The Effect of Pomegranate Peel Powder Supplementation on the Physical and Chemical Characteristics of Goat Kids' Meat

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Abstract

The current study was carried out to examine how dietary supplementation with pomegranate peel powder (PGPP), a natural antioxidant, affected the postmortem lipid oxidation, free fatty acids, cooking loss, drip loss, and myoglobin content of goat meat during refrigerated storage. TBARS and free fatty acids were assessed on days 1, 4, and 7, whereas myoglobin content, cooking loss, and drip loss were evaluated using samples collected on day 1. Twelve weaned male goat kids (weighing 15±0.5 kg) were housed in individual pens, randomly assigned into three treatment groups, and fed diets containing 0, 20, or 40 g•kg−1 DM of pomegranate peel powder, respectively. After 12 weeks of fattening, all goat kids were slaughtered and the *Biceps femoris* (BF) muscles were removed for analysis. Pomegranate peel powder (PGPP) was found to have no significant effect on meat myoglobin ($P > 0.05$), muscle lipid oxidation ($P = 0.545$), or drip loss ($P > 0.05$) when added to the animal diets. However, meat samples from animals fed diets containing 4% PGPP had numerically higher myoglobin content and drip loss values. Furthermore, during the storage period, a significant ($P < 0.001$) increase was found in free fatty acids and TBARS in meat samples. Dietary PGPP supplementation significantly affected cooking loss, with the highest value observed in meat samples from animals fed diets containing 4% PGPP (37.7%), followed by diets containing 2% PGPP (32.2%) and control (30.5%). The treatment × storage-period interaction for TBARS was significant ($P = 0.037$), indicating that the response to PGPP differed across storage periods. Overall, PGPP supplementation did not significantly affect the overall treatment means of FFA or TBARS, whereas storage period significantly increased both FFA and TBARS, and dietary PGPP significantly affected cooking loss.

Key words: Pomegranate peel; goat kid meat; TBARS; free fatty acids; drip loss; cooking loss; myoglobin

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1. Introduction

Meat represents one of the most beneficial providers of nutrition for humans and is consumed extensively worldwide because it provides valuable nutritional components like essential amino acids, lipids, carbohydrates, vitamins, and minerals [56]. Lipid and protein oxidation during the processing, manufacture, and chilled/frozen storage of meat products has a substantial impact on the storage life and nutritional quality of meat and its derivatives [20, 16]. A significant issue affecting meat's nutritional value and rheological properties, and potentially contributing to the formation of harmful compounds, is lipid oxidation. Besides, lipid oxidation can change the meat's color and often yields undesirable off-flavors, odors, and tastes [39]. Meat's high intramuscular polyunsaturated fatty acid concentration makes it vulnerable to lipid oxidation [35]. Although polyunsaturated fatty acids found in meat have well-defined health benefits, they are also readily oxidized [58], especially when meat is processed, cooked, and stored [24, 63]. Antioxidants have been widely studied to stop lipid oxidation of meat along with the resulting byproducts during preservation [14, 17, 58, 30]. Pomegranate peel is rich in phenolic compounds, flavonoids, and other bioactive compounds with antioxidant properties [52, 7]. Use of pomegranate peel and other pomegranate by-products has been demonstrated in several trials to improve the quality of meat from sheep, goat, beef, and broiler chicks, including by reducing the oxidative changes [4, 19, 21, 41, 53]. Pomegranate rind and seed powders have also been reported to have antioxidant effects in goat meat [15]. However, fewer studies have examined the effects of dietary pomegranate peel powder supplementation in goat kids on meat quality and lipid oxidation during refrigerated storage. This study evaluated the effects of dietary pomegranate peel powder, a natural antioxidant supplement, on the physical and chemical characteristics of goat kid meat, including lipid oxidation, free fatty acids, cooking loss, drip loss, and myoglobin content during storage.

