

Research Article

# Assessment of Silage Quality and In Vitro Digestibility in Alfalfa and Pomegranate Pomace Based Silages

## Yonca ve Nar Posası Silajlarında Silaj Kalitesinin ve İn Vitro Sindirilebilirliğin Değerlendirilmesi

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**Abstract**

This study evaluated the effects of pomegranate pomace supplementation on silage quality and in vitro digestibility of alfalfa, a forage crop that is challenging to ensile. For this purpose, three groups were formulated: the alfalfa silage group (A) without pomegranate pomace, a mixed group (APP) containing 50% alfalfa and 50% pomegranate pomace, and a pomegranate pomace silage group (PP) without alfalfa. Each group was ensiled in six jars for 45 days. Based on physical characteristics, the silages were classified as 'satisfactory' (A) and 'excellent-good' (APP and PP). In the APP group, pomegranate pomace supplementation increased the dry matter content ( $p<0.001$ ) and Flieg score ( $p<0.001$ ) of alfalfa silage, while significantly reducing ammonia nitrogen ( $p<0.05$ ) and pH ( $p<0.05$ ) levels. The crude ash contents of the A, APP, and PP groups were 11.33%, 7.94%, and 4.72%, respectively, while the ether extract contents were 3.58%, 2.74%, and 1.08%. Statistically significant differences were found among the groups in terms of neutral detergent fiber ( $p<0.001$ ), acid detergent fiber ( $p<0.001$ ), acid detergent lignin ( $p<0.01$ ), and crude cellulose ( $p<0.001$ ) contents. The in vitro dry matter digestibility values of A, APP, and PP silages were determined as 69.79%, 64.23%, and 61.13%, respectively, while in vitro organic matter digestibility values were 66.75%, 59.94%, and 59.77%, respectively. In conclusion, pomegranate pomace supplementation significantly enhanced fermentation characteristics and overall silage quality. These findings suggest that tannin-rich pomegranate pomace could be a promising additive in high-protein forage silages such as alfalfa, and its effects should be further evaluated at different inclusion levels in animal feeding trials.

**Keywords:** Alfalfa Silage, In Vitro Digestibility, Pomegranate Pomace, Silage Quality

**Öz**

Bu çalışma, silolanması güç olan yoncaya nar posası ilavesinin silaj kalitesi ve in vitro sindirilebilirlik üzerine etkilerini değerlendirmek amacıyla yürütülmüştür. Bu kapsamda üç grup oluşturulmuştur: nar posası içermeyen yonca silajı grubu (A), %50 yonca + %50 nar posası içeren silaj grubu (APP) ve yalnızca nar posası silajı grubu (PP). Her grup altışar kavanozda 45 gün süreyle silolanmıştır. Fiziksel özelliklere göre yapılan sınıflandırmada A grubu 'tatmin edici', APP ve PP grupları ise 'çok iyi' kalite olarak belirlenmiştir. APP grubunda nar posası ilavesi, yonca silajının kuru madde içeriğini ( $p<0.001$ ) ve Flieg skorunu ( $p<0.001$ ) artırırken, amonyak azotu ( $p<0.05$ ) ve pH ( $p<0.05$ ) düzeylerini önemli ölçüde azaltmıştır. A, APP ve PP gruplarının ham kül düzeyleri sırasıyla %11.33, %7.94 ve %4.72; ham yağ düzeyleri ise %3.58, %2.74 ve %1.08 olarak belirlenmiştir. Nötral deterjan lifi ( $p<0.001$ ), asit deterjan lifi ( $p<0.001$ ), asit deterjan lignini ( $p<0.01$ ) ve ham selüloz ( $p<0.001$ ) içerikleri bakımından gruplar arasında istatistiksel olarak anlamlı farklılıklar tespit edilmiştir. A, APP ve PP gruplarının in vitro kuru madde sindirilebilirlik değerleri sırasıyla %69.79, %64.23 ve %61.13; organik madde sindirilebilirlik değerleri ise %66.75, %59.94 ve %59.77 olarak bulunmuştur. Sonuç olarak, nar posası ilavesi silajın fermentasyon özelliklerini ve genel kalitesini önemli ölçüde iyileştirmiştir. Bu bulgular, tanen açısından zengin nar posasının yüksek proteinli yem bitkilerinden elde edilen silajlarda umut verici bir katkı maddesi olduğunu ve farklı düzeylerde hayvan denemeleriyle daha ayrıntılı olarak değerlendirilmesi gerektiğini ortaya koymaktadır.

