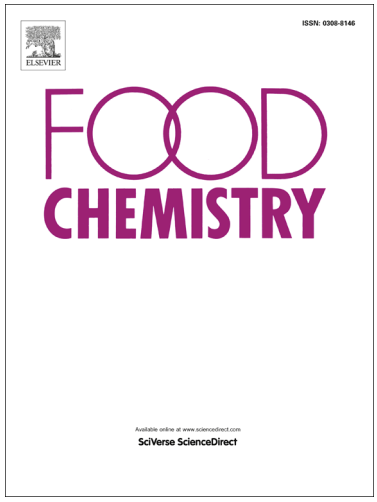

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Meat composition, fatty acid profile and oxidative stability of meat from broilers supplemented with pomegranate (*Punica granatum* L.) by-products

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RUNNING TITLE: Feeding pomegranate by-products to improve broiler meat quality

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ABSTRACT

The effects of diets supplemented with four levels (0, 0.5, 1.0 and 2.0%) of pomegranate by-product (PB) on meat composition, fatty acid profile and oxidative stability of broiler meat were evaluated. The crude protein and moisture contents increased, whereas ether extract in breast and thigh meat and cholesterol in breast meat decreased in response to dietary PB supplementation ($p < 0.05$). In breast and thigh meat, the sum of saturated fatty acids was lower, while the sum of mono-unsaturated and n-3 fatty acids were higher, alongside lower n-6/n-3 ratio in the 1.0 and 2.0% PB supplemented group ($p < 0.05$). The TBARS values and pH of breast and thigh meat were reduced in the PB supplemented groups ($p < 0.05$). Overall, the results presented herein indicate that supplementation of diets with up to 2% pomegranate by-products improved the meat composition, fatty acid profile and reduced lipid oxidation of broiler meat.

Keywords:

Broiler chickens

Fatty acids

Meat composition

Pomegranate by-products

TBARS

1. Introduction

The production and consumption of chicken meat has become very popular worldwide owing to its desirable nutritional characteristics, such as high protein, low fat and relatively high concentrations of polyunsaturated fatty acids (PUFAs) compared to beef or pork (Brenes and Roura, 2010). In addition, the reported health benefits of PUFAs, especially n-3 PUFAs, on human health have created a trend toward replacement of saturated fats with unsaturated fats in poultry products through dietary manipulation. However, the higher level of PUFAs in muscle membranes increases the susceptibility of oxidative deterioration of lipid (Engberg et al., 1996), which impairs the organoleptic characteristics and shortens the shelf-life of meat and meat products. Lipid peroxidation also produces free radicals (peroxyl and hydroxyl radicals), which are reportedly associated with carcinogenesis, mutagenesis and aging (Yagi, 1987). In addition to the proportion of PUFA in muscle membranes, the amount of reactive oxygen species produced and the levels of endogenous or nutritional antioxidants also influence the lipid oxidation process (Brenes et al., 2008). Antioxidants are incorporated within cell membranes, where they scavenge the active forms of reactive oxygen species involved in the initiation step or progression of oxidation, and thus maintain the overall quality of meat (Descalzo & Sancho, 2008). Therefore, there is a need to increase the antioxidation capacity of muscles that can be achieved by supplementation of the diet with antioxidants.

Synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, and tertiary butyl hydroquinone were previously used to retard or minimize oxidative deterioration by scavenging free radicals or diminishing the formation of lipid radicals (Fasseas et al., 2008). However, owing to their possible carcinogenicity (Mahdavi and Salunkhe, 1995) and residues found in meat products and the environment, considerable interest has risen in the use of natural antioxidants as bio-preservatives, such as tocopherols,

carotenoids, flavonoids, and phenolic acids. Bio-preservatives are mainly derived from plant extracts, and the majority of plants produce these compounds as secondary metabolites (Vaithiyanathan *et al*., 2011). Pomegranate (*Punica granatum* L.), is a fruit that belongs to the *Punicaceae* family, has been used for years in folk medicine for a number of therapeutic purposes. About 50% of the total fruit weight corresponds to the peel, which is an important source of several bioactive compounds including hydrolysable tannins (ellagitannin, punicalagin, punicalin and pedunculagin) (Kanatt et al., 2010), flavonoids, anthocyanins and other phenolic compounds (Li et al., 2006). The aqueous and alcoholic extract of pomegranate peel contains 122.33 ± 6.42 and 176 ± 5.29 mg/g gallic acid equivalent phenolic content, 135.33 ± 8.08 and 81.33 ±6.1 mg/g quercetin equivalent flavonoid and 81.66 ± 3.51 and 114.23 ± 12.16 mg/g tannic acid equivalent tannins, respectively (Rajan et al., 2011). The biological significance of these compounds is immense due to the enormous reducing power and scavenging of free radicals (Rajan et al., 2011). Pomegranate peel also contains several minerals, primarily K, N, Ca, P, Mg, Fe and Na (Mirdehghan and Rahemi, 2007), as well as complex polysaccharides (Jahfar et al., 2003). The seeds comprise 10% of the total fruit weight and are a rich source of total lipids with a high concentration of PUFAs (linolenic and linoleic acid) and other fatty acids (Fadavi et al., 2006). The seeds also contain protein, vitamins, minerals, pectin, sugars, isoflavones (mainly genistein), and polyphenols, as well as serving as the richest plant source of estrogens and other steroids (Prakash and Prakash, 2011).

Although the antioxidant activity of pomegranate juice and peel extract has been demonstrated by many scientific studies on fresh and processed chicken meat (Naveena et al., 2008; Vaithiyanathan et al., 2011), there is currently no published research regarding the effects of supplementing broiler diets with pomegranate. Therefore, in this study, we investigated whether feeding pomegranate by-product (a combination of peels, rinds and

seeds) to broilers improves meat quality, specifically, meat composition, fatty acid profile and oxidative stability without any negative effect on growth performance.

2. Materials and methods

2.1. Preparation of pomegranate by-product

Pomegranate by-product (PB) (Goheung-gun cultivar, Korea) was obtained from a juice manufacturing company which included ~80% of peel and rind and ~20% of seed. The by-products were then dried in a forced air oven (Doori TEC, Doori TEC, FA, Co., Ltd) at 80°C for 3 days and subsequently grinded into powder using a milling machine to pass through a 0.15-mm sieve, tightly packed in polythene plastic bags, sealed and kept at room temperature until required. The fresh and dried PB was analyzed in triplicate for crude protein (CP), ether extract (EE), moisture and ash as described by Association of Official Analytical Chemists (AOAC, 2000). The fatty acid composition was determined by a direct method for fatty acid methyl ester (FAME) synthesis using a gas chromatograph (GC). The pH was measured using a digital pH meter (Docu-pH+ meter, Sartorius, USA).

