



Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)

<https://dergipark.org.tr/en/pub/yyutbd>



ISSN: 1308-7576

e-ISSN: 1308-7584

Research Article

Effect of Different Additives and Ratios on Silage Quality Characteristics of Common Reed Growing in Drainage Channels

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Article Info

Received: 13.02.2025

Accepted: 24.02.2024

Online published: 15.06.2025

DOI: 10.29133/yyutbd.1639210

Keywords

Fermentation characteristics,
Nutritional and chemical
contents,
Phragmites australis,
Silage additives

Abstract: This study aims to determine some nutritional, chemical, and fermentation properties of Common reed silage using different additives (alfalfa grass, crushed corn, sugar beet molasses, and 1:1 alfalfa grass + sugar beet molasses) and their ratios (0%, 5%, 10% and 15%). Additives were added based on fresh weight to the Common reed grass, which was cut and chopped (1.0 cm sizes) at the beginning of the panicle. Then it was bagged in a vacuum device and left to ferment at 25±2 °C for 60 days. Analyse results showed that all additives increased crude protein, raw ash (RA), dry matter (except molasses), Fleig score, and lactic acid content (except alfalfa) of Common reed silage compared to the control but decreased the pH (except alfalfa), ammonia production, neutral detergent fiber, and acid detergent fiber contents. A similar situation (except RA) occurred as the levels of additives increased. On the other hand, molasses, crushed corn, and increasing levels of these additives increased the lactic acid content of the silage, while decreasing the acetic, propionic, and butyric acid contents. As a result, a quality silo material can be obtained from the Common reed by applying 5% alfalfa grass + 5% molasses.

To Cite: Temel, S, Keskin, B, Uluca, H, 2025. Effect of Different Additives and Ratios on Silage Quality Characteristics of Common Reed Growing in Drainage Channels. *Yuzuncu Yil University Journal of Agricultural Sciences*, 35(2): 360-368.
DOI: <https://doi.org/10.29133/yyutbd.1639210>

1. Introduction

In a healthy diet, animal products should be consumed in sufficient amounts as well as plant-based food sources. This can be achieved by providing sufficient and balanced nutrition for animals, too. However, due to the quality roughage deficit experienced (especially during transition periods and winter months when the herbaceous species in the vegetation dry up), animals cannot be fed adequately, and desired levels of animal products cannot be obtained (Temel and Şahin, 2011; Yavuz et al., 2020). In order to eliminate this problem, the use of corn, sorghum, alfalfa, and many other forage plants as silage has been seen as an important advantage in recent years. However, the amounts of silage obtained from known forage plant species are not at a level that can meet the needs of animals. In this sense, the use of plants that grow spontaneously in idle lands and have low nutritional content when used as green or dry grass has been seen as an alternative solution. Because coarse-structured plants, which are less preferred by animals, can be important feed materials rich in nutrients by ensiling. As a matter of fact, studies conducted, have revealed that silages prepared by adding additives in appropriate proportions to

low-quality feed materials are easily consumed by animals (Özinan, 2017; Güner and Temel, 2022; Keskin and Aksoy, 2024).

Phragmites australis can grow easily in heavy clayey, sandy, and salty soils, marshes and reeds, ponds, ditches, and all kinds of moist land (Mal and Narine, 2004). It can produce high amounts of biomass (16.32-28.35 tons ha⁻¹) per unit area during the growing season (Temel et al., 2023). In addition, the biomass it produces has a high nutritional content (in terms of nitrogen, NDF, potassium, manganese, and magnesium). Due to these properties, it has been shown that the plant can play an important role in closing the missing roughage gap and in the nutrition of ruminants as an alternative roughage source (hay, straw and silage) (Baran et al., 2002; El-Talty et al., 2015; Tanaka et al., 2016; Kadi et al., 2018; Aydoğan and Demiroğlu Topçu, 2022; Büyükkılıç Beyzi et al., 2022). However, the coarse structure of the plant can relatively limit the consumption preference and nutritional composition of the biomass it produces by animals. For this reason, it has been seen as an important advantage to evaluate the plant as silage rather than as fresh or dry grass. As a matter of fact, it has been shown that the common reed silage obtained without using any additives beforehand produces a higher quality feed material than its use as grass (Temel et al., 2023). However, it has been observed that the plant's pure silage is not at the desired quality level (Büyükkılıç Beyzi et al., 2022). For this reason, the idea of obtaining a higher quality silo feed from the plant by using different additives has been deemed important. For this purpose, studies were carried out to improve the nutritional, chemical, and fermentation properties of common reed silage by using different additives (glucose, lactic acid bacteria, urea, formic acid, NaOH, molasses, barley flour, enzyme, and mulberry leaves) and their ratios (Asano et al., 2018; Hussain, 2018; Saeed et al., 2019; Yeşil and Güney, 2023; Kazemi et al., 2024). In particular, alfalfa grass, crushed corn, and sugar beet molasses have been seen as preferred additives to increase the energy and nutrient content of silage feed (Gülümser et al., 2019). However, it has been observed that the studies carried out on the subject are limited.