**Anahtar Kelimeler:** İn Vitro Sindirilebilirlik, Nar Posası, Silaj Kalitesi, Yonca Silajı

## Introduction

Alfalfa (*Medicago sativa* L.) is a valuable legume forage with high crude protein (CP), mineral and organic acid contents, good digestibility, and high palatability. Its deep root system and strong nitrogen-fixing capacity also contribute to soil improvement (Shi et al. 2023). Owing to its nutritional value, alfalfa is widely used as hay, silage or grass silage, particularly in diets of lactating cows (Schmidt et al. 2009; Jung et al. 2024). However, alfalfa is difficult to ensile because of its high buffering capacity, low concentrations of water-soluble carbohydrates and relatively high moisture content (Ogunade et al. 2018; Sun et al. 2021). These conditions favor the growth of undesirable microorganisms, including *Clostridia*, *Bacilli* and *Enterobacter*, leading to excessive proteolysis, butyric acid formation, elevated pH, reduced dry matter (DM) intake and potential milk quality problems (Shi et al. 2023; Sun et al. 2023). For this reason, wilting and mixing alfalfa with other forage materials are commonly recommended to improve its ensiling characteristics. Previous studies have also reported that tannins can reduce ruminal protein degradation and shift nitrogen excretion toward the fecal route (Ünver et al. 2014; Besharati et al. 2022).

Pomegranate (*Punica granatum* L.) is a perennial fruit species of the family Lythraceae, with approximately 48% of the fruit weight consisting of peel and the remaining 52% constituting the edible portion. Industrial processing generates considerable amounts of pomace, composed mainly of peel and seeds, as nearly half of the fruit weight is non-edible. Of the edible part, about 78% is juice and 22% is seed (Kara et al. 2018). Pomegranate peel contains 28–30% tannins (Gerçekcioğlu et al. 2015), and both the peel and seeds are rich in polyphenolic compounds with strong antioxidant and antimicrobial activities (Zarei et al. 2011). Owing to its availability and nutrient content, pomegranate pomace has been proposed as an alternative feed resource in animal nutrition (İflazoğlu Mutlu and Güler 2021). Although it is often discarded or dried for fuel use in rural areas, its high moisture content complicates long-term storage (Canbolat et al. 2014). Pomegranate pomace typically contains 20–24% DM and relatively high concentrations of water-soluble carbohydrates (Canbolat et al. 2014), while the nutrient composition of pomegranate peel has been reported as 8.4% CP, 55.4% non-structural carbohydrates, 34.5% neutral detergent fiber (NDF), 16.7% lignin and 0.84–1.0% condensed tannins (Kara 2016).

Tannins are naturally occurring plant compounds involved in defense against pathogenic microorganisms and viruses (Huang et al. 2024). They can form reversible and irreversible complexes with proteins, polysaccharides, alkaloids, nucleic acids and minerals (Huang et al. 2024; Iqbal and Poór 2025). Tannins are widely distributed in many plant species-including acacia, oak, carob, tea, mimosa, chestnut, pomegranate and grape-and legumes are also recognized as important tannin sources (Kara et al. 2018; Hoque et al. 2025). Because alfalfa contains high levels of protein, proteolysis becomes pronounced during silage fermentation, resulting in elevated pH and increased butyric acid production. Previous studies have suggested that the inclusion of tannin-rich plant materials in alfalfa silage may reduce protein degradation and butyric acid formation and consequently improve overall fermentation quality (Ünver et al. 2014).

Tannins have recently gained attention as functional silage additives because of their ability to limit excessive protein degradation during fermentation and in the ruminant digestive tract. However, their effective use generally requires substrates rich in water-soluble carbohydrates to ensure adequate lactic acid fermentation (Besharati et al. 2022; Tatlı Seven et al. 2024). Pomegranate pomace is notable in this respect, as it contains substantial amounts of tannins and organic acids. Unlike previous studies that

primarily focused on tannin extracts or single by-products (Ünver et al. 2014; Besharati et al. 2022; Tatlı Seven et al. 2025), this study evaluates pomegranate pomace including peel fractions as a co-ensiling material with alfalfa and examines both fermentation quality and in vitro digestibility together. These characteristics suggest that its tannins may reduce proteolysis and butyric acid formation, while its organic acids could support a more favorable fermentation profile. Based on this rationale, the present study investigated whether the inclusion of pomegranate pomace in alfalfa silage would improve fermentation characteristics, reduce protein degradation, and influence nutrient composition and in vitro digestibility.

## Materials and Methods

### *Silage Preparation*

Alfalfa (*Medicago sativa* L.) was used as the primary forage material for silage production. The crop was harvested on October 5, 2022, at approximately 10% bloom stage from the farmland of a local enterprise located in the Yurtbaşı district of Elazığ, Türkiye, using a sickle bar. After mowing, the forage was wilted under sunlight for 4 hours, chopped into 2–3 cm lengths, and prepared for ensiling. Pomegranate pomace, obtained as a by-product of pomegranate molasses production in Hatay province, was chopped into small pieces prior to ensiling and had a DM content of approximately 30%, indicating that it was partially dried before use.

