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Original article

The impact of wheat straw and alfalfa additives on quality and *in vitro* digestibility of pumpkin (*Cucurbita pepo*) waste silage

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ABSTRACT

Background: Seed pumpkin waste is a significant portion of the vast quantities of vegetable waste generated annually, which has the potential to be used as feed for livestock. The disposal of pumpkin waste, which is unsuitable for human consumption, has the potential to reduce conflict between humans and cattle for agricultural land. This study examined the quality, aerobic stability, and *in vitro* organic matter digestion of silage generated by combining seed pumpkin waste with wheat straw and alfalfa in varying proportions.
Methods: The consisted of seven different mixtures of pumpkin seed waste with wheat straw and alfalfa. The mixtures were, control (100% pumpkin), $P_{85}S_{15}$ (85% pumpkin, 15% wheat straw), $P_{80}S_{20}$ (80% pumpkin, 20% wheat straw), $P_{75}S_{25}$ (75% pumpkin, 25% wheat straw), $P_{70}S_{15}A_{15}$ (70% pumpkin, 15% wheat straw, 15% alfalfa), $P_{60}S_{20}A_{20}$ (60% pumpkin, 20% wheat straw, 20% alfalfa), and $P_{50}S_{25}A_{25}$ (50% pumpkin, 25% wheat straw, 25% alfalfa). These mixtures were compressed into 1.5-liter glass jars and then ensiled. The silages were opened after a fermentation period of 60 days. The pH, ammonia nitrogen and volatile fatty acids were determined after the fermentation period.
Result: The pH values of the silage mixtures varied between 3.57 and 4.19. Aerobic stability values (CO_2 formation) increased with the addition of straw and alfalfa. Fleig score calculations were ranked as 'good' and 'very good'.
Conclusion: It is concluded that pumpkin waste, which has high moisture content, can be used as a source of roughage in animal feeding when the silage is made with the additives of wheat straw and alfalfa. The use of pumpkins as silage is of great significance in Turkey, as it serves to reduce environmental pollution and offers an alternative source of ruminant roughage. This may be achieved by making a silage mixture that incorporates pumpkins, wheat straw, and alfalfa.

1. Introduction

The increasing global population has led to an increased need for food resources, particularly those derived from animal sources such as meat and dairy products. Hence, it is important to increase the intake of dietary fiber in animals. The rising costs of animal feed have generated a growing interest in the exploration of cost-effective alternative feed sources that have no detrimental impact on animal well-being (Crosby-Galván et al. 2018). Similarly, the increasing rate of population growth and improvements in dietary habits have given rise to several significant societal issues. These include the shortage of food, intensified conflict for arable land in terms of food production and animal feed, and a substantial surge in agricultural waste (Du et al., 2021; Li et al., 2022; Song et al., 2022). Agricultural waste is extensively used in the feeding of farm animals. These wastes are products left after the harvest of various plants and it is possible to evaluate them as a source of roughage in ruminant feeding (Du et al., 2021; Li et al., 2022).

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The annual quantity of agricultural waste in Turkey amounts to 17 million tons (Aksay & Tabak, 2022). The predominant pumpkin species cultivated in Turkey is *Cucurbita pepo* (Seymen et al., 2012). It is mostly cultivated for seed production and once the seeds are removed from the pumpkin, the remaining flesh enclosed inside the shell is often discarded. The discarded portion accounts for ~92–95% of the seed pumpkin (Hashemi & Razzaghzadeh, 2007). The discarding leads to substantial quantity of waste generation. Typically, the waste material is allowed to undergo decomposition inside the harvested fields, rendering them inadequately used. Consequently, there is a pressing need for study pertaining to waste use, which holds significant environmental and economic significance. A large portion of the remaining 95% of the pumpkin (*Cucurbita pepo*), which is a roughage source, is waste after the seeds are separated (Hashemi and Razzaghzadeh 2007). The preparation of pumpkin waste silage has significance in terms of waste reduction, provision of nutrient-rich animal feed, cost savings, and the promotion of sustainable agricultural methods. This approach offers farmers a pragmatic means of optimizing resource use while simultaneously making a beneficial impact on both their agricultural practices and the surrounding ecosystem. However, high moisture contents in pumpkin waste and pathogen infestation are a major hurdle in its use as a silage.