2.2. Experimental design, dietary treatments and management

A total of 320 one-day-old Ross 308 male broiler chicks were purchased from a commercial hatchery, weighed and randomly allocated into four treatment groups with ten replicate pens of eight birds (4 diets × 10 replicates) in a completely randomized design. The dietary treatments were as follows: (1) basal diet without any supplementation (PB 0%); (2) basal diet plus 0.5% PB; (3) basal diet plus 1.0% PB; (4) basal diet plus 2.0% PB. The basal

diet was formulated to meet the Nutrient Requirements of Poultry (NRC, 1994, Washington DC, USA) and was applied during a starter (0-21 d) and finisher (22-35 d) period. All diets were in mashed form. The ingredients, chemical composition, and vitamin and mineral content of the basal diets are shown in Table 1.

Broilers were kept in a closed, ventilated, wire-floor caged broiler house (76 cm long × 60 cm wide × 40 cm high/cage) at a stoking density of 570 cm^2/bird. The cages had a linear feeder in the front and a nipple drinker in the back to provide *ad libitum* feed intake and free access to water. House temperature was set and maintained at 34°C for 1 to 7 d, after which it was gradually reduced to 23°C at a rate of 3°C per week and then maintained at this temperature until the end of the experiment. The relative humidity was maintained at around 50% throughout the experiment. Continuous lighting was provided throughout the experimental period, and no vaccination or medication program was followed. Chicks were inspected daily and dead birds were removed following recoding of mortality (pen, date and body weight). Feed intake and body weight (BW) were recorded every week by replicate and the average daily feed intake (ADFI), average daily gain (ADG), and FCR (feed to gain ratio) per cage were then calculated by period and for the entire experimental period.

2.3. Sampling and analysis of meat

2.3.1. *Slaughter and sampling procedure*

At 36 days of age, two broilers from each replicate cage (20 birds per treatment) were randomly selected and slaughtered by cutting their jugular veins. The breast and thigh meat were then excised from the carcass by removing the skin, bones and connective tissue. The breast and thigh meat samples from each bird were weighed and ground separately with a meat grinder, after which they were divided into two parts, one for oxidative stability analysis

and another for proximate composition, cholesterol, trace mineral and fatty acid composition analysis. The samples were poured into plastic sample bottles, after which those for oxidative rancidity analysis were refrigerated at 4°C and samples for other analyses were stored at -20°C.

2.3.2. *Analysis of meat proximate composition, cholesterol and trace minerals*

The moisture (930.15), total ash (942.05), CP (990.03), and EE (991.36) contents of the breast and thigh meat samples were analyzed in triplicate according to the method described by the AOAC (2000).

The cholesterol determination was derived from fat, which was separated via extraction of 5 g of minced meat (mixed with reference material; 0.5 ml of 5α-cholesterol) with a chloroform and methanol mixture (2:1 vol:vol; Folch et al., 1957). Cholesterol was separated from fat after saponification with KOH and extraction with ethyl ether by the modified method described by King et al. (1998). The samples were then subjected to chromatographic analysis in a DS 6200 gas chromatograph (Donam Co., Seongnam, Gyeonggi-do, Korea) with a flame ionization detector and a Hewlett Packard HP-5 capillary column (J&W Scientific, USA) 30 m in length with a 0.32 mm internal diameter and 0.25 μm polyethylene glycol-film thickness. Nitrogen was used as the carrier gas. The initial oven temperature was held at 250°C for 2 min, increased by 15°C/min to 290°C (held for 10 min), and then by 10°C/min to a final temperature of 310°C (held for 10 min). The other chromatographic conditions were as follows: injector and detector temperatures, 280°C; split ratio, 50:1; sample volume injected, 2μl. Cholesterol content was expressed as mg/100g meat.

Trace mineral contents were determined using an Atomic Absorption Spectrophotometer (AA-6200, Korea). Specifically, 2.5 g of sample was placed in a crucible and dried at 105°C. Following drying, the sample was burned in a muffle furnace at 550°C until it became

grayish white, after which the crucible was allowed to cool in a desiccator. Next, 1-2 drops of distilled water (DW) were added to the crucible along with 10 ml of primary reagent (HCl: DW=1:1). The crucible was then placed on a hot plate stirrer for evaporation, after which 10 ml of secondary solution (HCl:DW=1:3) was added. Following evaporation, 100 ml of sample was filtered with DW through Whatman No. 6 filter paper. After diluting commercial standard solutions (1000 ppm) of Ca and Fe by 0.5, 1.0 and 2.0 ppm and magnesium (Mg) and sodium (Na) by 0.1, 0.2 and 0.4 ppm, absorbance levels were measured by comparison with a calibration curve and the results expressed as mg/100g of meat.

2.3.3. Meat fatty acid composition assay

The fatty acids compositions of breast and thigh meat were determined by a direct method for fatty acid methyl ester (FAME) synthesis using a slight modification of the method described by O'Fallon et al. (2007). Briefly, 1 g of minced meat was placed into a 15-ml Falcon tube, after which 0.7 ml of 10 N KOH in water and 6.3 ml of methanol were added. The tube was then incubated in a 55°C water bath for 1.5 h with vigorous hand-shaking for 10 S every 30 min to properly permeate, dissolve and hydrolyze the sample. After cooling to below room temperature in a cold tap water bath, 0.58 ml of 24 N H_2SO_4 in water was added. The tube was then mixed by inversion, after which K_2SO_4 precipitated. The sample with the precipitate was incubated again in a 55°C water bath for 1.5 h with vigorous hand-shaking for 10 S every 30 min. After FAME synthesis, the tube was cooled in a cold water bath. Next, 3ml of hexane were added and the tube was vortex-mixed for 5 min on a multitube vortexer. The tube was then centrifuged for 5 min at 3000 × g (HANIL, Combi-514R, Korea), after which the top (hexane) layer containing the FAME was dehydrated through the anhydrous Na_2SO_4. The extracted and dehydrated hexane was concentrated to 1.5 ml and placed into a GC vial for analysis.

