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Determination of Silage Quality of Alfalfa and Annual Ryegrass Mixture

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Abstract
Animal feed often includes cereal straw and forage crops. Silage is a traditional process practiced ensiliation of seasonally harvested forage or some crops, ensuring a constant supply of feed for animals. In this study, which was carried out to determine the quality of silages made of alfalfa and annual ryegrass in plain and different mixture ratios in Bilecik conditions. This study was carried out to determine the silage quality of alfalfa and Italian ryegrass mixtures (100:0, 80:20, 60:40, 40:60, 20:80 and 0:100). The harvested plants were cut into 2 cm size after wilting a little and filled into 2 kg vacuum silage packages considering the mixing ratios. The silage samples, whose air was removed by vacuum packaging machine and their mouths were closed, were stored at 25±2 ºC for 45 days. In treatments were determined physical observations, dry matter rate, pH, crude protein rate, acid detergent fiber rate, neutral detergent fiber rate, relative feed value, acetic acid, lactic acid and some mineral contents. As a result, it was determined that annual ryegrass added to alfalfa at different rates positively affected silage quality. Considering all the examined characters, it was found that adding 40 – 80 % annual ryegrass to the alfalfa silage as better results in terms of silage quality.

Research Article

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1. Introduction

Feed costs are an important factor determining profitability in livestock enterprises. As a matter of fact, the cost of feed constitutes 70% of the total cost of an enterprise (Şengül, 2022). Therefore, the lower roughage cost of an enterprise, the higher the profitability rate. For this reason, it is necessary to harmonize plant and animal production within the same enterprise (İnan et al., 2022). The roughage requirement for animal husbandry is provided from meadows and pastures and sources such as herbage, dry herbage and silage obtained from field agriculture. When the weather is hot, meadows and pastures come to the fore as a source of roughage, while as the weather starts to get colder, grasses that are cut and dried from the field areas and silage become common as a source of roughage to be consumed in winter months. Especially recently, the increase in silage production in Türkiye is an indication of the increasing importance given to silage (Tutar and Kökten, 2023). The main purpose of silage production is to store the green forage material with high nutritional value with the least loss of nutrients. Crude protein content, dry matter ratio and carbohydrate content of silage plants are important factors in silage making. Legumes have high protein and low carbohydrates. While this reduces the production of lactic acid, which is a desirable acid in silage, the pH is not at the desired level since it also neutralizes the acid produced during fermentation. On the other hand, the protein content of annual ryegrass is low. For this reason, it is important for silage quality to mix legumes and cereals in a certain ratio. Wang et al. (2022) was conducted to determine the effects of alfalfa-sweet sorghum mixture silages on blood and rumen physiological status and rumen microbiota in sheep. They reported that adding 40 % sweet sorghum to alfalfa was the best mixture in improving serum antioxidant capacity, dry matter intake, rumen fermentation and cellulolytic bacteria abundance of lambs. Özyazıcı et al. (2022) conducted a study to determine the quality of Fenugreek with oat and rye mixture silages, they reported that the "25% oat + 75% fenugreek" mixture was superior to silages of the other mixtures when considered the chemical parameters. Another study reported that high moisture alfalfa silages should be ensiled with at least 30% peanut vine to increase the fermentation quality and nutritional value (Sun et al., 2023). In another study investigating some effects such as chemical composition, fermentation quality and bacterial communities of silages consisting of mixtures of alfalfa with perennial ryegrass in different proportions, it was reported that adding 70% perennial ryegrass to alfalfa increased the fermentation quality and high quality silage was obtained (Fan et al., 2022). In this study, it was aimed to determine the silage quality of mixtures of alfalfa and annual ryegrass at different rates.

2. Material and Methods

This study was carried out to determine the silage quality of alfalfa and Italian ryegrass mixtures (100:0, 80:20, 60:40, 40:60, 20:80 and 0:100). Nimet variety in alfalfa and İlkadım variety in Italian ryegrass were used as material, and alfalfa was harvested at 10% flowering period and Italian ryegrass was harvested during full bloom period in this study. In general, the harvest times of the two plants were close to each other. The harvested plants were cut into 2 cm size (Başaran et al., 2018) after wilting a little and filled into 2 kg vacuum silage packages considering the mixing ratios. The silage samples, whose air was removed by vacuum packaging machine and their mouths were closed, were stored at 25±2 ^{o}C for 45 days (Karadeniz and Saruhan, 2021).