The use of mixed silage, a prevalent agricultural waste feed method, allows for the full utilization of agricultural wastes and the production of silage of superior quality (Larsen et al., 2017). Alfalfa is a preeminent forage crop because of its abundant supply of vitamins, minerals, protein, and other essential elements. Additionally, alfalfa exhibits notable attributes such as high productivity, favorable taste, and exceptional digestibility, with a range of 70% to 80%. tudies have shown that the incorporation of alfalfa into silage, using mixed silage technology, may enhance both the efficacy of the silage process and the nutritional composition of the resulting feed (Wang et al., 2021; Zhang et al., 2017). Wheat straw is a frequently accessible and cost-effective fiber source generated through wheat production. Nevertheless, it has a sub-optimal digestion in the gastrointestinal tract of dairy cows (Kahyani et al., 2019). Therefore, mixing it with other silages could improve its digestibility and quality.

The ensiling has been widely regarded as a very efficient and environmentally benign technique for the treatment of organic waste inside ecosystems. The use of silages as cost-effective animal feeds has the potential to facilitate the advancement of sustainable livestock growth. Nevertheless, vegetable waste often exhibits elevated moisture content and a substantial presence of pathogens, potentially compromising the quality of the silage (Du et al., 2021). Pumpkin waste with a high moisture content has apparently been demonstrated to be a successful roughage alternative when maintained by ensiling and utilized as needed (Chavira, 2016). The pumpkin seed waste has a notably higher carbohydrate content, making it a viable option for use as animal feed after the process of silage production. The use of waste products with favorable nutritional characteristics as animal feed has the potential to mitigate environmental damage (Pirmohammadi et al., 2006). Different additives may be used to efficiently regulate the fermentation process to enhance the quality of silage. However, rare studies have been conducted to determine the possibilities for mix silage of pumpkin waste.

The current study investigated the possibilities of a mix silage comprising of mainly pumpkin waste, wheat straw and alfalfa. All of these were mixed in different quantities and nutrient composition, silage quality, volatile fatty acid compositions (lactic, acetic, propionic, and butyric acid), *in vitro* organic matter digestion (IVOMD) rate, and metabolic energy (ME) values of silages obtained mixing were determined. The results will help in the safe disposal of pumpkin waste and feed for animals.

2. Materials and methods

2.1. Experimental site

The experiment was conducted at Department of Animal Nutrition and Nutritional Disease, Faculty of Veterinary Medicine, Harran University, Eyyubiye, Sanliurfa, Turkey.

2.2. Treatments

Pumpkin (*Cucurbita pepo*) waste (fruit flesh left after seed collection), wheat straw, and alfalfa were used as silage materials in the study. There were 7 compositional groups included in the study, i.e., control (100% pumpkin), $P_{85}S_{15}$ (85% pumpkin + 15% wheat straw), $P_{80}S_{20}$ (80% pumpkin + 20% wheat straw), $P_{75}S_{25}$ (75% pumpkin + 25% wheat straw), $P_{70}S_{15}A_{15}$ (70% pumpkin + 15% wheat straw + 15% alfalfa), $P_{60}S_{20}A_{20}$ (60% pumpkin + 20% wheat straw + 20% alfalfa), and $P_{50}S_{25}A_{25}$ (50% pumpkin + 25% wheat straw + 25% alfalfa). The materials were collected from a nearby farmers' field and utilized in the study. The resulting mix silages were compressed in 1.5-liter glass jars with 4 replications for each treatment and ensiled in an airtight manner. The silages were kept for a period of 60 days in a cool and dark setting at room temperature.

At the completion of the 60-day fermentation period, the silages were opened, and their pH levels were assessed (Polan et al., 1998). Silage samples were examined for their ammonia nitrogen concentration (Broderick & Kang, 1980). Lactic acid and volatile fatty acids (acetic, propionic, and butyric acid) analyses were performed according to the method reported by Suzuki & Lund (1980). Dry matter (DM), raw ash (RA), and crude protein (CP) in the silage materials in were analyzed according to the AOAC (2005). Acid detergent insoluble fiber (ADF) and neutral detergent insoluble fiber (NDF) were analyzed on an Ankom device.