2. Materials and Methods

2.1. Animals and experimental design

This experiment was conducted at the University of Duhok, Animal Project, Animal Production Department, and College of Agricultural Engineering Science. Twelve 3–4-month-old weaned male goat kids weighing 15±0.5 kg were used [54]. The goat kids were randomly assigned to three treatment groups (diet groups) for a period of 12 weeks. Each treatment group consisted of four animals. Each individual goat kid was considered the experimental unit. The diet composition and chemical analysis were similar to previous work reported by Sulaiman et al. [54]. The first treatment, which was the control group (C), included no pomegranate peel powder (0% PGPP). The second group was provided the same diet as the control plus 2% PGPP (20 g/kg of dry matter (DM)), whereas the third group received an identical diet plus 4% PGPP (40 g/kg of DM). The pomegranate peel powder was produced from locally grown pomegranate fruit. Its peel (without pith) was sun-dried and ground to a fine powder. All animals were fed a two-part, concentrated diet that was weighed and provided to goat kids twice a day at 9:00 and 17:00. Ad libitum wheat straw was offered at a rate of 300 g/head/day with plentiful clean water. The diet was designed to suit the requirements of fattening goat kids (live body weight 15-25 kg) according to AFRC 1993 [2].

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2.2. Slaughter and sample preparation

The goat kids were weighed and slaughtered at the end of the 12-week experiment. Each goat kid's hot carcass weight was measured 30 minutes after slaughter. The mean weights of animals at slaughter ranged from 24.1 ± 0.93 kg for the control group, 22.2 ± 0.93 kg for 2% PGPP and 23 ± 0.93 kg for 4% PGPP. The carcass was refrigerated at 4°C and processed according to [13]. The carcasses were dissected the next day, 24 hours post-slaughter, and the *biceps femoris* (BF) muscle was excised, vacuum-packed, and kept at -20°C. For myoglobin measurement, about 250 g of BF muscle was taken from the right leg of each carcass, at 24 hours post-slaughter (day 1), packaged, then kept at−20°C. About 100 g of the BF muscle was used for cooking loss (50 g) and drip loss (50 g) from samples collected at 24 hours post-slaughter (day 1). The remaining 100 g of the BF muscle was minced and stored in polyethylene bags, and the same meat portion was assessed after 1, 4 and 7 days of storage at 2-4°C for assessing lipid oxidation (TBARS) and free fatty acid values.

2.3. Myoglobin

The concentration of myoglobin (Mb) was quantified using the method described by Krzywicki [32]. After mincing the meat sample, 5 g was added to a test tube along with 25 ml of cold buffered phosphate (pH 6–8). After two minutes of homogenization, the mixture was chilled for an hour at 4°C. The mixture was centrifuged for 10 minutes at 5000 rpm, and the supernatant was then filtered using Whatman No. 1 filter paper. The absorbance at 700 and 525 nm was then determined using a Jenway 6300 spectrophotometer (UK). The following formula was used to estimate the myoglobin level: Myoglobin (mg/g meat) = (A525 - A700) × 2.303 × dilution factor / weight of sample (g).

2.4. Drip Loss

Meat drip loss was calculated using the approach of [22]. A 50 g sample of meat was weighed, placed in a net bag inside a plastic container, and kept at 4°C for 24 hours. The meat sample was then taken out, wiped with paper towels, and weighed once again. The following calculation was then used to determine the drip loss: Drip loss (%) = [(Initial weight of sample (g) − Final weight of sample (g)) / Initial weight of sample (g)] × 100.

2.5. Cooking loss

Cooking loss was calculated using the method of [49]. The meat sample was taken out of the freezer at −20°C and allowed to thaw for 24 hours at 2–4°C. The initial weight was determined by weighing about fifty grams of thawed meat. After being vacuum-packed, the sample was cooked under pressure in a water bath at 70°C for an hour. The cooked meat sample, previously wrapped, was cooled beneath running water for half an hour. The weight of cooked meat was then calculated by weighing the meat sample after it had been dried using paper towels. The following equation was used to determine cooking loss: Cooking loss (%) = [(Initial meat weight (g) − Cooked meat weight (g)) / Initial meat weight (g)] × 100.