The objective of the present study was to determine some nutritional, chemical, and fermentation properties of Common reed silage using alfalfa grass, crushed corn, sugar beet molasses, and 1:1 alfalfa grass + sugar beet molasses in different ratios.

2. Material and Methods

The samples were performed in Iğdir located (at 39°59'21"N, 44°03'19"E) in the northeastern part of Türkiye in 2022. In the study, the Common reed plant that grows naturally in the drainage channels was used as silage material. Ground crushed corn and sugar beet molasses were preferred as carbohydrate sources to increase the fermentation quality of silage. In addition, to increase the crude protein content of the silage, alfalfa grass mowed and dried at 1/10 flowering stage was used.

The plants were mowed at a stubble height of 0.1 mm at the beginning of the panicle in the designated area (3 replications and each replication covered an area of 3 m²) in August 2022 and were shredded with a silage machine to a diameter of 2-3 mm without withering. In a previous study conducted on the subject, although the dry matter ratio (64.80%) at the beginning of the panicle was very high compared to other development periods (I. development period; 44.80% and II. development period; 60.32%), it was determined that NDF and ADF content were low, and crude protein, dry matter digestibility, and relative feed value were high (Temel et al., 2023). For these reasons, the panicle beginning period was preferred. However, since the dry matter ratio of the common reed plant is high during the panicle beginning period, 30% water was added from the dry matter calculation to reduce the dry matter ratio and thus, the starting material (control) was created. Then, the determined additives (alfalfa, molasses, and crushed corn) and their ratios (5%, 10%, and 15%) were calculated on the wet material and added to the starting material, and the samples placed in airtight nylon bags were passed through a vacuum device and subjected to fermentation for 60 days. In the study, dry matter ratios of silage samples were determined according to AOAC (1990). The amount of 20 g silage sample and 180 mL pure water were added into the blender and mixed until the mixture became homogeneous. Then, the pH of the samples obtained from the filtrate was measured by a pH meter, and also the amount of ammonia by distillation and titration using the Kjeldahl method (AOAC, 1990). Fleig score was calculated using the equation developed by Kılıç (1986), and then the resulting values were used to reveal the quality class of the silage samples using the scale of "0-20: bad, 21-40: moderate, 41-60: satisfactory, 61-80: good and 81-100: very good".

$$\text{Fleig score} = 220 + (2 * \text{DM}\% - 15) - \text{pH} \quad (1)$$

Crude protein contents of ground silage samples were determined by the Micro Kjeldhal method (AOAC, 1997), and NDF and ADF ratios were analyzed in the ANKOM fiber analyzer using the method developed by Van Soest et al. (1991). 1.0 grams of ground silage samples were taken and kept in a raw ash furnace at 550 °C for 8 hours. The samples that turned gray ash were cooled in a desiccator until they reached room temperature and then the samples were weighed on a precision scale. Then, the weighing values were proportioned with a simple equation and the percentage raw ash ratios were calculated (AOAC, 1990).