The DM contents of both alfalfa and pomegranate pomace were determined in a preliminary analysis by drying the samples at 60 °C for 48 hours. Three silage formulations were prepared on a fresh weight basis: 100% alfalfa (A), 50% alfalfa + 50% pomegranate pomace (APP), and 100% pomegranate pomace (PP). The mixtures were tightly packed into 1.8-L vacuum-sealed glass jars and hermetically closed to ensure anaerobic conditions. In total, 18 jars (six per group) were prepared and stored at room temperature (20–24 °C) for 45 days. At the end of the ensiling period, the top layer of each jar was discarded, and the remaining contents were transferred into a clean container, thoroughly mixed, and then subsampled for subsequent physical and chemical analyses.

### *Physical Evaluations*

The physical characteristics of the silage samples were evaluated according to the procedure described by Başaran et al. (2018). In addition, the Flieg score, an indicator of silage quality, was calculated using a widely accepted formula reported in earlier studies (AOAC 2006; Başaran et al. 2018):

$$\text{Flieg Score} = 220 + (2 \times \% \text{DM} - 15) - (40 \times \text{pH})$$

### *Chemical Analysis*

Following the 45-day ensiling period, silage samples of alfalfa and pomegranate pomace were retrieved from the jars, oven-dried at 60 °C for 72 hours, and ground to pass through a 1 mm sieve. The DM, crude ash (CA) (Protherm PLF 120/7, Türkiye), organic matter (OM), ether extract (EE) (Gerhardt, Germany), and CP (Vapodest, Gerhardt, Germany) contents were determined using standard procedures outlined by AOAC (2006). Fiber fractions, including NDF, acid detergent fiber (ADF), acid detergent lignin (ADL), and crude fiber (CF), were analyzed using an Ankom 220 Fiber Analyzer (Ankom Technology, Macedon, NY, USA) according to the method of Van Soest et al. (1991). Cellulose was calculated as the difference between ADF and ADL, whereas hemicellulose was calculated as the difference between NDF and ADF. For the determination of ammonia nitrogen (NH<sub>3</sub>-N), 20 g of silage was weighed into stomacher filter bags, and 180 mL of distilled water was added. The mixture was homogenized for 5 minutes using a stomacher (Colworth Stomacher 400, UK) and then filtered. The pH of the filtrate was measured using a calibrated digital pH meter (Hanna pH 211, Hanna Instruments Italia Srl, Padova, Italy). Subsequently, 100 mL of the filtrate was subjected to distillation and titration based on the Kjeldahl method for NH<sub>3</sub>-N analysis (Tatlı Seven et al.

2024). The metabolizable energy (ME) content of the silages was estimated according to the equation proposed by Kirchgessner et al. (1977):  $ME \text{ (kcal/kg DM)} = 3309.5 - 35.64 \times CF$

### ***In Vitro True Degradability (IVTD)***

On day 45 of the ensiling period, in vitro true degradability (IVTD) analyses of the silage samples were conducted using a Daisy II Incubator (Ankom Technology, Macedon, NY, USA) according to the procedure originally described by Tilley and Terry (1963). For the analyses, ground samples (1 mm particle size) were weighed (0.5 g) into pre-treated Ankom F57 filter bags. The bags were sealed and incubated in digestion jars containing buffered rumen fluid medium maintained at 39 °C under continuous agitation. Rumen fluid for the incubation was obtained from two Simmental bulls of similar age and body weight maintained at the ELKAS Meat Integrated Facility in Elazığ, Türkiye. The animals were fed a standard finishing ration consisting of 80% concentrate (barley, wheat bran, and commercial beef concentrate) and 20% forage (alfalfa hay, wheat straw, and dried sugar beet pulp). To maintain anaerobic conditions, CO<sub>2</sub> was continuously flushed into the system, and incubation was carried out for 48 hours. After incubation, the bags were rinsed, dried, and weighed. The in vitro true DM degradability (IVTDMD) and in vitro true organic matter degradability (IVTOMD) values of the silage samples were calculated using the equations provided in the Ankom protocol (ANKOM 2020).

### ***Statistical Analysis***

All statistical analyses were performed using the SPSS software package. One-way analysis of variance (ANOVA) was used to evaluate differences among treatment groups. When significant effects were observed, mean comparisons were conducted using Duncan's multiple range test. Statistical significance was declared at  $p < 0.05$ , and all results were expressed as mean values with their corresponding standard errors.