The fatty acid composition of the FAME was determined using a Gas Chromatograph (Agilent, 7890A series, USA) equipped with a flame ionization detector and a Hewlett Packard HP-88 capillary column (J&W Scientific, USA) with a length of 60 m, a 0.52 mm internal diameter and a 0.20 μm polyethylene glycol-film thickness. Samples were injected by an auto-sampler (Agilent Technologies 7693, USA). The initial oven temperature was 125°C, which was held for 1 min, then increased to 145°C at 10°C/min, where it was held for 26 min, then further increased to 220°C at 2°C/min, where it was held for 2 min. Purified air and H_2 were applied at a flow rate of 400 mL/min and 40 mL/min as the carrier gas, while helium was applied at 40 mL/min as the makeup gas. Both the injector and detector temperature were set at 260°C and the split ratio was 30:1. Fatty acids were identified by comparison of their retention times with those of a standard FAME mixture (Supelco^{TM} 37 Component FAME Mix, 10 mg/ml in CH_2Cl_2. Catalogue Number 47885-U. Supelco, North Harriso Road, Bellefonte, PA 16823-0048, USA). Sums and ratios useful for evaluating nutritional value and healthiness of the fatty acid profile were also determined; specifically, the sum of saturated fatty acids (∑SFA), monounsaturated fatty acids (∑MUFA), polyunsaturated fatty acids (∑PUFA), n-3 fatty acid (∑n-3) and n-6 fatty acids (∑n-6) and the ratios of USFA to SFA (USFA/SFA), PUFA to SFA (PUFA/SFA), n-6 to n-3 (n-3/n-6) and hypocholesterolaemic to hypercholesterolaemic (H/H) fatty acid ratio. The H/H ratio was determined as follows:

H/H = [(sum of C18:1 *cis*-9, C18:2 n-6, C20:4n-6, C18:3 n-3, C20:3n-6, C20:5 n-3, and C22:6 n-3)/(sum of C14:0 and C16:0)] (Santos-Silva et al., 2002).

2.3.4. *Oxidative rancidity and meat pH analysis*

To determine the oxidative stability of broiler breast and thigh meat, meat samples were preserved in a refrigerator at 4°C, after which the thiobarbituric acid reactive substances

(TBARS) values of 1, 3, 5, 7, 14, 21, and 28 day old meat were determined. Briefly, 4 g of meat were homogenized with a homogenizer (Ultra-Turrax T-25 Basic, IKA Werke, GMBH & CO. KG, Staufen, Germany) at full speed for 1.5 min with 10 ml of a solution containing 20% trichloroacetic acid (TCA) in 2 M phosphoric acid and 10 ml of distilled water. The mixture was then filtered through Hyundai Micro No. 60 (Hyundai Micro Co., Ltd.) filter paper. Equal amounts of the filtrate (2 ml) and 2-thiobarbituric acid (98% 4, 6 Dihydroxy-2-mercaptopyrimidine, 0.005 M in DW) were heated in a shaking water bath at 80°C for 30 min. After cooling, the absorbance was measured at 530 nm with a VIS-Spectrophotometer (Libra S22, Biochrom Ltd. Cambridge, England). The amount of TBARS was expressed as micromoles of malondialdihyde (MDA) per 100 g of meat.

The pH of the meat samples was determined by blending 2 g of meat with 18 ml of distilled water for 1.5 min in a homogenizer. The pH values were measured using a standardized electrode attached to a digital pH meter (Docu-pH+ meter, Sartorius, USA).

2.4. Statistical analyses

All data were subjected to ANOVA using the General Linear Models (GLM) function of the Statistical Analysis System (SAS, 2003, Version 9.1, SAS Institute, Cary, NC, USA). Each cage was used as the experimental unit for growth performance parameters (BW, ADG, ADFI and FCR), whereas an individual bird served as the experimental unit for meat proximate composition, fatty acid profile and oxidative stability of meat. The statistical model used to test the effects of treatment on growth performance, meat composition, fatty acid profiles and TBARS was:

$Y_{ij} = \mu + \alpha_i + e_{ij}$.

Where, Y_{ij} = the response variable, μ = the general mean, α_i = the effect of dietary treatments and e_{ij} = the random error. Student's t test with a probability level of $P \leq 0.05$ was used for comparison between mean values.

3. Results

3.1. Composition and pH of PB

The experimental PB contained 6.80% CP, 2.5% EE, 80.9% moisture and 5.81% ash, while after drying the nutrient content was as follows: 6.55% CP, 3.80% EE, 6.02% moisture and 1.06% ash. The fatty acid composition of fresh and dried PB is presented as supplementary Table 1. No significant differences were recorded among the proportion of fatty acids in fresh and dried PB, except α-linolenic acid (C18:3n3) and DGLA (C20:3n6) which were higher in fresh PB in comparison to dried ($p < 0.05$). The pH of the fresh PB 3.15 to 3.20, whereas dried PB had a pH of 3.56 to 3.59.

3.2. Broiler performance

Dietary supplementation of PB did not show significant effect on the ADG and ADFI of broiler throughout the experimental period (data not shown). However, the FCR was significantly reduced during the overall period when birds were fed 0.5% (1.70 vs. 1.64), 1.0% (1.70 vs. 1.65) and 2.0% (1.70 vs. 1.61) PB with basal diets ($p = 0.003$). The mortality of broilers was not affected by the dietary treatments (data not shown).

3.3. Meat proximate composition, cholesterol and trace minerals

As shown in Table 2, the moisture content was significantly higher in breast meat of broilers fed diet supplemented with 2.0% PB ($p < 0.05$) than in the 0% and 0.5% PB supplemented group. In addition, birds fed diets supplemented with 0.5, 1.0 and 2.0% PB had a higher CP content in breast meat ($p < 0.02$) than those in the non-supplemented group. The EE content of breast meat was reduced ($p < 0.02$) in response to higher doses of PB (1.0 and 2.0%) relative to the lower dose (0.5%). There was a significant reduction ($p < 0.05$) in cholesterol concentration of broiler breast meat from birds fed diet supplemented with PB relative to the non-supplemented group. Although the Ca contents did not differ among treatments, the Fe ($p = 0.0001$) and Na ($p < 0.02$) contents were higher in the breast meat of broilers fed diets supplemented with 0.5, 1.0 and 2.0% PB. Conversely, the Mg content was only higher in the 1.0% PB supplemented group ($p < 0.02$).