The physical properties of silage samples were determined according to the table that

based on the scoring method developed by the German Agricultural Organization (DLG) given by Yalçınkaya et al. (2012). Accordingly, the color was evaluated between 0–2, and 2 points were given for the silage that retained its color, 1 for the slightly changed silage, and 0 for the highly changed silage. Those with aromatic smells were scored 14, those with mild escalation 8, those with strong escalation 4, those with ammonia and very sour smells 2, and those with rotten mold smells 0 points. Structurally intact silage received 4, slightly deteriorated silages 2, heavily degraded and moldy silages 1, rotten and heavily contaminated silages scored 0. As a result of the scores obtained from the physical observations, the feed quality classes of the silages were determined. According to this; Silage with 0-4 points was in the bad class (useless), 5-9 points were in the low class, 10-13 points were in the middle, 14-17 points were in the good class, and 18-20 points were in the very good class.

After the forty-five-day fermentation period, 20 g samples were taken from the silages that were opened, 100 ml of distilled water was added to it, and it was mixed thoroughly with the help of a blender and filtered through filter paper (Başaran et al., 2018). The pH of the silages was measured with the help of a digital pH meter. After determining the wet weight of the fresh silage samples, they were placed in an oven and dried at 105 ^{o}C until they reached a constant weight, and the dry sample weight was proportioned to the wet sample weight. Dry matter ratio was determined as the dry sample weight was proportioned to the wet sample weight. Flieg scores were calculated with the help of the following formula for the silage samples whose dry matter and pH values were determined. Silage quality classes were determined according to the calculated Flieg score. Accordingly, the Flieg score was classified as bad between 0-20, low between 21-40, moderate between 41-60, good between 61-80 and very good between 81-100.

Flieg Points: 220+(2 x % Dry Matter – 15) – 40 x pH (İptaş et al., 2009; Uygur, 2023).

In the study, the samples dried at 65 °C until they reach a constant weight in order to determine the protein ratios of the silages were ground in a mill with a sieve diameter of 1 mm in the laboratory and made ready for analysis. Then, the total N values of these samples were determined by the Kjehldahl method and the protein ratios were determined by multiplying the nitrogen values with the coefficient of 6.25. In the samples prepared for protein analysis, the ADF and NDF values were found in by using the ANKOM 200 Fiber Analyzer device according to Van Soest method (Van Soest et al., 1991; Kutlu, 2008). Relative feed value (RFV) was determined the following formula (Khalilian et al., 2022).

RFV=[(88.9-(0.779*ADF))x(120/NDF)x0.775]

Relative Feed Value to determine the market prices of forage crops and divided the forage crops into 6 quality classes. According to this, if RFV is greater than 151, it is the best quality, if it is 125-151, very good (1. class), if 103-124 is good (2. class), if 87-102 is medium (3. class), 75-86 is bad quality (4. class) and if 75 is unacceptable (5. class) (Önal Aşcı and Acar, 2018).

Lactic acid and acetic acid analysis, high performance liquid chromatography (HPLC), nutrients (potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg)) Inductively Coupled Plasma-Mass Spectrometer (ICP-) MS) was determined using the instrument.

The data obtained from the study except for physical properties were analyzed according to the randomized plots experimental design and using the SPSS 20.0 statistical package program and the differences between the averages were

revealed with the Duncan multiple comparison test.

3. Results and Discussion

Color, smell, structure and total scores of alfalfa and ryegrass silages made as pure and mixed, and the values of forage quality classes are given in Table 1.

Apart from many chemical methods used to determine the silage quality, it is possible to have information about the quality of silage by looking at the color, smell and structure characteristics, which are organisms that are simpler, low speed and can be applied under all conditions (Aykan and Saruhan, 2018). It is stated that the color of the silo feed varies from light green to light brown or darker tones depending on the plant from which it is made (Uygur, 2023). It is expected that the color of the silage feed should have preserved the color at the time of ensiling in a silage that has been well ensiled and has not experienced storage. The values in which the colors in the residues of the silages made in the study do not cause any loss (Table 1). It was determined that the colors of all silages made were in the desired structure. Smell is very important quality trait of silage. Because, a slightly sweet, acid smell may indicate a good fermentation has occurred (Anonymous, 2023). Pure ryegrass, % 40A+% 60RG and % 20A+% 80RG silages have performed very good in terms of silage structure and smell. For these reasons, these silages were taken places in very good quality class.