## **Results**

The nutrient compositions of alfalfa and pomegranate pomace used in the study are presented in Table 1, while the nutrient compositions, NH<sub>3</sub>-N concentrations, and pH values of the alfalfa silage (A), mixed silage containing 50% alfalfa and 50% pomegranate pomace (APP), and pomegranate pomace silage (PP) are shown in Table 2. The effect of pomegranate pomace supplementation on the DM content of alfalfa silage was statistically significant ( $p < 0.001$ ). The highest DM content was observed in the PP group (26.43%). CA contents of fresh alfalfa and pomegranate pomace were determined as 10.75% and 3.49% in DM, respectively. CA content of silage groups varied between 4.72 and 11.33. When CA contents of silages were examined, it was found that there was a statistically significant difference between A, APP and PP groups ( $p < 0.001$ ). OM contents of A, APP and PP silages were determined as 81.50%, 87.06% and 90.90%, respectively, and the highest OM content was in PP group. A statistically significant difference was found between OM values among silage groups ( $p < 0.001$ ). The EE values of fresh alfalfa and pomegranate pomace were 2.72% and 1.21% of DM, respectively (Table 2). In the silages, EE contents were 3.58% in A, 2.74% in APP, and 1.08% in PP, with statistically significant differences among the groups ( $p < 0.001$ ).

The CP contents of fresh alfalfa and pomegranate pomace were 16.25% and 3.94% of DM, respectively. In the silages, CP contents were 21.32% in A, 14.95% in APP, and 4.29% in PP, with statistically significant differences among the groups ( $p < 0.001$ ). According to Table 2, the pH values of the silages ranged from 3.97 to 4.89, and the inclusion of pomegranate pomace significantly reduced the pH of alfalfa silage ( $p < 0.001$ ). The ammonia nitrogen (NH<sub>3</sub>-N) values were 7.41%, 5.23%, and 3.16% in the A, APP, and PP silages, respectively, and also differed significantly among the groups ( $p = 0.028$ ). The ME content was 2535 kcal/kg DM for alfalfa and 2816 kcal/kg DM for pomegranate pomace.

**Table 1.** Nutritional characteristics of alfalfa and pomegranate pomace used in silage production

Parameters	Pomegranate pomace	Alfalfa
DM, %	30.18	24.82
CA, %	3.49	10.75
CP, %	3.94	16.25
EE, %	1.21	2.72
CF, %	13.83	21.72
NDF, %	16.84	28.64
ADF, %	13.25	21.85
ADL, %	3.80	5.90
ME, kcal kg <sup>-1</sup> *	2816	2535

DM: Dry matter; CA: Crude ash; CP: Crude protein; EE: Ether extract; CF: Crude fiber; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; ADL: Acid detergent lignin; ME: Metabolizable energy; \*Calculated.

**Table 2.** Nutrient contents, NH<sub>3</sub>-N concentrations and pH values of alfalfa and pomegranate pomace silages

Parameters	Groups			p
	A	APP	PP	
DM, %	21.18±0.23 <sup>C</sup>	22.63±0.40 <sup>B</sup>	26.43±0.37 <sup>A</sup>	0.001
CA, %	11.33±0.34 <sup>A</sup>	7.94±0.38 <sup>B</sup>	4.72±0.11 <sup>C</sup>	0.001
OM, %	81.50±0.30 <sup>C</sup>	87.06±0.24 <sup>B</sup>	90.90±0.43 <sup>A</sup>	0.001
EE, %	3.58±0.14 <sup>A</sup>	2.74±0.15 <sup>B</sup>	1.08±0.07 <sup>C</sup>	0.001
CP, %	21.32±0.19 <sup>A</sup>	14.95±0.80 <sup>B</sup>	4.29±0.13 <sup>C</sup>	0.001
pH	4.89±0.03 <sup>A</sup>	4.60±0.01 <sup>B</sup>	3.97±0.07 <sup>C</sup>	0.001
NH <sub>3</sub> -N, %	7.41±0.14 <sup>A</sup>	5.23±0.14 <sup>B</sup>	3.16±0.12 <sup>C</sup>	0.028

DM: Dry matter; CA: Crude ash; OM: Organic matter; EE: Ether extract; CP: Crude protein; NH<sub>3</sub>-N: Ammonia nitrogen; A: 100% Alfalfa silage; APP: 50% Alfalfa + 50% Pomegranate pomace; PP: 100% Pomegranate pomace. Data are presented as mean ± standard error. A, B, C values with different letters in the same rows are significantly different (p < 0.05).

The scoring, quality classification, and Flieg scores of the silages for A, APP, and PP groups are presented in Table 3. Based on physical properties (odor, appearance, and color), the A group was classified as “satisfactory,” whereas the APP and PP groups were classified as “excellent–good”. The Flieg scores, calculated according to DM and pH values, were 51.44 for A, 66.37 for APP, and 99.12 for PP, with statistically significant differences among the groups (p<0.001).

