and DHA in eggs and broiler thigh meat (Cherian et al. 1996). Our study illustrates a plausible approach to increase the storage of long-chain n-3 PUFA content of broiler meat through dietary supplementation of 0.2 or 0.3 g kg⁻¹ PPE and 200 mg kg⁻¹ α-Toc and it enabled reasonable consumer acceptability scores to be maintained even after storage times.

The n-6/n-3 ratio in broiler thigh meat was decreased by dietary supplementation of PP, PPE and α-Toc. These results can be attractive to the consumers as low n-6/n-3 ratio has a positive effect on human health, mainly it protects against cardiovascular disease (Krauss et al. 2001).

Polyphenolic compounds in PPE are distributed, retained and remain functional in the muscle (Sáyago-Ayerdi et al. 2009; Saleh et al. 2017). Nagendra Prasad et al. (2009) suggested that polyphenol content showed highest relations with total antioxidant capacity ($R^2 = 0.9773$). Our findings also suggest that polyphenols present in PPE were absorbed at sufficient amounts to contribute to the protection of PUFA LC n-3 in membranes and to modulate the antioxidant activity in serum and muscle tissue.

Two indicators (DPPH and TBARs) were used to evaluate the antioxidative effect of dietary PP, PPE and α-Toc in thigh meat. The DPPH was used as a free radical to evaluate antioxidant activity present in natural source of compounds (Schwarz et al. 2009). A solution of DPPH, stable free radical, is mixed with an antioxidant that can donate a hydrogen atom to form a
stable DPPH-H molecule. Phenolic compounds present in natural plant oils react with lipid and hydroxyl radicals and convert them into stable products (Naveena et al. 2008). The antioxidant compounds present in pomegranate have already been identified as gallic acid, ellagic acid, proanthocyanidins, etc. The phenolic property of pomegranate peel may act in a similar way as reductions by donating electrons and reacting with free radicals to convert them to more stable products and terminating free radical chain reactions (Negi and Jayaprakasha 2003; Saleh et al. 2017).

The TBARS values of all kinds of meat increased along with shelf life. Supplementing diets with antioxidants revealed less peroxidation by producing lower amounts of TBARS. LC PUFA n-3 family with the potential to generate several types of free radicals can accelerate lipid peroxidation (Bou et al. 2004). These results are in line with other studies depicting that the lipid oxidation is decreased in broilers fed with various PUFA levels and α-Toc acetate. This was inhibited due to the antioxidant activity of α-Toc acetate and pomegranate by-products (Rymer and Given 2010; Sonia et al. 2015). The large amount of phenolics present in PP and PPE diets may cause its strong antioxidant ability, extend the shelf life and improve the quality of meat products. Sáyago-Ayerdi et al. (2009) indicated that the inhibition of TBARS values in meat could be due to a protective effect derived from the polyphenols present in added grape pomace which may act similarly to vitamin E on the lipid bilayers in the meat. Goni et al. (2007) demonstrated that grape pomace concentrate supplementation was equal in antioxidant potential to vitamin E. Based on observations of Rajani et al. (2011), diet containing 15 g kg⁻¹ pomegranate peel was able to reduce the TBARS occurrence in meat of birds in comparison to control birds.

In this experiment, the inclusion of α-Toc and PPE was more effective than PP in reducing the oxidation. However, PPE (0.2 and 0.3 g kg⁻¹) roughly had similar ability to α-Toc in reducing the oxidation. This indicates that PPE had higher availability than PP in broiler diet. Another reason that needs to be mentioned is the higher digestibility of hydrolysable tannins than condensed tannins in PPE diets. However, other compounds or mechanisms may be responsible for this antioxidant activity.

Using TBARS concentration as an index of absorption of polyphenols and based on the digestibility values obtained in the current experiment, this study showed that polyphenolic antioxidant compounds in PPE were dispersed, retained and remained functional in the muscle. Because there is no precise sensitive method available so far for the identification of the antioxidant constituents deposited in muscle, the presence of these compounds cannot yet be directly demonstrated.

Conclusions

In conclusion, the broiler thigh meat may be successfully enriched with long chain polyunsaturated fatty acids n-3 and its antioxidant potential and functional quality characteristics may be improved by diet supplementation with 0.2 and 0.3 g kg⁻¹ PPE. Moreover, the PPE supplementation is as effective as α-tocopherolacetate one in enriching broiler meat and exerts much more pronounced effect than PP.

Disclosure statement

No potential conflict of interest was reported by the authors.

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