In thigh meat (Table 2), the moisture content was higher in the 1.0 and 2.0% PB supplemented groups ($P < 0.03$); however, no difference was recorded for crude ash. The crude protein contents were higher in thigh meat of broilers fed diets supplemented with 0.5, 1.0 and 2.0% PB ($p = 0.002$), whereas the ether extract was lower ($p = 0.006$) than the non-supplemented group. Although there were no significant effects observed across treatments, birds fed 2.0% PB supplemented diet had a tendency to have lower cholesterol levels in thigh meat. The Na content was higher in thigh meat of the 1.0 and 2.0% PB supplemented group, whereas the Mg content was only higher in response to 2% PB supplementation ($p < 0.02$).

3.4. Meat fatty acid composition

The effects of dietary treatments on fatty acid compositions of broiler breast and thigh meat are shown in Table 3 and 4, respectively. Dietary supplementation with 1.0 and 2.0%

PB resulted in a significant reduction in the proportion of stearic acid ($p = 0.001$) and ∑SFA ($p = 0.003$) in broiler breast meat. Among individual MUFA, the oleic acid content was significantly higher ($p = 0.004$) in the 1.0% PB supplemented group, whereas the ∑MUFA was higher in both the 1.0 and 2.0% PB supplemented group ($p = 0.004$). The proportion of eicosapentanoic acid ($p = 0.001$) and ∑n-3 fatty acids ($p < 0.01$) in breast meat of broilers fed 0.5, 1.0 and 2.0% PB supplemented diets were higher than those of broilers fed non-supplemented diets; however, no difference was recorded for individual or ∑n-6 fatty acids. In addition, 1.0 and 2.0% PB diets significantly increased the ratio of USFA/SFA ($p = 0.007$). Although there were no significant differences, birds fed 0.5 and 2.0% PB supplemented diets tended to have higher PUFA/SFA ($p < 0.08$) and n-6/n-3 ($p < 0.06$) ratios in their breast meat.

In thigh meat of broilers fed diets supplemented with 1.0 and 2.0%, PB significantly reduced the palmitic acid ($p < 0.01$), stearic acid ($p < 0.01$) and ∑SFA ($p = 0.001$) contents compared to the non-supplemented group. In contrast, the ∑MUFA content was higher ($p = 0.001$) in the 1.0 and 2.0% PB supplemented group, as was the concentration of oleic acid ($p = 0.0003$) and eicosaenoic acid ($p < 0.05$). In addition, the α-linolenic acid ($p = 0.005$) concentration was higher in the 1.0 and 2.0% PB supplemented group, along with the ∑n-3 fatty acid ($p < 0.01$). Supplementation of the broiler diet with 2% PB led to a significant reduction in the proportion of dihomo-gama-linolenic acid (DGLA) ($p < 0.002$), arachidonic acid ($p < 0.0001$) and ∑n-6 fatty acids in broiler thigh meat relative to the non-supplemented group. The USFA/SFA ratio was increased ($p = 0.0006$) in response to 1.0 and 2.0% PB, whereas the n-6/n-3 ratio was reduced ($p = 0.002$) in response to 2% PB supplementation. The H/H ratio was increased in both breast ($p < 0.05$) and thigh ($p = 0.003$) meat in response to dietary 1.0 and 2.0% PB supplementation.

3.5. Oxidative stability and meat pH

The effects of dietary PB on the oxidative stability of broiler breast and thigh meat are shown in Fig. 1 (a, b). Dietary PB had no significant effects on the TBARS values of breast meat up to day 5 ($p > 0.05$). From d 7 to d 28, significantly lower TBARS values were observed in breast meat of broilers fed diet supplemented with 0.5, 1.0 and 2.0% PB ($p < 0.05$) relative to the non-supplemented group. Dietary supplementation of PB significantly reduced the TBARS values of broiler thigh meat in all measurement periods ($p < 0.05$).

The pH of breast meat was significantly lower ($p < 0.05$) in all of the PB supplemented groups after 14 days of storage [Fig. 2 (a)] compared to the non-supplemented group. At d 14 and 21, the reduction in thigh meat pH was significant in response to 1.0 and 2.0% PB supplementation, whereas, at d 28 it was significantly lower ($p < 0.05$) in all of the PB supplemented meat samples [Fig. 2 (b)] compared to control.

4. Discussion

The by-product of pomegranate fruit after juice extraction, is a rich source of polyphenols (149.91 ± 15.28 mg/g), with hydrolysable and condensed tannin (Kim et al., 2013). The biological effects of tannins include reduced feed intake and digestibility of protein when present in non-ruminant (Jansman, 1993) and ruminant (Reed, 1995) diets, ultimately leading to depressed animal performance. In the present study, supplementing diets with three levels of PB had no effect on the ADG and ADFI of broilers. In partial consistent, Rajani et al. (2011) reported no significant changes in broiler growth performance fed diet supplemented with pomegranate peel. This can be explained by a reduction in the content of tannins in oven dried pomegranate peel (Shabtay et al., 2008) and therefore reduced the astringency of the included diet. The reason of concomitant improvement in FCR

of the PB consuming broilers suggest that plant polyphenols may improve utilization of diet energy by manipulating the gut microflora to achieve better performance (Jami et al., 2012). Added benefit from the protein content of PB together with manipulation of gut microflora may increase the protein digestibility and the meat protein of broiler supplemented with PB. Previously, Jami et al. (2012) also reported increased protein digestibility and milk protein yield in lactating cow fed concentrated pomegranate peel extract. Pomegranate peels contain considerable amounts of ellagic acid (1421.6 ± 28.62 mg/100 g) (Kim et al., 2013). Lei et al. (2007) reported that ellagic acid from pomegranate leaf extract can inhibit the pancreatic lipase activity, thereby reducing abnormal lipid metabolism. Hadrich et al. (2014) assayed the inhibitory effects of methanol and ethanol extracts of pomegranate peel on porcine pancreatic lipase and reported a marked reduction in lipase activity. The lower ether extract content in breast and thigh meat of broilers in response to PB supplementation can be explained by modulation of lipid metabolism in response to the reduced lipase activity. Increasing meat moisture with increased levels of PB may be due to the inverse relationship between meat moisture and fat content, which are directly related with meat juiciness. In addition, the cholesterol content of breast meat was also lower in broilers fed PB supplemented diet. Plant sterols such as β-sitosterol, campesterol, and stigmasterol are naturally occurring compounds that have structures similar to, yet slightly different from cholesterol. These steroids can compete with absorption of dietary cholesterol, as well as inhibit the re-absorption of endogenous cholesterol in the gastrointestinal tract (John and Sorokin, 2007). Pomegranate seed is particularly rich in the above steroids (Prakash and Prakash, 2011), which may be responsible for lowering the cholesterol content in broiler meat. Mirdehghan and Rahemi (2007) found significant amounts of different trace minerals in pomegranate peels including Fe, Mg and Na, which may increase their content in breast and thigh meat of broilers.