**Table 3.** Evaluation of silage based on physical organoleptic characteristics and Flieg score

Groups	Odor	External Appearance	Color	Total Score	Flieg Score
A	7.6	3.2	1	13	51.44±1.17 <sup>C</sup>
APP	14	4	2	20	66.37±0.72 <sup>B</sup>
PP	13.3	4	2	19.3	99.12±3.37 <sup>A</sup>
p < 0.001					

A: 100% Alfalfa silage; APP: 50% Alfalfa + 50% Pomegranate pomace; PP: 100% Pomegranate pomace. Quality grades are classified as excellent-good (16-20 points); satisfactory (10-15 points); moderate (5-9 points); and very poor (0-4 points). Data are presented as mean ± standard error. Values with different letters in the same columns (A–C) are significantly different (p < 0.001).

The NDF, ADF, ADL, cellulose, and hemicellulose values of the silage groups are presented in Table 4. NDF contents were 27.14% in A, 20.70% in APP, and 24.05% in PP, with statistically significant differences among the groups (p<0.001). When the ADF values of the silages were examined (Table 4), they were found to be 20.83% in A, 14.88% in APP, and 17.27% in PP. A statistically significant difference was observed among the groups with respect to ADF values (p<0.001). Similarly, the ADL values were 4.75% in A, 3.50% in APP, and 4.06% in PP. The ADL value of group A differed significantly from those of the other groups (p=0.003). It was determined that the cellulose values of the silage groups presented in Table 4 ranged between 12.87% and 21.12%, while hemicellulose values ranged between 5.82% and 6.78%. The cellulose contents of the A, APP, and PP silages were 21.12%, 12.87%, and 16.18%, respectively. Similarly, hemicellulose values were 6.31%, 5.82%, and 6.78%. A statistically significant difference was found among all groups with respect to cellulose content (p<0.01). In contrast, hemicellulose values showed a statistically significant difference only between the APP and PP groups (p<0.05).

**Table 4.** NDF, ADF, ADL, cellulose, and hemicellulose values of alfalfa and pomegranate pomace silages

Parameters	Groups			p
	A	APP	PP	
NDF, %	27.14±0.22 <sup>A</sup>	20.70±0.53 <sup>C</sup>	24.05±0.27 <sup>B</sup>	0.001
ADF, %	20.83±0.17 <sup>A</sup>	14.88±0.46 <sup>C</sup>	17.27±0.24 <sup>B</sup>	0.001
ADL, %	4.75 ± 0.11 <sup>A</sup>	3.50±0.25 <sup>B</sup>	4.06±0.06 <sup>B</sup>	0.003
Cellulose, %	21.12 ± 1.67 <sup>A</sup>	12.87±0.39 <sup>C</sup>	16.18±0.12 <sup>B</sup>	0.001
Hemicellulose, %	6.31 ± 0.13 <sup>AB</sup>	5.82±0.18 <sup>B</sup>	6.78±0.30 <sup>A</sup>	0.042

NDF: Neutral detergent fiber; ADF: Acid detergent fiber; ADL: Acid detergent lignin; A: 100% Alfalfa silage; APP: 50% Alfalfa + 50% Pomegranate pomace; PP: 100% Pomegranate pomace. Data are presented as mean ± standard error. A, B, C values with different letters in the same rows are significantly different (p < 0.05).

The in vitro true digestibility values of the silage groups are presented in Table 5. The in vitro DM digestibility values of groups A, APP, and PP were 69.79%, 64.23%, and 61.13%, respectively. The in vitro OM digestibility values were 66.75% in A, 59.94% in APP, and 59.77% in PP. Statistically significant differences were observed among all silage groups for in vitro DM digestibility ( $p < 0.001$ ). Similarly, in vitro OM digestibility values also differed significantly among the A, APP, and PP groups ( $p < 0.001$ ).

**Table 5.** In vitro true degradability values of alfalfa and pomegranate pomace silages

Groups	IVDMD, %	IVOMD, %
A	69.79±0.36 <sup>A</sup>	66.75±0.72 <sup>A</sup>
APP	64.23±0.32 <sup>B</sup>	59.94±0.57 <sup>B</sup>
PP	61.13±0.73 <sup>C</sup>	59.77±0.81 <sup>B</sup>
<i>p</i>	0.001	0.001

A: 100% Alfalfa silage; APP: 50% Alfalfa + 50% Pomegranate pomace; PP: 100% Pomegranate pomace; IVDMD: In vitro DM degradability; IVOMD: In vitro OM degradability. Data are presented as mean ± standard error. Values with different letters in the same columns are significantly different ( $p < 0.001$ ).

## Discussion

Numerous studies have investigated the utilization of fruit and vegetable industry by-products as alternative silage materials and their potential contributions to ruminant nutrition (Köksal et al. 2021; Jalal et al. 2023). In line with this context, the present study specifically examined the effects of pomegranate pomace, a by-product of the fruit processing industry, on the nutrient composition, in vitro nutrient digestibility, and overall silage quality of alfalfa.