The extraction methods determined by Canale et al. (1984) and Saad-Allah and Youssef (2018) were revised to determine organic acids. Then, the amounts of acetic, propionic, lactic and butyric acid in the silage extracts placed in the vial were determined by HPLC-DAD device (mobile phase 0.02 N H₂SO₄ in water, syringe volume 10 microliters (μl), flow rate 0.6 ml/min, column temperature 50 °C, detector wavelength 210/4 nm, column type HI-Plex H, 300 x 7.7 mm, column part no PL1170-6830, operating system isocratic and detector pressure 36.5 bar).

Since the additive applications were made under controlled conditions (laboratory), the data obtained from the study were subjected to analysis of variance (ANOVA) according to the completely randomized design in the IBM SPSS 17.0 statistical package program. The differences between the applications that were significant were determined by Duncan's multiple comparison test at 1% probability.

3. Results and Discussion

The effects of additives added at different rates on silage dry matter, pH, ammonia production, and Fleig score were found statistically significant ($p < 0.01$) (Table 1).

3.1. Silage dry matter, pH, Ammonia production, and Fleig score

When Table 1 was examined, the highest dry matter ratio was measured in silo feed with 15% alfalfa and 15% alfalfa + 15% molasses addition, while the lowest ratio was measured in common reed silage with 5% molasses addition following 10% and 15% molasses applications. These results showed that other additives and applications except molasses increased the silage dry matter ratio compared to the control.

Table 1. The DM, pH, ammonia, and Fleig score of Common reed silage

Applications	DM (%)	pH	Ammonia (%)	Fleig score
0% Control	43.56 d	4.81 c	6.30 a	84.58 f
5% AG	48.40 b	5.08 b	5.68 ab	83.73 fg
10% AG	48.82 b	5.17 a	5.50 abc	80.83 g
15% AG	49.87 a	5.22 a	5.17 abc	81.07 g
5% SBM	42.40 e	4.11 g	4.99 abc	110.40 cd
10% SBM	42.84 de	4.08 g	3.94 cd	112.34 c
15% SBM	43.14 de	3.89 h	3.33 d	120.54 b
5% CC	48.11 b	4.72 d	5.34 abc	97.28 e
10% CC	48.17 b	4.42 ef	5.21 abc	109.42 cd
15% CC	48.72 b	4.06 g	4.99 abc	124.90 a
5% AG+ 5% SBM	46.72 c	4.38 f	4.52 bcd	108.23 d
10% AG + 10% SBM	48.31 b	4.41 f	4.08 bcd	110.08 cd
15% AG + 15% SBM	49.86 a	4.48 e	3.95 cd	110.65 cd
F value and significant	174.46**	893.80**	5.12**	403.41**

Means shown by different letters in the same column are significantly different by Duncan at $p < 0.01$. AG; Alfalfa grass, SBM; Sugar beet molasses, CC; Crushed corn, DM; Dry matter.

This may be due to the fact that alfalfa hay and crushed corn have higher dry matter content than molasses. In fact, the average dry matter ratio of alfalfa hay is 91.1%, corn crushed is 87.0% and beet molasses is 74.0% (Cacan et al. 2012). In addition, studies conducted with different feed materials have reported that molasses and crushed corn used as additives increased the dry matter ratio of the silage obtained (Keskin and Aksoy, 2024).

The pH value of the common reed silage varied between 3.89-5.22 (Table 1). When Table 1 is examined, only alfalfa grass increased the pH of the silage compared to the control, while other additives decreased the pH of the silage. Similar results were obtained in previous studies conducted with different species (Acar and Bostan, 2016; Kardes et al., 2023). This may be due to the high soluble sugar content of molasses and crushed corn, while the low content of alfalfa. On the other hand, to prevent proteolysis, which breaks down proteins into ammonia and causes silage deterioration in silo feeds, the pH of the silage is desired to fall below 4 (four) (Virtanen, 1993). In this study, it was observed that the application of 15% molasses added to common reed decreased the silage pH below 4 (four). In addition, considering the high dry matter ratios of the silages obtained, it can be said that other applications except alfalfa are suitable in terms of silage pH. As a matter of fact, in low moisture silages, the pH does not drop much and remains around pH 4.9. In addition, the fact that the pH does not decrease in this type of silo feed does not pose a problem in terms of silage quality (Açıkgoz, 2001).