Fleig scores of the silages were calculated with equation 1.

$$\text{Fleig Score} = [220 + (2 \times \text{DM\%} - 15) - 40 \times \text{pH}] \tag{1}$$

Based on the Fleig score categorization, scores falling within the range of 0 to 20 are categorized as 'poor', scores ranging from 21 to 40 are classified as 'low', scores between 41 and 60 are considered 'moderate', scores ranging from 61 to 80 are labeled as 'good', and scores falling within the range of 81 to 100 are categorized as 'very good'.

The aerobic stability tests were conducted in accordance with Ashbell et al. (1991). The silages collected in the research were examined for *in vitro* organic matter digestibility (IVOMD) and metabolic energy (ME) content by following (Menke & Steingass, 1988). The equations 2 and 3 published by Menke & Steingass (1988)were used to determine the ME and IVOMD, respectively.

$$\text{ME (MJ/Kg DM)} = 2.20 + 0.136\text{GP} + 0.057\text{CP} + 0.002\text{CP2} \tag{2}$$

In the equation, ME = metabolic energy, GP = 24-hour gas production (ml), and CP = Crude protein.

$$\text{OMD (\%)} = 14.88 + 0.889\text{GP} + 0.45\text{CP} + 0.065\,\text{RA} \tag{3}$$

In the equation, OMD = organic matter digestion, GP = gas production, CP = crude protein, and RA = raw ash content.

2.3. Statistical method

The collected data on the studied traits were subjected to one-way analysis of variance (ANOVA) (Steel et al., 1997). The normality and homogeneity of variance in the data were tested prior to ANOVS. The analysis was performed on SPSS statistical software (IBM Spss Inc., 2012). Duncan's test was used to assess the differences among the groups where ANOVA indicated significant differences.

3. Results

The dry matter (DM), raw ash (RA), crude protein (CP), acid detergent insoluble fiber (ADF), neutral detergent insoluble fiber (NDF), *in vitro* organic matter digestibility (IVOMD), and metabolic energy (ME) values of the pumpkin, wheat straw, and alfalfa mixtures used as silage material are given in Table 1. Wheat straw had the highest DM, while it was lowest in the pumpkin. Similarly, pumpkin had higher RA compared with wheat straw and alfalfa. Alfalfa and pumpkin had higher values for CP, whereas wheat had the lowest CP. Alfalfa and pumpkin had similar ADF, while wheat straw had higher ADF. The highest and the lowest NDF was noted for wheat straw and pumpkin, respectively (Table 1).

The nutrient values of the silages obtained by adding straw and alfalfa at different rates to the pumpkin are given in Table 2. The DM content of the control, $P_{85}S_{15}$, $P_{80}S_{20}$, $P_{75}S_{25}$, $P_{70}S_{15}A_{15}$, $P_{60}S_{20}A_{20}$, and $P_{50}S_{25}A_{25}$ were 7.01%, 17.60%, 23.03%, 24.19%, 19.30%, 27.52%, and 29.35%, respectively. The RA contents varied between 8.96% and 11.32 ($P < 0.05$). The highest CP content (13.89%) was observed for control, while the lowest value (6.24%) was found in $P_{75}S_{25}$. The ADF values ranged between 39.70% and 46.14% and NDF values differed between 48.47% and 73.11% ($P < 0.001$). The IVOMD values were highest in control group 861.78%9 and the lowest (44.26%) in $P_{50}S_{25}A_{25}$ ($P < 0.001$). The highest ME value was noted for control (21.73 MJ/kg DM), while the lowest (3.39 MJ/kg DM) was in $P_{75}S_{25}$ ($P < 0.01$).

The results of the fermentation are shown in Table 3. The pH levels in the silage mixtures were 3.57 to 4.19 ($P < 0.001$). High NH3-N/TN was noted for control (28.95%), whereas ammonia nitrogen value decreased with increasing additions (P 0.001). The control group had the greatest CO_2 value for aerobic stability (21.73% g/kg DM), whereas $P_{75}S_{25}$ had the lowest (3.39% g/kg DM) value in this regard ($P < 0.001$). Scores on the Fleig test ranged from 77.32 to 102.66 (P 0.001). The control group had the greatest lactic acid value (60.67 g/kg DM), one of the most crucial requirements for silages, whereas the $P_{50}S_{25}A_{25}$ group had the lowest value (13.86 g/kg DM) ($P < 0.001$). The value of acetic acid ranged from 4.44 g/kg DM to 13.02 g/kg DM (P 0.001). There was no evidence of propionic acid in any of the groups. The control silage had the highest butyric acid levels at 2.73 g/kg DM ($P < 0.001$).