Table 1. Some physical traits determined in pure and mixture of alfalfa and ryegrass silage

Mixtures	Color	Smell	Structure	Total	Quality Class
Alfalfa	2.0	6.7	2.0	12.0	Medium
Ryegrass	2.0	14.0	4.0	20.0	Very good
%80A+%20RG	2.0	8.0	2.0	12.0	Medium
%60A+%40RG	1.7	8.0	2.0	11.7	Medium
%40A+%60RG	2.0	14.0	4.0	20.0	Very good
%20A+%80RG	2.0	14.0	4.0	20.0	Very good

A: Alfalfa; RG: Annual Ryegrass;

Dry matter, pH, Flieg and quality class of alfalfa and ryegrass silages made as pure and mixed are given in Table 2. Adding ryegrass to alfalfa silage increased dry matter and flieg point of silages and decreased pH. The increase in DM content is probably due to the restriction of growth and development of a certain group of microorganisms in silage. As a result, the loss of nutrients in the silage is less (Besharati et al., 2023). Silage is a fermented feed that have been preserved by fermentation to the point of acidification. Therefore, pH is used to evaluate fermentation. Silage pH is an important parameter used to determine silage quality. The pH content should be between 4.6 – 4.8 in a quality silage (Filya, 2001). In these study, the highest pH was obtained from pure alfalfa (5.2), and the lowest was obtained from mixtures (4.6) except for % 80A+% 20RG. Mixtures of % 60A+% 40RG, % 40A+% 60RG and % 20A+% 80RG are among these values. According to Flieg score, silages were in the medium, good and very good quality class. The best quality silage was determined in mixture of % 60A+% 40RG, % 40A+% 60RG and % 20A+% 80RG.

Table 2. Some quality traits determined in pure and mixture of alfalfa and ryegrass silage

Mixtures	Dry Matter (%)**	pH**	Flieg**	Quality Class
Alfalfa	28.3 c	5.2 a	53.2 d	Medium
Ryegrass	32.9 a	4.8 bc	80.1 b	Good
%80A+%20RG	30.7 b	4.9 b	70.0 c	Good
%60A+%40RG	33.1 a	4.6 d	88.2 a	Very good
%40A+%60RG	31.0 b	4.6 cd	82.9 ab	Very good
%20A+%80RG	31.6 ab	4.6 cd	83.9 ab	Very good

*A: Alfalfa; RG: Annual Ryegrass, ** No significant difference at the p≤0.01 level were determined between the means shown with the same letter and same columns*

Crude protein (CP) rate, Acid detergent fiber (ADF) rate, Neutral detergent fiber (NDF) rate, Relative feed value, Acetic (AA) and Lactic acid (LA) value of alfalfa and ryegrass silages made as pure and mixed are given in Table 3. The effect of mixtures on CP, ADF, NDF, RFV, AA and LA contents of silages was very significant (P<0.01). One of the important characteristics evaluated as a quality criterion in forages are the protein content rate of the feed. The highest protein content was determined in alfalfa silage with 20.1 % because it is a legume. However, the protein content of the other mixtures was in the good quality class, except for pure annual ryegrass. As the alfalfa ratio decreased in silages, protein ratios also decreased (Can et al., 2020; Mut et al., 2020; Görü and Seydoşoğlu, 2021; Demiroğlu Topçu and Kahya, 2023). ADF and NDF contents determined in feeds are related to feed digestibility and are an indicator of the cellulose, hemicellulose and lignin content rate of the feed (Bakhtiyari et al., 2020). Generally, forage contains relatively high crude fiber content which can be indicated by the contents of ADF and NDF of the forage (Farhadi et al., 2022). In our study shows the changes in ADF and NDF under different mixtures. ADF and NDF ratios of silages varied between 30.6-35.3 % and 42.0-57.1 % respectively. As the alfalfa content decreased in silage, the ADF and NDF rates increased. RFV varied between 143.7-100.0 and the highest value was determined in pure alfalfa silage with 143.7. According to RFV values, silage quality in pure alfalfa, 80%+20%RG and 60%+40%RG treatments was in the premium class (Önal Aşçı and Acar, 2018).