Ammonia production of common reed silage varied between 3.33% and 6.30% (Table 1). The highest ammonia production (6.30%) was determined in common reed silage where no additives were used (control), and the lowest ammonia production was determined in 15% molasses application. On the other hand, it was observed that additives reduced ammonia production of common reed silage compared to control. In fact, in studies conducted with different species, it was reported that the additives used (molasses, corn grits, wheat bran, and barley grain) reduced the ammonia production of the silage obtained compared to the control (Bingol et al., 2009; Keskin and Aksoy 2024). In addition, increasing additive rates reduced the ammonia production of the silages. For example, while ammonia production in 5% alfalfa, 5% molasses, 5% crushed corn, and 5% alfalfa + 5% molasses applications was 5.68%, 4.99%, 5.34%, and 4.52%, respectively, these rates were measured as 5.17%, 3.33%, 4.99%, and 3.95%, respectively, in the highest dose of 15% applications. These results showed that the additive that reduced silage ammonia production the most was molasses following the alfalfa + molasses application.

When Table 1 was examined, it was seen that the Fleig score of the silages with control and alfalfa additive was good, and the Felig score of the silages with other additives was of the best quality. In general, while the alfalfa additive decreased the silage Felig score compared to the control, the other additives increased the silage Fleig score. In addition, increasing alfalfa additive doses decreased silage Fleig score, while increasing the doses of molasses, crushed corn, and alfalfa grass + molasses additives increased silage Felig score. This may be because the alfalfa additive increases silage pH compared to other additives. Indeed, in the current study, the highest Fleig scores were determined in the applications of 15% molasses and 15% crushed corn with low silage pH, while the lowest Fleig scores were calculated in the applications of 10% alfalfa and 15% alfalfa with high silage pH. Because the Fleig score is calculated by using the pH value and dry matter ratio (DM) of the silage material, and according to this equation, high silage pH decreases the Fleig score, and vice versa, increases it.

3.2. Crude protein, NDF, ADF, and Raw ash contents

Compared to the control, the additives used and their increasing doses caused significant increases in the crude protein content of common reed silage (Table 2). Accordingly, the highest silage crude protein content was measured in 15% alfalfa + 15% molasses application with 9.95%. The second significant increases in silage crude protein content were determined in 15% alfalfa (9.43%), 5% alfalfa + 5% molasses (9.28%), and 10% alfalfa + 10% molasses (9.75%) additive applications. These results showed that especially the alfalfa additive had a significant effect on the increase in silage crude protein content. This is an expected result. Because alfalfa grass has a high crude protein content (Keskin et al., 2021). As a matter of fact, in studies conducted with different forage materials, it has been reported that the presence of legume species in silage mixtures and increasing their proportions cause significant increases in the crude protein content of the silage obtained (Kaymak et al., 2021; Kardeş et al., 2023).

In the common reed silage, the NDF ratio varied between 51.31% and 70.74% and all additives applied decreased the silage NDF ratios compared to the control. In addition, as the additive ratios

increased, the silage NDF contents decreased (Table 2). Accordingly, the highest NDF ratio was determined in the control group where no additives were used (70.74%), and the lowest NDF content was determined in the 10% alfalfa + 10% molasses and 15% alfalfa + 15% molasses applications in the same statistical group. In a previous study, the NDF content of common reed silage harvested at the beginning of the panicle and without any additives was determined as 69.02% (Temel et al., 2023). Molasses is generally a material with a high water-soluble carbohydrate content and can provide the necessary energy for microorganisms that break down hemicellulose and cellulose, and as a result, it increases the activity of cellulolytic bacteria. On the other hand, NDF content is generally low due to the lack of cell wall substances (cellulose and hemicellulose) in alfalfa hay (Keskin et al., 2021; Erbeyli et al., 2022). For these reasons, the addition of alfalfa hay + molasses may have further reduced the NDF content of common reed silage. Indeed, Kazemi et al. (2024) stated that molasses addition and increasing doses compared to control decreased the NDF content of common reed silage. These results support the findings of the current study.