4. Discussion

The ensiling of pumpkin waste is challenging due to their relatively low dry matter (DM) concentration. The use of certain additives is necessary to maintain the nutritional content, promote fermentation, and prolong the longevity of silage with low DM content. The present study used several combinations of wheat straw and alfalfa to enhance the DM content of pumpkin waste. Dry wheat and straw are often used in the process of ensiling to reduce the moisture content of feed materials that possess a high-water content, such as pumpkin (İ. Ülger et al., 2020).

The current investigation revealed that DM content in pumpkin was 5.34%. Several studies have reported different DM levels for *Cucurbita pepo* and *Cucurbita argyrosperma*. Ülger et al. (2020) found a DM content of 8.98% for *C. pepo*, whereas Lorenzo-Hernández et al. (2019) reported a DM content of 13.71% for *C. argyrosperma*. Overall, the DM content of the pumpkin (*C. pepo*) used in the present study (5.34%) was lower than other studies. In our study, the CP of pumpkin was 15.55%, which was higher than the value (8.17%) reported byÜlger et al. (2020), lower than the value (18.2%) reported by Łozicki et al. (2015) for pumpkin (*C. maxima* D.), and similar to the value (15.40%) determined by Lorenzo-Hernández et al. (2019). The ADF content of the pumpkin used in the present study (32.10% DM) was lower than the value (38.84% DM) found by Ülger et al. (2020), and (24.21% DM) by Lorenzo-Hernández et al. (2019), whereas it was higher than that (18% DM) found by Łozicki et al. (2015).

Table 1
The raw nutrient contents, *in vitro* organic matter digestion and metabolic energy values of pumpkin, wheat straw, and alfalfa used as silage materials.

	DM	RA	CP	ADF	NDF	IVOMD	ME
Pumpkin	5.34	15.49	15.55	32.10	43.84	62.53	9.15
Wheat straw	88.79	9.08	5.32	45.82	78.41	50.78	7.55
Alfalfa	29.79	9.29	18.34	33.43	51.28	62.42	9.26

DM = dry matter (%), RA = raw ash (DM %), CP = crude protein (DM %), ADF = acid detergent insoluble fiber (DM %), NDF = neutral detergent insoluble fiber (DM %), IVOMD = *in vitro* organic matter digestibility (DM %), ME = metabolic energy (MJ/kg DM).

Table 2
The effects of different additives on various quality attributes of silages obtained after mixing of pumpkin, wheat straw and alfalfa.

Treatment	DM	RA	CP	ADF	NDF	IVOMD	ME
Control	7.01^{e}	11.32^{a}	13.89^{a}	39.70^{d}	48.47^{d}	61.78^{a}	21.73^{a}
$P_{85}S_{15}$	17.60^{c}	9.54^{b}	7.82^{d}	46.14^{a}	72.49^{a}	52.24^{b}	17.68^{b}
$P_{80}S_{20}$	23.03^{d}	9.27^{b}	6.87^{e}	44.09^{b}	72.57^{a}	52.86^{b}	3.77^{b}
$P_{75}S_{25}$	24.19^{bc}	10.15^{ab}	6.24^{e}	43.26^{bc}	73.11^{a}	51.75^{b}	3.39^{b}
$P_{70}S_{15}A_{15}$	19.30^{d}	9.26^{b}	9.97^{b}	41.49^{cd}	67.28^{bc}	50.01^{b}	5.50^{b}
$P_{60}S_{20}A_{20}$	27.52^{ab}	8.96^{b}	9.96^{b}	43.14^{bc}	66.05^{c}	52.50^{b}	4.36^{b}
$P_{50}S_{25}A_{25}$	29.35^{a}	9.09^{b}	8.90^{c}	42.00^{bc}	68.45^{b}	44.26^{c}	3.95^{c}
SEM	1.39592	0.20777	0.46563	0.42704	1.54965	1.07859	0.16843
P < 0.05	***	*	***	***	***	***	**