When the DM contents of the silages were evaluated, it was observed that the inclusion of pomegranate pomace significantly increased the DM content of alfalfa silage ( $p < 0.001$ ). The elevated DM content in the APP group can be explained by the higher DM concentration of pomegranate pomace (26.43%) relative to that of alfalfa (21.18%), as presented in Table 2. Nevertheless, the evaluation of potential dose–response effects is limited by the use of a single inclusion level (50%) of pomegranate pomace in the present study, and future studies investigating different inclusion rates would be required to identify the optimal supplementation level for alfalfa silage.

Supporting the present findings, Erişçi et al. (2022) reported that the supplementation of alfalfa silage with apple pomace (DM, 17.25%) resulted in decreased silage DM content as the level of apple pomace increased. In that study, apple pomace was incorporated into alfalfa silage (DM, 19.44%) supplemented with 5% wheat straw at inclusion rates of 0%, 5%, and 10%. In a related study, Kamalak et al. (2014) demonstrated that increasing the level of oak tannin extract (DM, 91.95%) in fresh alfalfa silage (DM, 21.13%) led to a linear increase in silage DM content, with each unit increase in tannin extract resulting in a 1.1125-unit increase in DM content.

With respect to the CA content of the silages, the incorporation of pomegranate pomace exerted a statistically significant effect (Table 2). As the proportion of pomegranate pomace increased in the silage mixtures, a gradual reduction in CA content was observed. This reduction can be attributed to the considerably lower CA concentration of pomegranate pomace (3.49%) compared to that of alfalfa (10.75%). These results suggest that the ash level of the additive material directly influenced the overall ash

concentration of the silages. Supporting this observation, Şakalar and Kamalak (2016) reported that supplementation of fresh alfalfa (CA, 10.88%) with molassed dried sugar beet pulp (CA, 4.58%) significantly decreased the CA content of the silages across all tested inclusion levels (1.5%, 3.0%, 4.5%, and 6.0%) ( $P < 0.001$ ).

When the OM contents of the silages were evaluated, statistically significant differences were observed among the treatment groups ( $p < 0.001$ ). The lowest OM content was measured in the A group (81.50%), whereas the highest value was obtained in the PP group (90.90%). This variation in OM content can be attributed to differences in the CA concentrations among the groups, since lower CA levels correspond to higher OM contents. In line with this interpretation, Malhatun Çotuk and Soycañ Öneñ (2017) reported that supplementation of alfalfa (CA, 9.71%) with wheat bran (CA, 8.05%) and pudding residue (CA, 1.04%) reduced the CA concentration of the resulting silages, which consequently led to increased OM levels.

The results regarding the EE contents of the silages are presented in Table 2, where statistically significant differences were observed among the groups ( $p < 0.001$ ). The EE contents of fresh alfalfa and pomegranate pomace were 2.72% and 1.21%, respectively. A progressive decrease in EE content was observed in the silages as the proportion of pomegranate pomace increased, which can be explained by the lower EE concentration of pomegranate pomace relative to that of alfalfa. Supporting this finding, Canbolat et al. (2010) investigated the use of grape pomace as a carbohydrate source in alfalfa silage and incorporated grape pomace at different inclusion rates (0, 40, 80, 120, 160, and 200 g/kg DM). They reported that, due to the higher EE content of grape pomace (9.37%) compared with alfalfa (6.34%), increasing levels of grape pomace supplementation significantly elevated the EE content of the silages ( $p < 0.05$ ).

The CP contents of the silage groups were 21.32% in A, 14.95% in APP, and 4.29% in PP, with statistically significant differences observed among the groups ( $p < 0.001$ ). This reduction in CP content across the groups can be explained by the disparity between the CP concentrations of fresh alfalfa (16.25%) and pomegranate pomace (3.94%). In agreement with this finding, Canbolat et al. (2010) reported that the incorporation of grape pomace into alfalfa silage at varying inclusion levels (0, 40, 80, 120, 160, and 200 g/kg DM) significantly decreased the CP content of the silages ( $p < 0.05$ ). In their study, CP concentrations ranged between 18.60% and 17.11%, with the lowest value recorded in silages containing 200 g/kg DM of grape pomace. The decline in CP levels was attributed to the lower CP concentration of grape pomace (14.21%) compared to that of alfalfa (19.35% on a DM basis).