2.6. Thiobarbituric Acid Reactive Substances (TBARS)

Lipid oxidation in meat samples was measured using the TBARS assay, which was initially reported by Buege and Aust [11]. A 15 ml test tube was filled with around 0.5 g of

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meat mince and 2.5 ml of TBARS stock solution (one liter of a solution containing 150 g of trichloroacetic acid and 3.75 g of thiobarbituric acid in 0.25 N HCl). The sample was vortexed for 15 seconds and placed into a water bath at 95°C for 15 minutes, or until it turned pink. The tubes were centrifuged (K Centrifuge PLC Series, Taiwan) at 3000 × g for 10 minutes after a brief cooling period. The filtrate was then put into a 1 ml cuvette, and the absorbance at 532 nm was measured using a spectrophotometer (Jenway, UK) in comparison to a blank (0.5 ml of deionized water and 2.5 ml of stock solution). Using a malondialdehyde standard curve, the amount of TBARS in the meat sample was calculated as mg MDA equivalents/kg fresh meat.

2.7. Free Fatty Acid Value

The free fatty acid level was determined by titration following the principle described by [48] and the procedure used for meat samples by [5]. A sample of about 2.5 g of meat was homogenized in 15 ml of chloroform for one minute before being filtered through Whatman No. 1 filter paper. After mixing 10 ml of the filtered sample with 5 drops of 1% ethanolic phenolphthalein, the combination was titrated using a 0.01 N ethanolic KOH solution. The following formula was applied in determining the value of free fatty acids: Free fatty acid value (%) = [(Titration volume (ml) × normality of KOH × 28.2) / initial meat sample weight (g)] × 100.

2.8. Statistical Analysis

The Genstat program (version 18, VSN International Ltd, UK) was used to analyze all of the study's data. The individual animal was considered the experimental unit, with four animals per treatment. Duplicate measurements (R1 and R2) obtained from the same animal and sample were averaged before statistical analysis. Cooking loss, drip loss, and myoglobin, which were measured at a single time point, were analyzed using a one-way ANOVA, whereas free fatty acids (FFA) and lipid oxidation (TBARS values), measured on days 1, 4, and 7, were analyzed using a repeated-measures analysis, with dietary treatment (0% control, 2% PGPP, and 4% PGPP) as the between-animal factor and storage period (1, 4, and 7 days) as the within-animal factor. Animal within dietary treatment was used as the appropriate error term for testing the dietary treatment effect. The effects of dietary treatment, storage period, and the dietary treatment × storage-period interaction were evaluated. Tukey's HSD test was applied to identify significant differences when the main effects and interactions were significant. Statistical significance was considered at $P < 0.05$.

3. Results and Discussion

3.1. The effect of pomegranate peel powder dietary supplements on meat lipid oxidation

TBARS is commonly utilized for evaluating the level of muscle lipid oxidation [44]. The results of lipid oxidation in meat from animals given pomegranate peel powder (PGPP) as a dietary supplement are shown in Figure 1. Dietary supplements containing the naturally occurring antioxidant PGPP had no significant overall effect on muscle lipid oxidation ($P = 0.545$). This indicates that, under the conditions of the present study, increasing dietary PGPP from 0 to 2% or 4% did not produce a consistent reduction in overall TBARS values. Comparable results were published by Karami et al. [26], whose work revealed that adding dietary antioxidants to Kacang goat muscle did not alter lipid oxidation. In contrast to the current findings, lower TBARS levels were reported in thigh and breast meat of broilers supplemented with pomegranate by-products [3]. The difference

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among studies may be related to the type and inclusion level of the pomegranate by-product, animal species, and experimental conditions. Furthermore, Gharib and Mahmmod [19] observed that longer frozen storage period substantially reduced the amount of TBA for each treatment.