Dietary SFA, especially stearic, myristic and palmitic acid have great importance due to their hypercholesterolemic properties, which are associated with coronary heart disease. Replacing SFA with MUFA results in a reduced LDL cholesterol and total/HDL cholesterol ratio (FAO/WHO, 2009). In our study, supplementation with 1.0 and 2.0% PB significantly reduced the stearic acid and ∑SFA contents of broiler breast and thigh meat. This can be explained by the increased concentration of oleic acid and ∑MUFA in broiler meat in view of the fact that stearic acid is more rapidly converted to oleic acid (Bruce and Salter, 1996). The n-3 and n-6 fatty acids also play important role in human nutrition, being both precursors of eicosanoids, prostaglandins, leucotriens, and thromboxanes, which regulate the cardiovascular system and immunological processes (Grashorn, 2007). The experimental PB was rich in unsaturated fatty acids (45.02 ± 0.28 g/100g), including n-3 fatty acid (11.76 ± 0.24 g/100g), which may increase the concentration of n-3 fatty acid in broiler breast and thigh meat, in this study. An increase in n-3 fatty acid in muscle may cause a corresponding decrease in n-6 fatty acid (DGLA, arachidonic acid and ∑n-6 fatty acid) in thigh meat because these two families of fatty acid compete for the same enzymes in their elongation and desaturation metabolism (Nuernberg et al., 2005).

The PUFA/SFA and n-6/n-3 ratio are normally used to assess the nutritional value of fat. Fats having a low PUFA/SFA (<0.4) and high n-6/n-3 (>5) ratio are considered unfavorable because they may induce an increase in cholesterolaemia (Santos-Silva et al., 2002). In this experiment, breast meat from PB supplemented birds had higher PUFA/SFA and lower n-6/n-3 ratios than the control, although no significant differences were observed. Dietary supplementation of PB also reduced the ratio of n-6/n-3 in thigh meat, indicating a beneficial effect of PB on broiler meat quality. However, the above indexes may not be adequate for evaluation of the nutritional value of fat owing to a lack of information regarding MUFA. Therefore, a better approach to the nutritional evaluation of fat should be

the utilization of indexes based on functional effects of fatty acids, such as the ratio between hypocholesterolaemic/hypercholesterolaemic fatty acid (H/H) (Santos-Silva et al., 2002). The values obtained for this ratio were higher in the breast meat of broilers fed 1.0 and 2.0% PB, indicating a positive effect of dietary PB on broiler meat.

The shelf life of aerobically packed fresh meat is very short due to spoilage by rapid microbial growth and oxidation of lipids and proteins. In our experiment, supplementation of broiler diets with different levels of PB significantly reduced the oxidation of lipids, as indicated by the lower TBARS value of broiler breast and thigh meat, consistent with the findings of Kanatt et al. (2010), Vaithiyanathan et al. (2011) and Rajan et al. (2011), indicating the presence of natural antioxidants. The antioxidant potential of pomegranate tannins (individuals and mixtures) and phenolic acids (ellagic acid and gallagic acid) has been reported by Reddy et al. (2007). Antioxidant activity is directly correlated with reducing power (Tanaka et al., 1988) and the presence of reductones, which can break the free radical chain reaction by donating a hydrogen atom. Pomegranate tannins and phenolics may act in a similar fashion as reductones by donating electrons to the free radicals produced during the first step in lipid oxidation and then converting them to a more stable product to terminate the free radical chain reaction (Vaithiyanathan et al., 2011). Kanatt et al. (2010) reported good reducing power and high 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl and superoxide ion radical scavenging capacity of pomegranate peel extract.

High meat pH is usually an indication of spoilage, results from the growth of putrefactive bacteria (Jałosińska and Wilczak, 2009). Hence, it is important that meat reaches as low a pH as possible to ensure shelf-life stability. Post rigor meat normally has a pH of 5.7 to 5.8 (Savell et al., 2005). Consistent with these findings, the pH of breast meat was 5.76 to 5.79, while that of thigh meat was 5.73 to 5.77 at the beginning of the experiment. In addition, after 14 day of storage, the pH values of meat samples obtained from PB supplemented birds were

lower than those of the control, indicating reduced spoilage of meat by microorganisms (Jałosińska and Wilczak, 2009). The antibacterial activity of aqueous extract of pomegranate peel against spoilage bacteria (*Pseudomonas stutzeri*) isolated from poultry meat was revealed by Devatkal et al. (2013), which may be due to the presence of tannins and phenolic acid in pomegranate peel (Reddy et al., 2007).

5. Conclusion

The results of the present study suggest that dietary supplementation of pomegranate by-product at additive levels of 1.0 or 2.0% improved the nutritional value of broiler meat by increasing the protein and trace mineral content, while reducing the ether extract and cholesterol contents. In addition to lower proportion of SFA, the unsaturated fatty acid profile of meat was improved in response to PB supplementation. The lower TBARS and pH value of breast and thigh meat from the PB supplemented group was a clear indication that PB is capable of reducing lipid oxidation and microbial growth in broiler meat under refrigerated storage. Based on these findings, it can be concluded that inclusion of pomegranate by-product to feed at levels that are not detrimental to performance may be a promising method to improve broiler meat quality.