Values with different letters within a column are statistically different from each other different. Significance (*:P < 0.05; **:P < 0.01:***:P < 0.001); DM = dry matter (%), RA = raw ash (DM %), CP = crude protein (DM %), ADF = acid detergent insoluble fiber (DM %), NDF = neutral detergent insoluble fiber (DM %), IVOMD = *in vitro* organic matter digestibility (DM %), ME = metabolic energy (MJ/kg DM). SEM = standard error of the means. Control = 100% pumpkin, $P_{85}S_{15}$ = 85% pumpkin + 15% wheat straw, $P_{80}S_{20}$ = 80% pumpkin + 20% wheat straw, $P_{75}S_{25}$ = 75% pumpkin + 25% wheat straw, $P_{70}S_{15}A_{15}$ = 70% pumpkin + 15% wheat straw + 15% alfalfa, $P_{60}S_{20}A_{20}$ = 60% pumpkin + 20% wheat straw + 20% alfalfa, and $P_{50}S_{25}A_{25}$ = 50% pumpkin + 25% wheat straw + 25% alfalfa.

Table 3
The effects of different additives on fermentation properties of silages obtained after mixing of pumpkin, wheat straw and alfalfa.

Treatment	pH	NH_3-N/TN	CO_2	Fleig	LA	AA	PA	BA
Control	3.57^c	28.95^a	21.73^a	77.32^e	60.67^a	13.02^a	NF	2.73^a
$P_{85}S_{15}$	3.71^{bc}	16.21^c	17.68^b	93.11^c	28.60^b	6.05^b	NF	1.38^b
$P_{80}S_{20}$	3.79^b	13.33^{de}	3.77 ^{cd}	97.59^b	18.01^c	4.81^b	NF	1.09^b
$P_{75}S_{25}$	3.86^b	13.16^e	3.39^e	102.66^a	18.57^c	5.01^b	NF	1.05^b
$P_{70}S_{15}A_{15}$	4.05^a	18.32^b	5.50^c	87.01^d	19.52^c	6.39^b	NF	1.29^b
$P_{60}S_{20}A_{20}$	4.18^a	15.02 ^{cd}	4.36 ^{cd}	94.13^{bc}	16.58^c	4.81^b	NF	1.14^b
$P_{50}S_{25}A_{25}$	4.19^a	12.32^e	3.95 ^{cd}	90.39 ^{cd}	13.86^c	4.44^b	NF	1.20^b
SEM	0.4638	1.04529	1.38205	1.50972	2.99192	0.57674	NF	0.12447
P < 0.05	***	***	***	***	***	***	NF	***

Values with different letters within a column are statistically different from each other different. Significance (*:P < 0.05; **:P < 0.01:***:P < 0.001). NH_3-N/TN = ammonia nitrogen ratio in total nitrogen (TN), NH_3% -N/TN, CO_2 = carbon dioxide formation (g/kg DM), LA = lactic acid (g/kg DM), AA = acetic acid (g/kg DM), PA = propionic acid (g/kg DM), BA = butyric acid (g/kg DM), NF = not found, SEM = standard error of the means. Control = 100% pumpkin, $P_{85}S_{15}$ = 85% pumpkin + 15% wheat straw, $P_{80}S_{20}$ = 80% pumpkin + 20% wheat straw, $P_{75}S_{25}$ = 75% pumpkin + 25% wheat straw, $P_{70}S_{15}A_{15}$ = 70% pumpkin + 15% wheat straw + 15% alfalfa, $P_{60}S_{20}A_{20}$ = 60% pumpkin + 20% wheat straw + 20% alfalfa, and $P_{50}S_{25}A_{25}$ = 50% pumpkin + 25% wheat straw + 25% alfalfa.

Based on the NDF content (43.84% DM) observed in our research, it is noteworthy that this value is comparatively lower than the findings reported by Ülger et al. (2020) (53.09% DM) and Lorenzo-Hernández et al. (2019) (48.01% DM). Conversely, our results indicate a greater NDF content than that reported by Łozicki et al. (2015) (21.7% DM). In relation to the DM contents derived from the silages, the $P_{50}S_{25}A_{25}$ group exhibited the greatest percentage (29.35%), while the control group had the lowest percentage (7.01%) (P < 0.001). The DM content has typically seen an increase because of the rise in the proportion of wheat straw and alfalfa in the composition. This could be linked with higher DM content in wheat straw and alfalfa. The DM contents of the pumpkin and straw mixture (95% pumpkin + 5% wheat straw) and the pumpkin and alfalfa mixture (95% pumpkin + 5% alfalfa) produced by Ülger et al. (2020) exhibited higher values, measuring 15.59% and 17.20% respectively. The difference between our research and the preceding study may be attributed to the inclusion of wheat straw and alfalfa, both of which possess a high DM content, as contributing factors towards improved DM of the mixed silages.