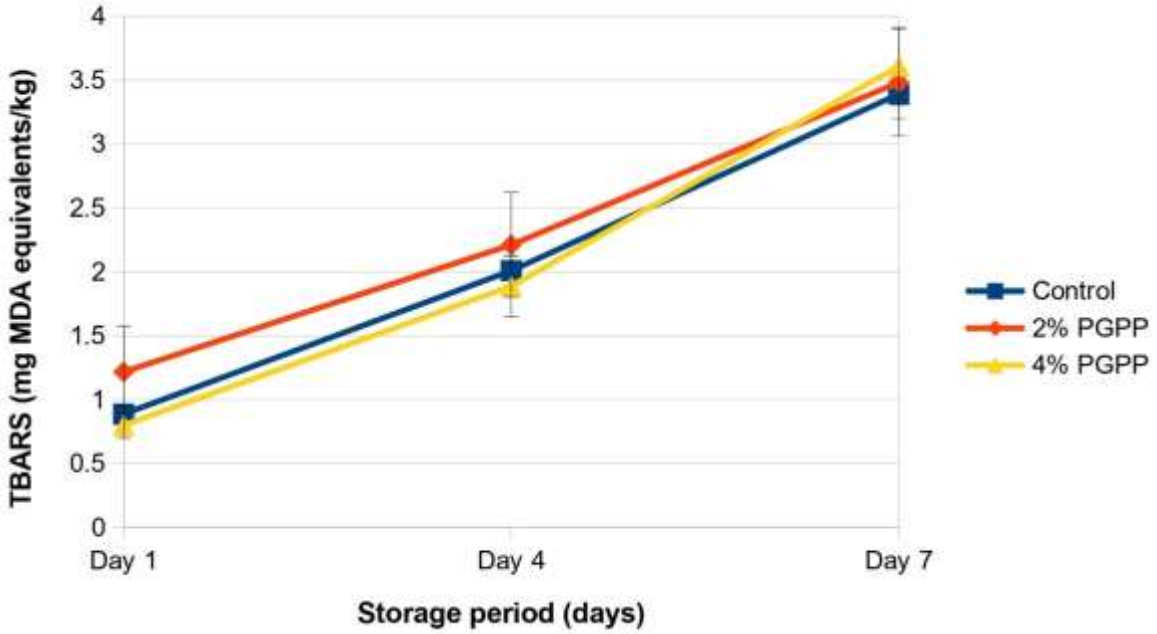


Figure 1. Effect of dietary supplementation with pomegranate peel powder (PGPP) on lipid oxidation, expressed as TBARS values (mg MDA equivalents/kg fresh meat), in goat muscle during three storage periods (days 1, 4, and 7).

Although pomegranate peel extracts contain flavonoids, ellagic acid, catechins, and gallic acid with recognized antioxidant properties [34, 62, 15], the absence of a significant overall treatment effect in the present study suggests that the antioxidant potential of PGPP did not translate into a measurable reduction in TBARS under the dietary and storage conditions used. The TBARS readings increased significantly over the seven-day storage period ($P < 0.001$), and the treatment × storage-period interaction was also significant ($P = 0.037$). The TBARS values increased from day 1 to day 7 in all treatment groups. At day 1, the TBARS values were 0.89, 1.22, and 0.80 mg MDA equivalents/kg fresh meat for the control, 2% PGPP, and 4% PGPP groups, respectively. These values increased to 2.01, 2.21, and 1.89 mg MDA equivalents/kg fresh meat, respectively, on day 4, and to 3.39, 3.48, and 3.61 mg MDA equivalents/kg fresh meat, respectively, on day 7 (Figure 1). Thus, although PGPP had no significant overall treatment effect, the significant treatment × storage-period interaction indicates that the response to PGPP was not consistent across storage periods. The changing pattern among treatments across storage periods may reflect differences in the availability or effectiveness of antioxidant compounds from PGPP during storage; however, the present study did not measure the phenolic composition or antioxidant activity of the supplemented diets or meat, and therefore this mechanism cannot be confirmed. The process induced oxidation of

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lipids may be reduced if antioxidants are fed to the animals [42], and the quantity of TBARS in muscle may be inversely related to the level of antioxidants [47]. Total polyphenol levels were higher in ensiled PP [51]. According to Kazemi and Valizadeh [28], pomegranate peels and seeds have high levels of phenolic compounds and potent antioxidant properties that scavenge free radicals. An increase in lipid oxidation values in meat samples over the storage period is a reliable sign that lipid oxidation has occurred, because hydroperoxides are broken down and malondialdehyde, another oxidized lipid product, is formed according to Bax et al. [9]. It has been noted that an increase in lipid oxidation products in meat indicate advanced lipid oxidation, which is most likely the cause of bad odors and flavors [29]. This might negatively affect the meat's nutritional value and rheological properties [9]. As a result, the quality and potential storage of life of meat may be reduced [43, 12].