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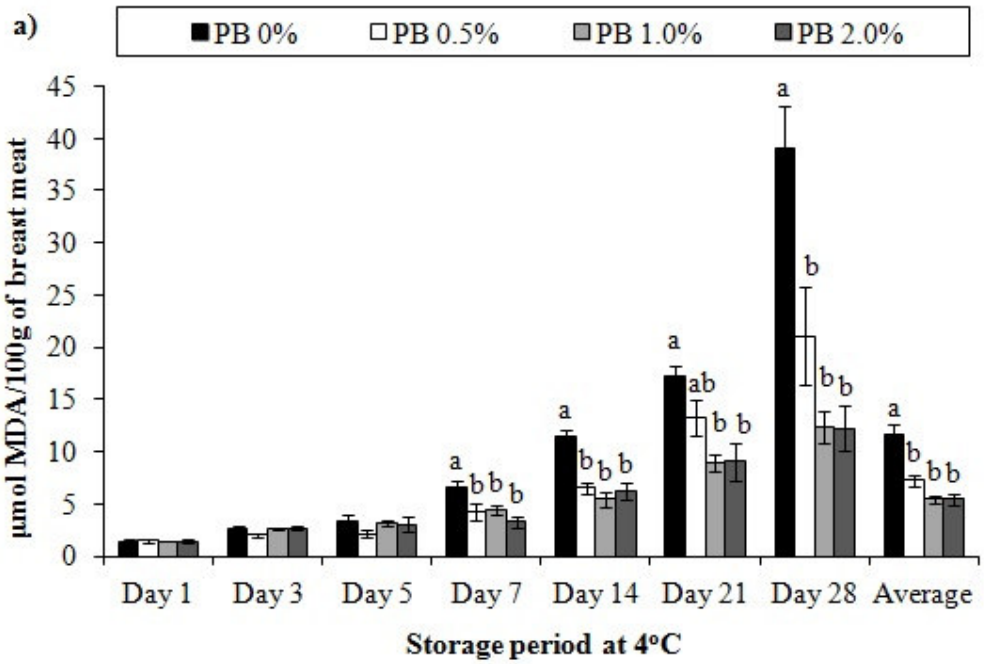
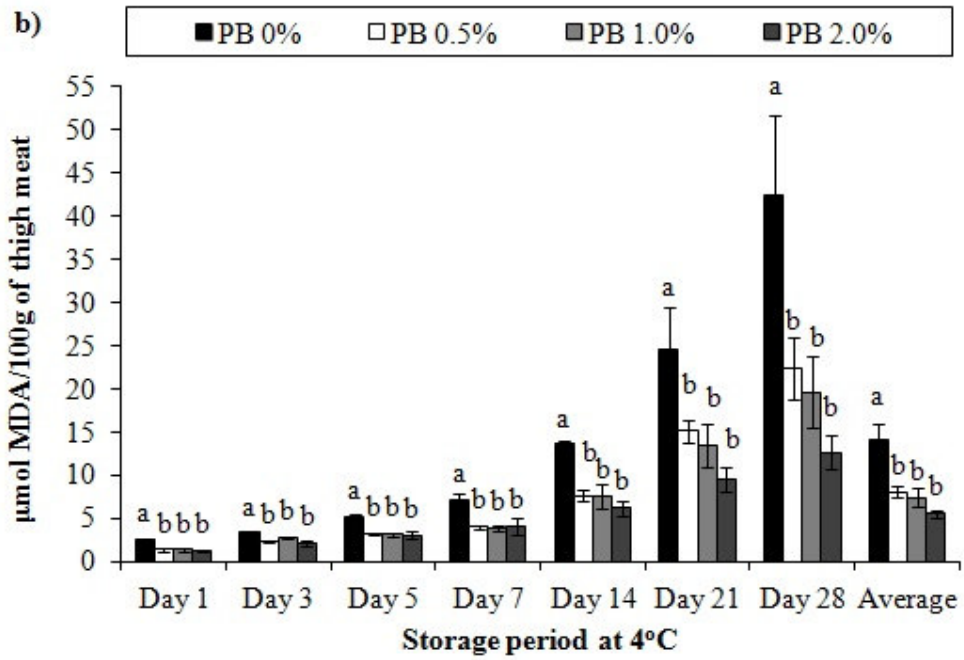
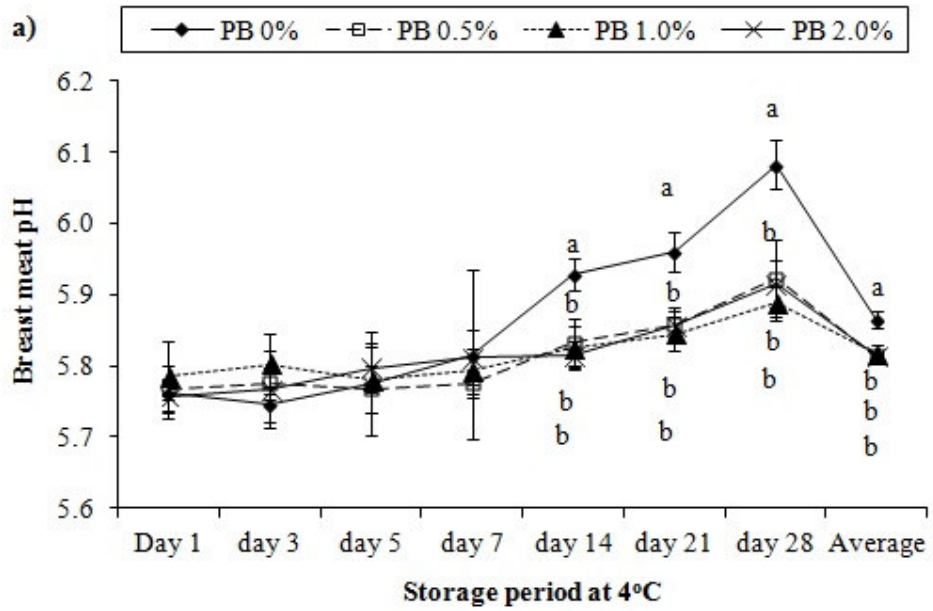
Figure captions

Fig. 1. Effects of diet supplemented with or without pomegranate by-products (PB) on TBARS values of broiler breast (a) and thigh (b) meat (35 d) during refrigerated storage.

Fig. 2. Effects of diet supplemented with or without pomegranate by-products (PB) on pH of broiler breast (a) and thigh (b) meat (35 d) during refrigerated storage.