Pirinç et al. (2020) discovered CP concentrations that were greater than the CP values observed in the present research for the control (14.14% DM). Halik et al. (2014) revealed that the CP values of the control silages (10.97% DM) and silages treated with 0.2% inoculant (*Lactobacillus plantarum*) (10.89% DM) were comparatively lower (ranging from 6.24% to 9.97% DM) than the CP value of the control silage (13.97% DM) observed in the present study. Ülger et al. (2020) reported that the CP values of the 95% pumpkin + 5% straw (5.52% DM) and 95% pumpkin + 5% alfalfa (9.66% DM) silages were close to the CP values observed in the current study. The addition of straw decreased the protein content, whereas the CP content increased with the addition of alfalfa. The lowest CP contents were obtained from the $P_{85}S_{15}$ (6.87 g/kg DM) and $P_{75}S_{25}$ (6.24% g/kg DM) groups (P < 0.001). The explanation for this phenomenon is attributed to the fact that straw has a low crude protein (CP) level and a high concentration of cellulose (Pirinç et al., 2020). On the other hand, alfalfa has high protein content (İ. Ülger et al., 2020). Statistically significant differences were recorded in ADF and NDF parameters across the silage groups included in the current study (P 0.001). The lowest values for both ADF and NDF were noted for the control group (39.70–48.47% DM). The ADF and NDF values increased with the addition of straw and alfalfa. The ADF (21.33%-21.54% DM) and NDF (31.43–30.73% DM) values of the silages reported by Halik et al. (2014) were lower than the values obtained in the current study. The ADF (43.66%-36.59% DM) and NDF (63.33%-49.56% DM) values reported by Ülger et al. (2020) were similar to the values found in the current study.

The IVOMD values of the silages were between 44.26% and 61.78% DM. In the study by Ülger et al. (2020), silages with 95% pumpkin + 5% straw (49.86% DM) and 95% pumpkin + 5% alfalfa (55.52% DM) demonstrated similar IVOMD values (P < 0.001). The present research suggests that the observed reduction in IVOMD values for straw and alfalfa might be attributed to their lower provision of protein that is beneficial for rumen microbes, as well as their higher content of NDF and ADF (Table 2). Numerous studies have indicated a good correlation between the augmentation of protein and energy levels in animal feeds, as well as the reduction of acid detergent fiber (ADF) and neutral detergent fiber (NDF) content, and the enhancement of gas generation (Canbolat & Karaman, 2009). The highest metabolic energy content was found in the control (21.73 MJ/kg) silage due to the high amount of *in vitro* gas and CP content released because of fermentation of pumpkin silage since the metabolic energies of the feeds were calculated by considering the 24-hour gas production values and CP content.

The pH values obtained in the present study were between 3.57 and 4.19 and increased due to the addition of alfalfa (Table 3). These pH values are within the range specified for high quality silages (pH = 3.80–4.30). The ammonia nitrogen values (12.32–28.95% NH_3N-TN) decreased with increasing additive content when compared to the control silage. The ammonia value of silage should be < 11% of the total nitrogen value (Carpemtero et al., 1969). Ammonia nitrogen (NH_3N-TN) is formed as a result of the degradation of proteins in the silo by clostridial bacteria during silage fermentation, and clostridial activity in the silo increases due to low DM level (Kung, 2010). The ammonia nitrogen values in the current study were over 11%.