The pH and ammonia nitrogen ( $\text{NH}_3\text{-N}$ ) values of the silage groups are presented in Table 2. The pH values ranged between 3.97 and 4.89, and the inclusion of pomegranate pomace significantly reduced the pH of alfalfa silage ( $p < 0.001$ ). This result is consistent with the findings of Kamalak et al. (2014), who reported that supplementation with oak tannin extract, due to its high tannin content, led to a reduction in silage pH, with each one-unit increase in oak tannin inclusion causing a 0.0813-unit decrease. Regarding  $\text{NH}_3\text{-N}$ , the values were 7.41% in A, 5.23% in APP, and 3.16% in PP, with statistically significant differences observed among the groups ( $p = 0.028$ ). The highest  $\text{NH}_3\text{-N}$  concentration was recorded in the untreated alfalfa silage, whereas supplementation with pomegranate pomace reduced  $\text{NH}_3\text{-N}$  levels in both APP and PP silages, indicating lower proteolysis. Similarly, Kamalak et al. (2014) reported that  $\text{NH}_3\text{-N}$  concentrations in silages ranged from 4.48% to 29.34%, with the highest values detected in the untreated control group. In that study, a one-unit increase in oak tannin inclusion was associated with a 4.169-unit reduction in  $\text{NH}_3\text{-N}$  levels. Accordingly, as organic acids (lactic, acetic, and butyric acids) were not directly

quantified, fermentation quality in the present study was evaluated indirectly based on pH, NH<sub>3</sub>-N, DM content, and Flieg score.

The physical properties and Flieg scores of the silages are presented in Table 3. Based on smell, appearance, and color, the A group received the lowest physical evaluation scores, whereas the APP group received the highest. The calculated Flieg scores were 51.44 for A, 66.37 for APP, and 99.12 for PP, with statistically significant differences among the groups ( $p < 0.001$ ). It is well established that achieving desirable pH and DM levels in silage contributes to higher Flieg scores (Ülger and Özdemir 2023). The improved Flieg score observed in the APP group may be attributed to the tannin content of pomegranate pomace, which likely exerted a positive effect on fermentation by reducing proteolysis and facilitating a lower pH. Proteolysis is recognized as a critical process during silage fermentation, whereby plant proteins are hydrolyzed into peptides, amino acids, and ammonia through the activity of plant proteases and microorganisms. The extent of proteolytic degradation, particularly in the early stages of ensiling, is strongly influenced by the acidity of the medium. Therefore, a rapid reduction in pH during the initial phase of fermentation is considered a key factor in preserving protein integrity (Bai et al. 2020; Leon-Tinoco 2025). Supporting this interpretation, Tabacco et al. (2006) examined the effects of commercially available hydrolyzable tannins derived from chestnut in alfalfa silage. After 78 days of fermentation, no butyric acid was detected in any of the treatments, indicating proper fermentation. The reported pH values were 4.47 in the control silage and 4.06, 4.36, and 4.34 in the silages supplemented with 2%, 4%, and 6% chestnut tannins, respectively. It was further noted that the pH values of the silages containing 4% and 6% tannins were not statistically different from that of the control group ( $p > 0.05$ ). Nevertheless, although tannins were discussed based on the known chemical composition of pomegranate pomace, tannin concentrations were not directly analyzed in the silages, and interpretations related to tannin effects should therefore be considered with caution.

The NDF, ADF, ADL, hemicellulose, and cellulose contents of the silage groups are presented in Table 4. In this study, it was observed that the inclusion of pomegranate pomace significantly reduced the NDF and ADF contents of alfalfa silage ( $p < 0.001$ ). This reduction can be attributed to the lower NDF (16.84%) and ADF (13.25%) concentrations of pomegranate pomace compared with those of alfalfa (NDF, 28.64%; ADF, 21.85%). Furthermore, the tannin content of pomegranate pomace may have partly contributed positively to silage fermentation by stimulating the activity of lactic acid bacteria and other anaerobic microorganisms, which could partly explain the hydrolysis of structural carbohydrates such as NDF, ADF, and hemicellulose. These findings indicate that the fiber composition of the additive material plays a decisive role in shaping the final fiber profile of the silage. In agreement with the present results, Bağuç and Aksu (2021) reported that supplementation of sugar beet pulp silage (NDF, 46.88%) with apple pomace, which contains substantially lower NDF levels (6.45%), significantly decreased the NDF content of the silages at inclusion rates of 5% and 10% ( $p < 0.01$ ). Similarly, Canbolat et al. (2010) demonstrated that the incorporation of grape pomace into alfalfa silage led to significant reductions in both NDF and ADF contents ( $p < 0.05$ ). This effect was attributed to the lower NDF (44.13%) and ADF (30.76%) levels of grape pomace compared with those of fresh alfalfa (NDF, 53.70%; ADF, 37.81%). In addition, grape pomace was reported to act as a carbohydrate source, which may have enhanced the activity of lactic acid bacteria, thereby facilitating the breakdown of structural cell wall components and improving fiber degradability during fermentation (Canbolat et al. 2010).

