Livestock Studies, 2024; 64(2), 46-50
http://doi.org/10.46897/livestockstudies.1534399

LIVESTOCK STUDIES

RESEARCH ARTICLE

The Effects of Biscuit on Fermentation Characteristics, Aerobic Stability and In Vitro Organic Matter Digestibility of Alfalfa Silage

Besime DOĞAN DAŞ[1,*], Duygu BUDAK[2], Aydın DAŞ[3]

[1]Harran University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Disease, Şanlıurfa, Türkiye.
[2]Aksaray University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Disease, Aksaray, Türkiye.
[3]Harran University, Faculty of Veterinary Medicine, Department of Zootecnichs, Şanlıurfa, Türkiye.
* Corresponding author

Article History
Received: 24 Jun 2024
Accepted: 05 Aug 2024

Corresponding Author
E-mail: bdas@harran.edu.tr
Tel: +905071916514

Keywords
Alfalfa
Biscuit
Digestibility
Methane production
Silage

Abstract

This study was conducted to determine the effects of addition of expired biscuit to alfalfa silage on silage quality, fermentation characteristics, aerobic stability and *in vitro* organic matter digestion. In the study, the silage group without additives constituted the control group, while the silages prepared by adding 1% biscuit, 2% biscuit and 4% biscuit constituted the experimental groups. Silages were opened after 70 days ensiling. There were significant differences among groups for dry matter (DM), ash, crude protein (CP), neutral detergent fiber (NDF), pH, carbon dioxide (CO_2) formation, *in vitro* organic matter digestion (IVOMD), metabolizable energy (ME) and in vitro methane gas (CH4) values of silages. The silage pH range of all silage groups was 5.12-5.82. When the CO_2 formation amounts of the silage groups were compared with the control group silages, it was determined that the amount of CO_2 released in the silages with increasing amounts of biscuits decreased ($P<0.05$) and IVOMD, ME and CH4 values increased ($P<0.05$). As a result, biscuits past their expiration date can be used as a silage additive in the ensiling of alfalfa. It was determined that it was appropriate to add 4% of expired biscuit waste in the ensiling of alfalfa.

Introduction

Animals meet their green fodder needs from meadows and pastures in certain periods of the year according to the specific vegetation conditions of each region. The productivity of animals is high in these 150-200 day periods and decreases in other periods. It is possible to minimize the difference in yield between seasons by providing sufficient quantity and quality of roughages rich in sap to animals outside these periods (Acar and Bostan, 2016).

Alfalfa has an important place in the rations of dairy cows, especially due to its contribution to the amount of cellulose and protein in rations. In order to ensure high milk yield, high quality alfalfa is primarily needed in enterprises. In dry climates, alfalfa can be preserved by drying. In humid climates, however, alfalfa harvest carries a significant risk of rain during drying in the field (Aksu and Yakışır, 2019). Therefore, the necessity of silage production arises in the green storage of green forages. Preservation of green forages by making silage during the seasons when they are abundant can ensure that the productivity of animals is at the same level throughout the year (Çetiner and Polat, 2022).

Successful ensiling of alfalfa without additives is difficult (Aksu *et al*., 2017). The high buffering capacity, low water-soluble carbohydrate content and low dry matter content of legumes make their ensiling difficult. In recent years, there have been studies in which various silage additives have been used to obtain high quality legume green grass silages by eliminating these negativities. These additives are inoculants to improve fermentation in alfalfa silage (Koç *et al*., 2017), sugar-based fermentable liquids (Denek *et al*., 2011), carbohydrate sources (Şakalar and Kamalak, 2016) and fermentation- limiting organic acid additives (Ke *et al*., 2017).

Due to the insufficiency of feed resources, the use of many industrial wastes as an alternative feed source in animal nutrition has come to mind. Foods that are used in human nutrition and whose shelf life has expired, cannot be offered for human consumption due to problems that occur during their production (spillage, damage to packaging, etc.), and problems arising during transportation and storage lead to the formation of significant amounts of food waste. This situation causes great economic losses for food production companies. When these foods are utilized in animal husbandry enterprises, both economic and environmental damage is prevented. Pasta, biscuits, cakes, wafer crumbs, instant soups, puddings, etc. are used as animal feed in many farms (Korkmaz and Önenç, 2017). However, there are few studies on their use in animal nutrition (Çotuk and Önenç, 2016; Aydın, 2023).