Table 2. The CP, NDF, ADF, and RA of Common reed silage

Applications	CP ratio (%)	NDF ratio (%)	ADF ratio (%)	RA ratio (%)
0% Control	6.82e	70.74a	47.98a	9.27f
5% AG	8.29b-e	68.26ab	45.69ab	9.81def
10% AG	8.82abc	65.06bcd	45.77ab	11.04ab
15% AG	9.43ab	64.72bcd	47.71a	11.06ab
5% SBM	7.77cde	62.14cb	45.53ab	11.14ab
10% SBM	8.28b-e	61.09cde	42.66bc	11.33ab
15% SBM	8.74a-d	61.05cde	40.47cde	11.63a
5% CC	7.27de	66.68abc	45.59ab	10.75abc
10% CC	7.57cde	63.30bcd	42.33cd	10.18cde
15% CC	7.71cde	60.09de	37.77ef	9.69ef
5% AG+ 5% SBM	9.28ab	56.41ef	36.88f	11.52a
10% AG + 10% SBM	9.75ab	54.58f	39.39def	11.35ab
15% AG + 15% SBM	9.95a	51.31f	40.31cde	10.55bcd
F value and significance	8.63**	18.22**	26.17**	14.88**

Means shown by different letters in the same column are significantly different by Duncan at $p < 0.01$. AG; Alfalfa grass, SBM; Sugar beet molasses, CC; Crushed corn, NDF; Neutral detergent fiber, ADF; Acid detergent fiber, CP; Crude protein, RA; Raw ash.

When Table 2 was examined, the ADF content of common reed silage varied between 36.88% and 47.98%. In general, additives decreased the ADF content of silage compared to the control (except alfalfa). The highest ADF rate was determined in the control (47.98%) and 15% alfalfa (47.71%) additive applications in the same statistical group, while the lowest ADF rate in silage was determined in the 15% corn application followed by 5% alfalfa + 5% molasses application. On the other hand, increasing molasses and corn crushing doses decreased the silage ADF rates, while increasing alfalfa and alfalfa + molasses applications increased the silage ADF rates. This may be due to the higher ADF content of alfalfa compared to other additives. Because the alfalfa plant contains more lignin than carbohydrate-rich cereals. This is because alfalfa has a leaf steam and nerves rich in lignin content (Fales and Fritz, 2007).

The raw ash content of common reed silage varied between 9.27% and 11.63% and all applied additives increased the raw ash content of silage compared to the control (Table 2). On the other hand, increasing alfalfa and molasses additive doses increased the raw ash content of silage, while increasing doses of corn and alfalfa + molasses additive decreased it. Accordingly, the highest raw ash ratio was obtained in 15% molasses and 5% alfalfa + 5% molasses applications and these two applications were statistically in the same group. The lowest raw ash content was determined in the control group following 15% corn crushing.

3.3. Lactic acid, acetic acid, propionic acid and butyric acid

The lactic acid content of the silo feed obtained varied between 0.843% and 5.320%, and the highest lactic acid content was determined in the 15% molasses additive application (5.320%) followed

by the 10% molasses application (4.823%) (Table 3). The lowest lactic acid rate was determined in the 15% alfalfa additive application and this was followed by the control (%), 5% alfalfa, 10% alfalfa, and 5% molasses additive applications in the same statistical group. These results showed that additives, except for the alfalfa additive, generally increased the lactic acid content of silage. This may be due to the high soluble sugar content of molasses and crushed corn. Because molasses and corn have high water-soluble carbohydrate (WSC) content in dry matter (Canbolat et al., 2010). In general, in silages, the pH should fall between 3.5 and 4.0 for fermentation to develop healthily and the lactic acid content should increase to 2.0% and above for the silage material to be stored for a long time without spoiling (McDonald et al., 1991). According to this information, it was observed that other additives, except for the control and alfalfa applications, provided the desired lactic acid rate.