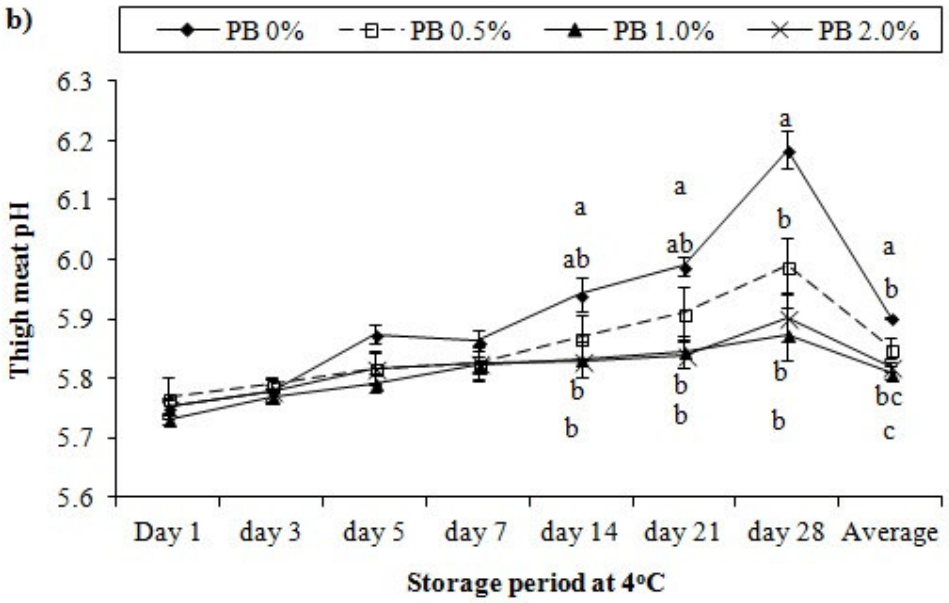


Table 1

Feed ingredients and chemical compositions of broiler diets.

Item	Starter diet (0 to 21 d)	Finisher diet (22 to 35 d)
Ingredients (%, as fed basis)		
Corn grain	57.58	60.64
Soybean meal	26.80	24.90
Corn gluten	5.00	3.50
Soybean oil	2.20	2.20
Animal fats	4.50	5.00
Common Salt	0.25	0.25
Dicalcium phosphate	2.14	2.00
Limestone	0.92	0.88
Vitamin-mineral premix[A]	0.30	0.30
Choline	0.08	0.07
L-lysine HCl (78%)	0.24	0.16
DL-Methionine	0.20	0.10
Calculated composition (% DM)		
ME (MJ/kg)	13.03	13.27
Moisture	12.07	13.08
Crude protein	20.89	19.12
Ether extract	4.65	2.43
Crude fiber	4.42	3.71
Crude ash	5.63	5.61
Calcium	1.05	0.81
Available phosphorus	0.55	0.45
Lysine	1.42	1.10
Methionine	0.49	0.45

[A] Vitamin-mineral mixture provided the following nutrients per kilogram of diet: vitamin A, 15,000 IU; vitamin D3, 1,500 IU; vitamin E, 20.0 mg; vitamin K3, 0.70 mg; vitamin B12, 0.02 mg; niacin, 22.5 mg; thiamine, 5.0 mg; folic acid, 0.70 mg; pyridoxine, 1.3 mg; riboflavin, 5 mg; pantothenic acid, 25 mg; choline chloride, 175 mg; Mn, 60 mg; Zn, 45 mg; I, 1.25 mg; Se, 0.4 mg; Cu, 10.0 mg; Fe, 72 mg; Co, 2.5 mg (Bayer Korea Ltd., Dongjak-Ku, Seoul, Korea).

Table 2

Effects of dietary pomegranate by-products (PB) on proximate composition, cholesterol and trace mineral content of broiler meat [A].

Parameter	Pomegranate by-product (PB)				SEM	P-value
	0%	0.5%	1.0%	2.0%		
Breast meat						
Crude protein (%)	26.21[b]	28.51[a]	28.19[a]	28.55[a]	0.48	0.016
Ether extract (%)	1.50[ab]	1.90[a]	1.33[b]	1.19[b]	0.12	0.015
Moisture (%)	72.38[b]	72.59[b]	72.82[ab]	73.29[a]	0.18	0.033
Crude ash (%)	1.45	1.40	1.39	1.40	0.02	0.359
Cholesterol (mg/100g)	77.44[a]	63.91[b]	64.83[b]	62.80[b]	3.32	0.048
Calcium (mg/100g)	3.25	3.36	3.48	3.43	0.13	0.658
Iron (mg/100g)	1.34[c]	1.62[b]	1.85[a]	2.00[a]	0.06	0.0001
Sodium (mg/100g)	37.79[b]	44.67[a]	46.75[a]	44.35[a]	1.50	0.016
Magnesium (mg/100g)	22.27[b]	23.53[b]	29.13[a]	25.92[ab]	1.30	0.018
Thigh meat						
Crude protein (%)	22.18[b]	24.24[a]	24.11[a]	23.44[a]	0.30	0.002
Ether extract (%)	4.07[a]	3.03[b]	3.27[b]	2.85[b]	0.18	0.006
Moisture (%)	67.88[b]	68.30[b]	71.93[a]	71.52[a]	0.86	0.026
Crude ash (%)	1.23	1.11	1.21	1.22	0.04	0.300
Cholesterol (mg/100g)	65.78	63.83	64.54	61.05	1.48	0.263
Calcium (mg/100g)	3.17	3.72	3.05	3.60	0.34	0.694
Iron (mg/100g)	1.75	1.60	1.54	1.71	0.17	0.827
Sodium (mg/100g)	59.82[b]	65.02[ab]	69.82[a]	69.63[a]	2.11	0.024
Magnesium (mg/100g)	26.81[b]	25.11[b]	26.50[b]	29.05[a]	0.68	0.016

[a,b] Values with different superscripts in the same row differ significantly ($p < 0.05$).

[A] Each value represents the mean of 10 replications with 2 birds/replication.

Table 3

Effects of dietary pomegranate by-products (PB) on fatty acid composition of broiler breast meat [A].