The CO_2 formation in the present study was 3.39–21.73 g/kg DM. As soon as the silage is taken out of the silo to be fed to the animals, the anaerobic conditions in the silo turn aerobic. Under these conditions, undesirable microorganisms (bacteria such as Clostridia, Enterobactericiae, Bacilli, and Listeria; yeasts of Candida, Hansenula, Pichia, Issatchenkia, and Saccharomyces species; and molds of Aspergillus, Fusarium, and Pencillium species) begin to multiply in the silo and cause the silage to deteriorate (McDonald et al., 1991). The most typical determinants of the process, which is often defined as aerobic deterioration in field conditions, are the development of yeast and mold fungi with the increase in silo temperature. During the feeding period, the microorganisms of concern consume the water-soluble carbohydrates (WSCs) and fermentation products such as lactic acid causing loss of DM and nutrients. As a result of aerobic activity, CO_2 and water are released in the silo, the temperature rises, and the silage begins to deteriorate. The silo's increased CO_2 production reduces the aerobic stability of the silages. Researchers found that silages made from low DM content materials also had poorer aerobic stability values. Straw and alfalfa were added to increase DM, which decreased CO_2 generation and improved aerobic stability in the current study (P 0.001).

An important criterion in determining silage quality is the pH values of silages. The Fleig score, which is an easy method for determining the quality of silo feeds, is calculated according to the pH and DM ratio and every factor affecting the pH and DM ratio also influences the Fleig score. When the desired pH and DM ratio in silo feed are provided, the Fleig score is also high. According to the pH value and DM ratios obtained in the present study, the Fleig score of some mixtures was above 100 points, while in some mixtures it was lower depending on the pH and DM value. The Fleig score can be from 0 to 100 points. However, in some studies, the Fleig scores obtained were 100 and above (Aykan & Saruhan, 2018). A high Fleig score indicates an increase in silage quality. As can be seen from the Fleig scores in our study, the quality was high in all silages.

The present study found that adding straw and alfalfa reduced the lactic acid value in the control silage, which was 60.67 g/kg DM, while increasing the quantity of lactic acid in the silage did not affect the lactic acid value (P 0.001). Łozicki et al. (2015) found that lactic acid levels in pumpkin silages without inoculants (56.4 g/kg DM) and with inoculants (64.3 g/kg DM) were equivalent to those in the control silage in the current experiment (P0.001) (2015).

It is thought that the reason for the high rate of lactic acid bacteria in the control silage is the high content of WSCs. Considering the acetic acid values, it was again found to be high (13.02 g/kg DM) in the control silage, while it decreased with the addition of straw and alfalfa and the lowest value was in the P50S25A25 (4.44 g/kg DM) group. Moreover, the acetic acid values of pumpkin silages without inoculants (22.4 g/kg DM) and with inoculants (25.5 g/kg DM) determined by Łozicki et al. (2015) are higher than the acetic acid values in the current study, while the values reported by Pirinç et al. (2020) for pumpkin (4.13 g/kg DM), pumpkin + acetic acid values of silages with 100 ppm inoculant (4.8% g/kg DM), pumpkin + 15% straw (4.85% g/kg DM), and pumpkin + 15% straw + 100 ppm inoculant (8.14 g/kg DM) added are similar ($P < 0.01$). Lactic acid is produced as the primary byproduct from WSC sources by the homofermentative lactic acid bacteria that are present in the medium during silage fermentation. Contrarily, heterofermentative lactic acid bacteria also generate CO_2, acetic acid, diacetyl, and ethyl alcohol as by-products in addition to lactic acid (Blandino et al., 2003). The high acetic acid concentration (13.2% g/kg DM) of the control group silages assessed in this research may have resulted from their low DM content and the increased effectiveness of heterofermentative bacteria in the silo (Kung, 2010). In the present study, propionic acid values were not determined. Butyric acid values were between 1.05% and 2.73 g/kg DM, and the highest value was found in the control silage ($P < 0.001$). A high-quality silage should have a low butyric acid level. Especially in silo materials with high protein and water content, ammonia and butyric acid are formed in very high amounts. It has been reported that if the pH is not stabilized at or below 4 and sufficient lactic acid is not formed during the first 2 weeks of ensiling, bacteria such as Clostridia that convert lactic acid and sugars to butyric acid may increase (Bolsen et al., 1996).

5. Conclusion

It is concluded that pumpkin (*C. pepo*) waste material could be used in making silage with the addition of wheat straw and alfalfa. While making silage from pumpkin waste, wheat straw and alfalfa can be added to increase the DM and cellulose contents. It is important that the pumpkin must be utilized as silage by preparing a mix silage consisting of pumpkin, wheat straw and alfalfa to prevent environmental pollution in Turkey and provide an alternative source of ruminant roughage.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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