3.2. The effect of pomegranate peel powder dietary supplements on free fatty acids in meat

The concentrations of free fatty acids in meat samples obtained from animals fed pomegranate peel powder (PGPP) supplements are shown in Table 1. Adding antioxidants (PGPP) to the animal diet had no effect on the amount of free fatty acid in the meat when compared to the control samples ($P = 0.214$). The lack of a significant treatment effect suggests that the dietary PGPP levels used in the present study did not substantially alter the processes responsible for lipid hydrolysis in the muscle. However, the storage time had a considerable ($P < 0.001$) impact on the quantity of free fatty acids. As a result, the concentration of free fatty acids increased significantly during storage. Day seven had the largest concentration of free fatty acids (Table 1). The concentration of free fatty acids in all meat samples showed no significant treatment × storage-period interaction ($P = 0.377$). Gharib and Mahmmod [19] found that PSE and PPE had a consistent rise in FFA concentration, which supports our findings. Enzymatic hydrolysis of esterified lipids results in an increase in FFA (lipolysis) [23]. This rise in FFA was consistent with the findings of other researchers [1, 57]. However, Uçak et al. [57] reported that the FFA levels of the groups that received rosemary extract and the control group differed significantly ($P < 0.01$). Elevated amounts of free fatty acids in meat samples are more likely to be associated with lipid decomposition, which is thought to be a reliable predictor of lipid stability over time [5]. This formation of fatty acids in meat is caused by enzymatic and microbial processes.

Table 1. Effect of dietary pomegranate peel powder (PGPP) supplementation and storage period on free fatty acid (FFA) values (%) in goat meat.

Effect	Control (0% PGPP)	2% PGPP	4% PGPP	P-value
Treatment	0.964 ± 0.055	1.028 ± 0.071	1.027 ± 0.085	**0.214**
Storage period				**<0.001**
Day 1	**0.735 ± 0.033**			
Day 4	**1.037 ± 0.034**			
Day 7	**1.248 ± 0.034**			
Treatment × Storage period				**0.377**
Day 1	0.776 ± 0.035	0.732 ± 0.029	0.697 ± 0.095	
Day 4	0.943 ± 0.034	1.086 ± 0.063	1.080 ± 0.058	
Day 7	1.174 ± 0.063	1.266 ± 0.045	1.304 ± 0.062	

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Values are presented as mean ± SEM. Treatment means represent the overall effect of dietary treatment across storage periods, whereas storage-period means represent the overall effect of storage across dietary treatments. Values under treatment × storage period represent treatment-specific means at each storage period. PGPP, pomegranate peel powder.

3.3. The effect of pomegranate peel powder dietary supplements on meat myoglobin

Figure 2 shows that the diets supplemented with PGPP had no significant ($P > 0.05$) effect on myoglobin levels when compared to the control samples. However, meat samples from animals given diets containing 4% PGPP had a numerically higher myoglobin content compared with 2% PGPP and control. The numerical increase at the 4% inclusion level, despite the absence of a significant treatment effect, does not establish a dose-dependent response and should therefore be interpreted cautiously. According to Selani et al. [50], pigment oxidation may be connected to the lipid oxidation process because free radicals produced from fatty acids might react with the iron atoms in myoglobin to alter the color of food while it is being stored. However, proteins and amino acids can react with peroxides or the outcomes of their degradation, resulting in deeper colors [6]. Research on meat products has shown that certain food-grade antioxidants can effectively increase the stability of oxymyoglobin by preventing metmyoglobin production, which helps maintain the meat products' desired bright red color [33, 37, 55, 27].