Table 3. Some quality traits determined in pure and mixture of alfalfa and ryegrass silage

Mixtures	CP (%)**	ADF (%)**	NDF (%)**	RFV**	AA (%)**	LA (%)**
Alfalfa	20.1 a	30.9 d	42.0 d	143.7 a	0.089 ab	1.30 d
Annual Ryegrass	12.0 e	35.3 a	57.1 a	100.0 d	0.109 a	2.09 cd
%80A+%20RG	18.3 b	30.6 d	43.9 c	137.8 b	0.076 b	2.27 bcd
%60A+%40RG	15.8 c	31.5 cd	47.6 b	125.7 c	0.075 b	3.24 abc
%40A+%60RG	15.1 d	32.5 bc	48.8 b	121.2 c	0.068 b	4.28 a
%20A+%80RG	15.0 d	33.4 b	56.8 a	103.0 d	0.066 b	3.53 ab

CP: Crude protein; ADF: Acid detergent fiber; NDF: Neutral detergent fiber; RFV: Relative Feed Value; AA: Acetic acid; LA: Lactic acid; A: Alfalfa; RG: Annual Ryegrass

** No significant difference at the p≤0.01 level were determined between the means shown with the same letter and same columns

In a good silage, lactic acid content of 2% and above is desired, while acetic acid is desired to be between 0.3-0.8% (Uygur, 2023). In present study, acetic acid varied between 0.0109 – 0.066 %, and lactic acid varied between 1.30-4.28 %. While acetic acid rate was within the desired values in all treatments, lactic acid content was determined to be very low in pure alfalfa. Low lactic acid content negatively affects the quality of silage. Therefore, it would be more suitable to make silage in a mixture with ryegrasses instead of pure alfalfa silage.

Potassium (K), calcium (Ca), phosphorus (P) and magnesium (Mg) rates of alfalfa and ryegrass silages made as pure and mixed are given in Figure 1. The effect of mixtures on K, Ca, P and Mg contents of silages was very significant ($P<0.01$). Potassium, calcium, phosphorus and magnesium contents of mixture was ranged from 3.58 to 2.88%, 1.31 to 0.65%, 0.46 to 0.44% and 0.27 to 0.17%. Kidambi et al. (1989) reported that P, Ca and Mg contents in forages must be 0.21%, 0.3% and 0.1% respectively. The values determined in all treatments in the present study were above the desired level.

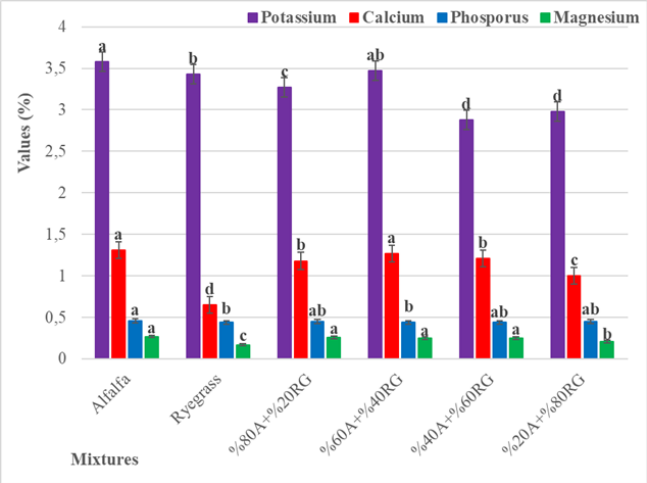

Figure 1. K, Ca, P and Mg contents determined in pure and mixture of alfalfa and ryegrass silage

4. Conclusion

In this study, which was carried out to determine the quality of silages made of alfalfa and annual ryegrass in plain and different mixture ratios in Bilecik conditions. As a result, it was determined that annual ryegrass added to alfalfa at different rates positively affected silage quality. Considering all the examined characters, it was found that adding 40 - 80 % annual ryegrass to the alfalfa silage as better results in terms of silage quality.

Authors' Contribution Declaration

Authors declare that they have contributed equally to the article and that they have seen/read and approved the final version of the article ready for publication.

Conflict of Interest Declaration

All authors declare that there is no conflict of interest for this study.

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