Parameter (g/100 g of total fatty acid)	Pomegranate by-product (PB)				SEM	P-value
	0%	0.5%	1.0%	2.0%		
Myristic acid (C14:0)	0.43	0.37	0.48	0.45	0.03	0.12
Palmitic acid (C16:0)	24.03	23.11	22.29	22.62	0.55	0.20
Palmitoleic acid (C16:1n7)	4.35	4.00	4.23	4.31	0.17	0.54
Stearic acid (C18:0)	10.73^{a}	9.63^{ab}	7.67^{c}	8.78^{bc}	0.35	0.001
Oleic acid (C18:1n9)	37.44^{b}	37.83^{b}	40.78^{a}	38.86^{b}	0.52	0.004
Linoleic acid (C18:2n6)	14.30	15.05	14.97	14.85	0.40	0.64
α-linolenic acid (C18:3n3)	1.35	1.78	1.71	1.70	0.11	0.06
Eicosanoic acid (C20:0)	0.38	0.36	0.39	0.46	0.04	0.50
Eicosaenoic acid (C20:1n9)	0.93	0.87	0.86	0.83	0.04	0.41
DGLA (C20:3n6)	1.16	1.15	0.93	1.09	0.10	039
Arachidonic acid (C20:4n6)	2.20	2.17	2.27	2.50	0.28	0.87
Eicosapentanoic acid (C20:5n3)	0.17^{b}	0.44^{a}	0.44^{a}	0.53^{a}	0.04	0.001
Docosahexaenoic acid (C22:6n3)	1.23	1.83	1.60	1.70	0.14	0.11
Tetracosaenoic acid (C24:1n9)	1.33	1.44	1.41	1.36	0.07	0.68
∑SFA[B]	35.57^{a}	33.47^{ab}	30.83^{c}	32.29^{bc}	0.64	0.003
∑MUFA[B]	43.94^{c}	44.53^{bc}	47.46^{a}	45.70^{b}	0.55	0.004
∑PUFA[B]	19.17	20.58	20.31	20.66	0.57	0.33
∑n-3[B]	2.74^{b}	4.04^{a}	3.75^{a}	3.92^{a}	0.19	0.006
∑n-6[B]	17.66	18.36	18.17	18.43	0.57	0.81
USFA/SFA	1.79^{c}	1.95^{bc}	2.21^{a}	2.06^{ab}	0.06	0.007
PUFA/SFA	0.54	0.62	0.66	0.64	0.03	0.074
n-6/n-3	6.89	4.57	4.87	4.73	0.47	0.057
H/H[B]	2.37^{b}	2.57^{ab}	2.77^{a}	2.66^{a}	0.08	0.05

[a,b,c]Values with different superscripts in the same row differ significantly ($p < 0.05$).

[A] Each value represents the mean of 10 replications with 2 birds/replication.

[B] ∑SFA = saturated fatty acid; ∑MUFA = mono-unsaturated fatty acid; ∑PUFA = poly-unsaturated fatty acid; ∑n-3 = total omega 3 fatty acid; ∑n-6 = total omega 6 fatty acid, H/H = hypocholesterolaemic to hypercholesterolaemic fatty acid ratio.

Table 4

Effects of dietary pomegranate by-products (PB) on fatty acid composition of broiler thigh meat [A].

Parameter (g/100 g of total fatty acid)	Pomegranate by-product (PB)				SEM	P-value
	0%	0.5%	1.0%	2.0%		
Myristic acid (C14:0)	1.11	0.99	1.09	1.12	0.05	0.26
Myristoleic acid (C14:1 n5)	0.32	0.30	0.31	0.34	0.02	0.48
Palmitic acid (C16:0)	25.04^{a}	24.78^{ab}	22.91^{c}	23.64^{bc}	0.41	0.01
Palmitoleic acid (C16:1 n7)	6.18	5.27	5.74	6.05	0.37	0.55
Heptadecenoic acid (C17:1)	0.15^{b}	0.21^{a}	0.20^{ab}	0.19^{ab}	0.01	0.07
Stearic acid (C18:0)	6.12^{ab}	6.81^{a}	6.03^{b}	5.48^{b}	0.23	0.01
Oleic acid (C18:1 n9)	39.18^{b}	40.01^{b}	42.03^{a}	43.06^{a}	0.42	0.0003
Linoleic acid (C18:2 n6)	15.59	15.43	15.04	14.01	0.45	0.15
α-linolenic acid (C18:3 n3)	1.70^{bc}	1.60^{c}	1.83^{ab}	1.96^{a}	0.06	0.005
Arachidic acid (C20:0)	1.06	0.96	0.97	0.97	0.03	0.25
Eicosenoic acid (C20:1 n9)	0.11^{b}	0.13^{ab}	0.14^{ab}	0.16^{a}	0.01	0.03
Eicosadienoic acid (C20:2 n6)	0.17	0.17	0.16	0.14	0.01	0.54
DGLA (C20:3 n6)	0.22^{a}	0.22^{a}	0.24^{a}	0.14^{b}	0.02	0.002
Arachidonic acid (C20:4 n6)	1.03^{a}	1.05^{a}	1.12^{a}	0.59^{b}	0.05	<.0001
Eicosapentanoic acid (C20:5 n3)	0.15	0.15	0.16	0.16	0.02	0.87
Docosahexaenoic acid (C22:6 n3)	1.65	1.71	1.76	1.86	0.07	0.31
Tetracosaenoic acid (C24:1 n9)	0.24^{a}	0.23^{a}	0.28^{a}	0.14^{b}	0.02	0.01
∑SFA[B]	33.32^{a}	33.54^{a}	31.00^{b}	31.21^{b}	0.38	0.001
∑MUFA[B]	46.17^{b}	46.15^{b}	48.69^{a}	49.94^{a}	0.55	0.001
∑PUFA[B]	20.51	20.31	20.31	18.85	0.45	0.13
∑n-3[B]	3.51^{bc}	3.45^{c}	3.75^{ab}	3.98^{a}	0.09	0.01
∑n-6[B]	17.00^{a}	16.87^{a}	16.56^{a}	14.88^{b}	0.45	0.04
USFA/SFA	2.00^{b}	1.98^{b}	2.23^{a}	2.21^{a}	0.04	0.0006
PUFA/SFA	0.62	0.61	0.66	0.61	0.02	0.31
n-6/n-3	4.86^{a}	4.91^{a}	4.43^{a}	3.74^{b}	0.18	0.002
H/H	2.28^{b}	2.34^{b}	2.60^{a}	2.50^{a}	0.05	0.003

[a,b] Values with different superscripts in the same row differ significantly ($p < 0.05$).

[A] Each value represents the mean of 10 replications with 2 birds/replication.

[B] ∑SFA = saturated fatty acid; ∑MUFA = mono-unsaturated fatty acid; ∑PUFA = poly-unsaturated fatty acid; ∑n-3 = total omega 3 fatty acid; ∑n-6 = total omega 6 fatty acid, H/H = hypocholesterolaemic to hypercholesterolaemic fatty acid ratio.

Highlights

- Diet supplemented with pomegranate by-product reduced the TBARS of broiler meat.
- Pomegranate by-product reduced the cholesterol and crude fat content of broiler meat.
- Pomegranate by-product improved the fatty acid profile of broiler meat.
- Pomegranate by-product had no negative effect on broiler performance.