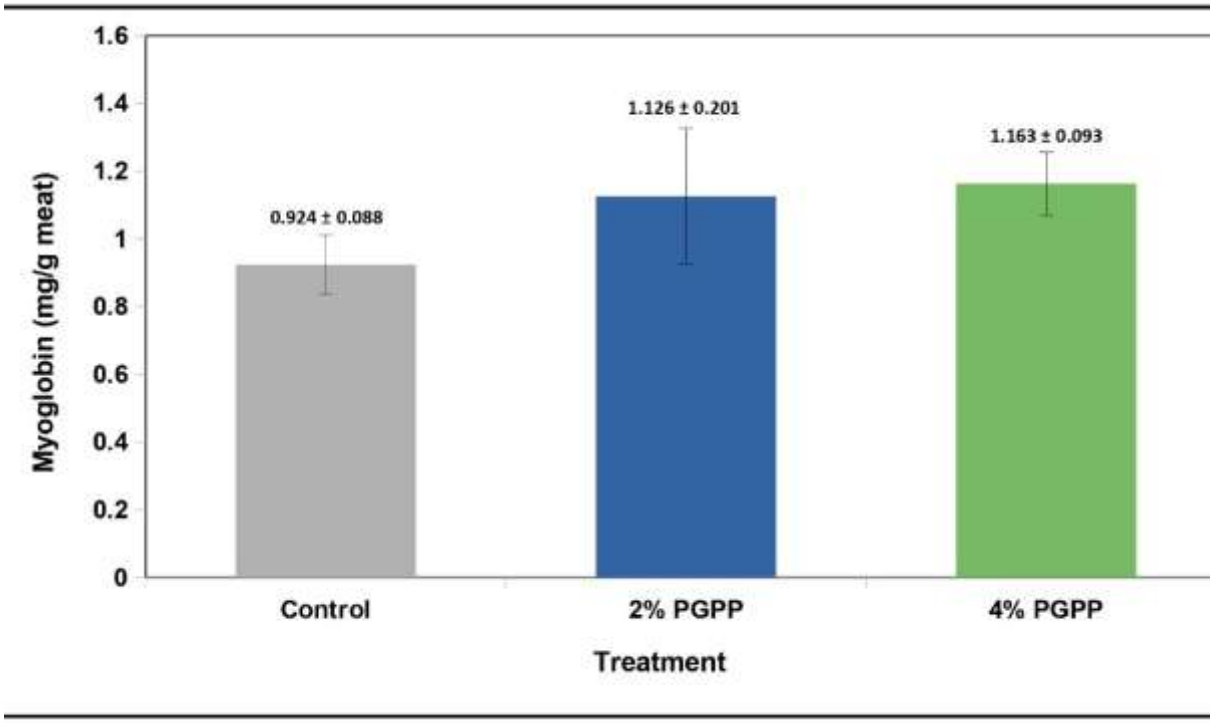


Figure 2. Effect of dietary pomegranate peel powder (PGPP) supplementation on myoglobin concentration in goat muscle using samples collected 24 hours post-slaughter (day 1). Values are

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presented as mean ± SEM (n = 4 animals per treatment). Control (T1), 0% PGPP; T2, 2% PGPP (20 g/kg DM); T3, 4% PGPP (40 g/kg DM). PGPP, pomegranate peel powder; DM, dry matter.

3.4. The effect of pomegranate peel powder dietary supplements on meat cooking loss

Dietary PGPP supplementation significantly affected cooking loss ($P < 0.001$), with cooking loss increasing as the PGPP inclusion level increased, from 30.5% in the control group to 32.2% and 37.7% in the 2% and 4% PGPP groups, respectively (Fig. 3). Cooking loss is the amount of water lost when meat is cooked thermally [59, 46], which denatures and oxidizes proteins [10]. Consequently, the capacity of meat proteins to retain water is reduced [59, 40]. The higher cooking loss observed at the 4% PGPP inclusion level may therefore indicate greater moisture loss during heating, although the mechanism underlying the response to dietary PGPP cannot be established from the present measurements. In contrast to the results of the current study, pomegranate seed pulp did not change the meat's cooking loss (45 minutes or 24 hours after slaughter) in Mahabadi kids [18]. On the other hand, Akuru et al. [4] concluded that adding 2 g/kg of pomegranate peel extract to the diet considerably reduced cooking loss in broiler breast meat, which contrasts with the present results. Pomegranate peel extract has increased water-binding ability [4], which boosts the meat's capacity to hold water and decreases thaw and cooking losses, according to an earlier study [36].