When the ADL contents of the silage groups were evaluated, values were recorded as 4.75% in A, 3.50% in APP, and 4.06% in PP, with statistically significant differences among the groups. The A group exhibited a higher ADL concentration compared with the other treatments ( $p=0.003$ ). Cellulose contents were measured as 21.12% in A, 12.87% in APP, and 16.18% in PP, also showing statistically significant differences among the groups ( $p<0.001$ ). With respect to hemicellulose, the contents were 6.31% in A, 5.82% in APP, and 6.78% in PP. The reductions observed in ADL, cellulose, and hemicellulose levels in the groups supplemented with pomegranate pomace cannot be attributed solely to tannins during the fermentation process. These effects may also be influenced by changes in the fermentation environment, including microbial activity, which could contribute to the partial hydrolysis of structural cell wall carbohydrates during ensiling (Bingöl et al. 2008; Tatlı Seven et al. 2024; Tatlı Seven et al. 2025).

The in vitro DM and OM digestibility values of the silage groups are presented in Table 5. The lowest digestibility values were recorded in the PP group, with DM and OM digestibility measured at 61.13% and 59.77%, respectively. In contrast, the A group exhibited the highest digestibility, reaching 69.79% for DM and 66.75% for OM. Statistically significant differences were observed in in vitro DM digestibility among all groups ( $p<0.001$ ). The inclusion of pomegranate pomace in alfalfa silage (APP group) resulted in a reduction in in vitro DM digestibility compared with the control (A) group. This reduction can be attributed to the inherently lower digestibility of pomegranate pomace and the presence of tannins, which are known to suppress proteolytic, cellulolytic, and overall fermentation activity in the rumen, which may partly impair nutrient digestibility (Paydaş 2019; Besharati et al. 2022). Contrary to these findings, Tatlı Seven et al. (2024) reported that alfalfa silage alone exhibited the lowest in vitro DM and OM digestibility values, while supplementation with sumac and a sumac+molasses combination significantly enhanced digestibility compared with alfalfa silage alone ( $p<0.001$ ). This improvement has been attributed to several synergistic factors, including the lower NDF and ADF contents (albeit only numerically) observed in the sumac + molasses group, the positive influence of molasses on the fermentation process through enhanced degradation of structural carbohydrates (Bingöl et al. 2008), and the favorable effect of sumac on silage fermentation due to its lower fiber content compared with alfalfa. Collectively, these factors contributed to the significant improvement in digestibility reported by Tatlı Seven et al. (2024). The discrepancy between the findings of that study and the present one is likely explained by differences in both the tannin content and the inclusion levels of the tannin-rich additives employed. Specifically, Tatlı Seven et al. (2024) incorporated 10% sumac into alfalfa silage, whereas the current study used 50% pomegranate pomace. Moreover, the chemical compositions of sumac and pomegranate pomace, particularly with respect to their tannin concentrations, differ substantially. Therefore, the variation in results may be attributed to differences in the type of additive, the tannin level provided, and the distinct effects on fermentation dynamics and nutrient digestibility depending on the applied inclusion rate. Good fermentation quality does not necessarily translate into higher in vitro digestibility, as tannins may exert different effects during ensiling and ruminal incubation (Paydaş 2019; Besharati et al. 2022; Tatlı Seven et al. 2024). Accordingly, as digestibility was evaluated solely under in vitro conditions, the absence of in vivo measurements or animal performance data should be considered an additional limitation when extrapolating the results to practical feeding conditions.

## Conclusion

The inclusion of pomegranate pomace in alfalfa silage was found to exert a significant influence on the nutritional composition and fermentation characteristics of the resulting silages. Compared with untreated alfalfa silage, the APP group demonstrated a clear increase in DM and OM contents, whereas CA and EE levels decreased significantly. Furthermore, the higher Flieg score and the lower pH and NH<sub>3</sub>-N values observed in the APP group relative to the A group provide evidence of reduced proteolysis and the establishment of a more favorable fermentation environment during ensiling. Collectively, these findings indicate that the incorporation of pomegranate pomace contributes positively to silage fermentation and is associated with changes in structural cell wall components, which should be interpreted as indirect effects of improved fermentation conditions rather than direct evidence of enhanced hydrolysis.

**Conflict of interest:** The authors have no conflicts of interest to report.

**Authors' Contributions:** SİM: Conceptualization, methodology, formal analysis, investigation, writing-original draft, writing-review & editing, supervision; PTS: Conceptualization, methodology, investigation, writing-review & editing; NYA: Formal analysis, investigation, writing-original draft; MŞÇ: Formal analysis, investigation, writing-review & editing; AAY: Formal analysis, investigation, writing original draft.

**Ethical approval:** This study is not subject to the permission of HADYEK in accordance with the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" 8 (k). The data, information and documents presented in this article were obtained within the framework of academic and ethical rules.

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