Table 3. The lactic, acetic, propionic, and butyric acid of Common reed silage

Applications	Lactic acid (%)	Acetic acid (%)	Propionic acid (%)	Butyric acid (%)
0% Control	1.383f	1.207c	0.093e	0.110c
5% AG	1.360f	1.317c	0.217d	0.220b
10% AG	1.220f	1.587b	0.377c	0.227b
15% AG	0.843g	1.747b	0.530a	0.280a
5% SBM	3.253c	0.743d	0.083ef	0.047d
10% SBM	4.823b	0.630de	0.070ef	0.020d
15% SBM	5.320a	0.447e	0.047f	0.020d
5% CC	1.483f	0.633de	0.060ef	0.023d
10% CC	2.347e	0.577de	0.047f	0.020d
15% CC	2.677d	0.553de	0.073ef	0.023d
5% AG+ 5% SBM	3.303c	1.210c	0.213d	0.193b
10% AG + 10% SBM	3.107c	1.753b	0.353c	0.123c
15% AG + 15% SBM	3.093c	1.973a	0.447b	0.113c
F value and significance	304.69**	97.03**	387.20**	62.85**

Means shown by different letters in the same column are significantly different by Duncan at $p < 0.01$. AG; Alfalfa grass, SBM; Sugar beet molasses, CC; Crushed corn.

When Table 3 was examined, it was seen that molasses and crushed corn silage decreased the acetic acid content compared to the control and also that the acetic acid content decreased as the ratio of these two additives increased. This may be due to the high soluble sugar content of molasses and corn crushed. Accordingly, the acetic acid ratio in molasses applications varied between 0.447% and 0.743%, and in cracked corn applications it varied between 0.553% and 0.633%. These results showed that acetic acid was in the required range (0.3-0.8%) in a good silage (Menke and Huss, 1975). On the other hand, alfalfa and alfalfa + molasses additives and also the increasing doses of these additives generally increased the acetic acid content of the silage (Table 3). This increase may have been due to the alfalfa hay used as an additive. Because the water-soluble carbohydrate content in legume forage plants is low and the buffer capacity is high. This also slows down the pH drop (McDonald et al., 1991; Albrect and Muck, 1991).

When Table 3 is examined, it was determined that alfalfa and alfalfa + molasses additives and increasing doses of these additives increased the silage propionic acid content compared to the control. However, it was determined that crushed corn and molasses additives decreased the silage propionic acid content. According to these data, the highest silage propionic acid content (0.530%) was determined in 15% alfalfa additive, the lowest propionic acid rate was determined in 15% molasses and 10% crushed corn applications, and these two applications were statistically in the same group.

In the present study, the Butyric acid content of the silage obtained varied between 0.020% and 0.280% (Table 3). It has been observed that these rates are at the required levels in silo feeds. In fact, in a quality silo feed, the butyric acid content is desired to be between 0.1% and 0.7% (Weinberg and Ashbell, 2003). When Table 3 was examined, it was seen that alfalfa and alfalfa + molasses applications increased the butyric acid content of the silage compared to the control, and the highest butyric acid rate was determined in the 15% alfalfa additive application. However, crushed corn and molasses additives reduced the butyric acid content of silage compared to the control and the butyric acid content remained

at the lowest level in these applications. This may be because molasses and crushed corn with high soluble sugar content cause more lactic acid formation in the silage. Indeed, when the appropriate pH and dry matter ratio are provided in silo feeds, lactic acid production is high and the undesirable butyric acid content is low (Canbolat et al., 2010).

4. Conclusion

When compared with the control, it was determined that all additives used and increasing doses generally increased the crude protein and raw ash content of the silo feed and decreased the ammonia, NDF, and ADF content. On the other hand, molasses, crushed corn (except alfalfa), and their increasing doses increased the Fleig score and lactic acid content of the silage, while decreasing the acetic acid, propionic acid, and butyric acid contents compared to the control. As a result of the study, it was concluded that Common reed can be evaluated as an alternative silo feed in animal feeding.

Ethical Statement

This study does not require Ethics Committee Approval.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Funding Statement

The study was approved by the University of Igdir, Scientific Research Project Unit, under project number ZIF0324A02.

Author Contributions

Authors contributed equally.

Acknowledgements

We wish to express the University of Igdir, Scientific Research Project Unit for their finance contributions.

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