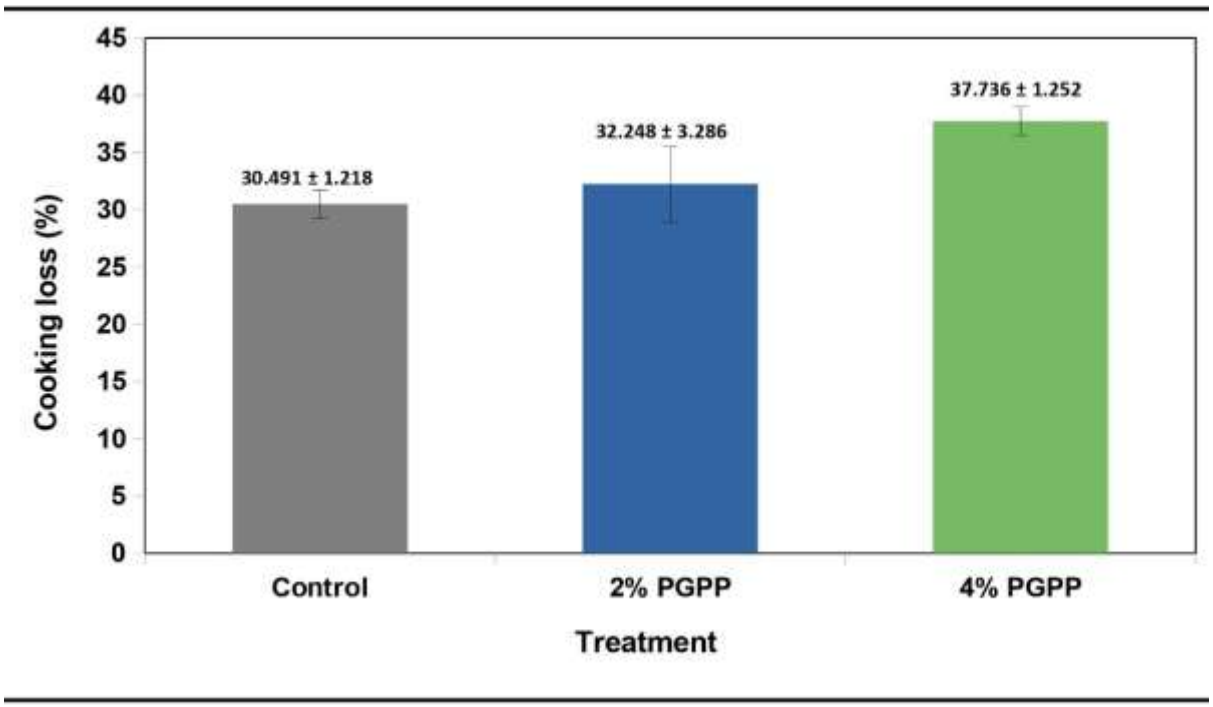


Figure 3. Effect of dietary pomegranate peel powder (PGPP) supplementation on cooking loss of goat muscle using samples collected 24 hours post-slaughter (day 1). Values are presented as mean ±

SEM (n = 4 animals per treatment). Control (T1), 0% PGPP; T2, 2% PGPP (20 g/kg DM); T3, 4% PGPP (40 g/kg DM). PGPP, pomegranate peel powder; DM, dry matter.

3.5. The effect of pomegranate peel powder dietary supplements on meat drip loss

There was a numerical increase in drip loss as dietary PGPP inclusion increased, but the differences were not statistically significant (P > 0.05) (Fig. 4). The present results correspond to the ones obtained by Kotsampasi et al. [31], who discovered no differences in the water, protein, and ash levels of lamb muscle samples supplemented with dietary pomegranate by-product silage. The meat samples from animals fed a diet containing 4% PGPP had the numerically highest drip loss value compared with those from animals fed 2% PGPP and the control diet (Fig. 4). The numerical increase in drip loss at higher PGPP inclusion may be related to changes in muscle protein structure and water-holding capacity; however, protein oxidation was not directly measured in the present study, and therefore this explanation remains speculative.

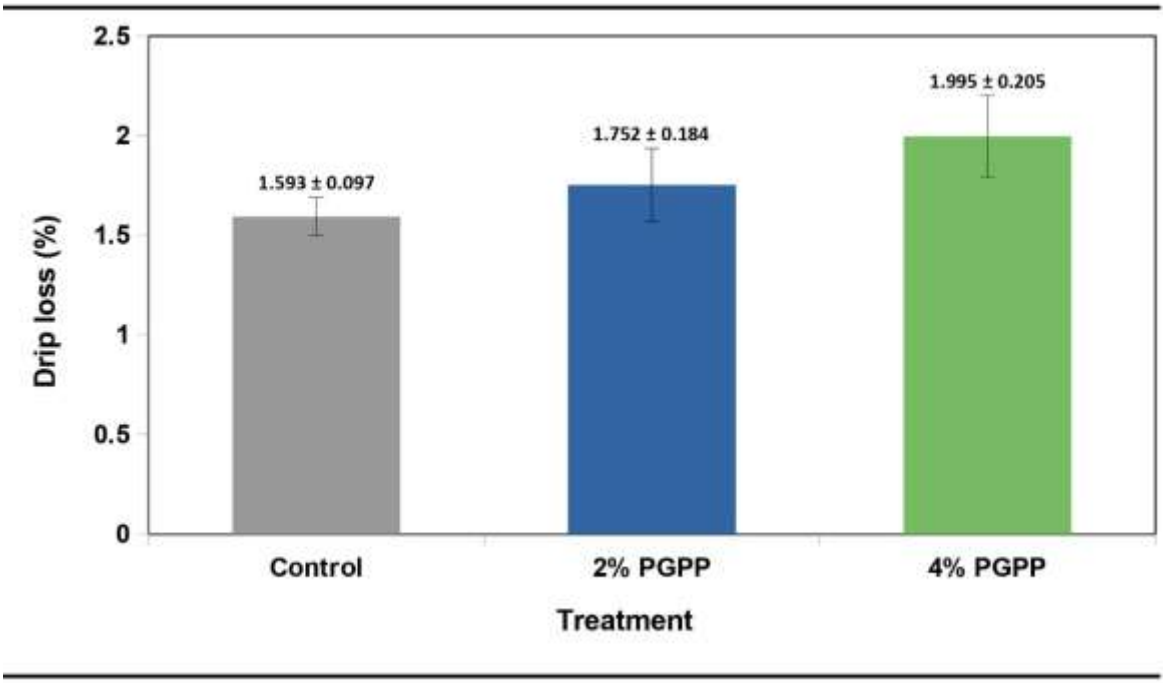


Figure 4. Effect of dietary pomegranate peel powder (PGPP) supplementation on drip loss of goat muscle using samples collected 24 hours post-slaughter (day 1). Values are presented as mean ± SEM (n = 4 animals per treatment). Control (T1), 0% PGPP; T2, 2% PGPP (20 g/kg DM); T3, 4% PGPP (40 g/kg DM). PGPP, pomegranate peel powder; DM, dry matter.

Changes in muscle protein solubility and water-holding capacity may influence drip loss. Wang et al. [61] reported that heat exposure reduced protein solubility and water-holding capacity and increased drip loss in broiler breast meat. Comparable results have been reported by Hama et al. [21] and Gharib and Mahmmod [19], who observed decreases in meat moisture with longer storage periods. Changes in muscle protein

structure may reduce water-holding capacity and contribute to moisture loss during storage [25]. Maqsood et al. [38] and Wang et al. [60] similarly reported increased drip loss with prolonged storage. These findings suggest that changes in muscle protein structure and water-holding capacity may contribute to drip loss during storage. Oxidative processes have also been reported to affect muscle protein structure and water-holding capacity during meat storage [45, 8]. In contrast, the meat of Ghezel lambs given pomegranate pulp had a higher moisture content than the control [41]. Similarly, Ahmed et al. [3] reported that chicken breast moisture levels were higher in birds receiving diets supplemented with pomegranate by-products than in the control group. The higher moisture content reported in these studies may be related to the phenolic compounds provided by the pomegranate by-products.

4. Conclusion

This study shows that pomegranate peel powder, a natural antioxidant, did not significantly reduce free fatty acid levels or overall TBARS values. However, throughout the storage time, the levels of FFA and TBARS increased significantly. The significant treatment × storage-period interaction for TBARS indicated that the response to PGPP differed across storage periods. Dietary PGPP supplementation significantly affected cooking loss, with animals given 4% PGPP showing the highest value. Although there was a tendency for drip loss and muscle myoglobin content to increase as PGPP was incorporated into the diet, these measured variations were not statistically significant. From a practical perspective, the present results do not support using PGPP at the tested inclusion levels specifically to improve lipid stability of goat meat under the storage conditions examined. The higher cooking loss observed at 4% PGPP should also be considered when evaluating this inclusion level for meat-quality applications. A limitation of our study was the relatively small sample size, with four animals per treatment, which may have limited the ability to detect some treatment effects and treatment × storage interactions. Therefore, the findings should be interpreted with this limitation in mind, and further studies with larger sample sizes are warranted to confirm the observed responses.

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