This study was conducted to determine the effects of adding expired biscuits to alfalfa silage (0%, 1%, 2% and 4%) on silage quality, fermentation properties, aerobic stability and *in vitro* organic matter digestion.

Materials and Method

In this study, alfalfa (Medicago sativa L.) plant was used as silage raw material. The alfalfa was obtained from a private enterprise during the flowering period. The alfalfa was withered and chopped in the silage machine to approximately 1.5-2.0 cm in size. The control group used in the study consisted of alfalfa without additives (0%), while experimental groups were formed with 1%, 2%, 4% biscuit waste additions. Expired sweet biscuits were obtained from a supermarket. Biscuits were used by grinding. The control and each experimental group were pressed into 1,5 liter glass jars as 5 replicates. Silages were opened after 70 days of fermentation at room temperature in a dark environment. Silages were opened after 70 days of fermentation in a dark environment. After the top 3-5 cm of the jars were discarded, 100 ml of pure water was added to 25 g of silage samples taken homogeneously and the pH value of the disintegrated silage liquid was quickly measured with a laboratory pH meter in just a few minutes and recorded. Aerobic stability test (determination of CO_2 production values) analysis of silage samples was performed according to the method reported by Ashbell *et al*. (1991). After the silages were opened, they were subjected to an aerobic stability test for 5 days. The pH of the silage samples on the 5th day of aerobic stability was measured and their CO_2 production was determined. In the research, abrasion-resistant, gas-tight 1.5 L polyethylene (PET) bottles were used to perform the aerobic stability test. To create a test unit, the pet bottle was cut into two: 1L and 0.5L. 1 cm diameter hole was drilled in the lid of the 1L PET bottle to ensure air circulation. Then, 0.5 L was placed on the cut section. Fresh silage samples between 250-300 g were placed on the upper part of the unit without compressing, and 100 ml of 20% potassium hydroxide (KOH) solution was placed on the lower part of the unit. The prepared unit in question was kept for 5 days. In this way, CO_2 gas, which is formed in silage samples as a result of aerobic activity and is 1.5 times denser than air, settled at the bottom and was kept at the bottom. 10 ml of the solution was taken and titrated with 1N 37% hydrochloric acid solution. The amount of HCl spent between pH 8.1-3.6 was determined and the amount of CO2 gas was calculated according to the equation below.

CO_2 (g/kg DM) = 0.044xTxV / (AxFMxDM)

T= Amount of 1 N HCl (37%) acid consumed in titration (ml).

V= Total volume of 20% KOH solution (ml).

A= Amount of KOH added to the bottom of the apparatus (ml).

FM= Weight of fresh material (kg).

DM= Dry matter amount of fresh material (g/kg).

Dry matter (DM), ash and crude protein (CP) analyses of the silages and raw materials (alfalfa and biscuit) obtained in the study were performed according to AOAC (2005), while ADF and NDF analyses were performed according to Van Soest *et al*. (1991).

In vitro organic matter digestibility (IVOMD), metabolizable energy (ME) and methane gas (CH4) contents of silages were determined according to the method reported by Menke *et al.* (1988). The IVOMD and ME values were calculated by using the production amounts of the gases produced by the silages at the end of 24 hours using the equation reported by Menke *et al.* (1979).

IVOMD (%) = 14.88 + 0.889xGP + 0.45xCP + 0.0651xCA

ME (MJ/kg DM) = 2.20 + 0.136xGP + 0.057xHP

GP = Net amount of gas released after 24 hours of incubation (ml).

CP= Crude protein content of feed (%, DM).

CA= Crude ash content of feed (%, DM).

The data obtained at the end of the research were evaluated by one-way analysis of variance (One Way Anova) and Duncan multiple comparison test was used to compare group means (Soysal, 1998). For this purpose, SPSS (2008) package program used.

RESULTS AND DISCUSSION

The nutrient analysis results of the alfalfa plant and biscuit used in the research are presented in Table 1.

The nutrient contents of alfalfa silages prepared by adding biscuit waste at different levels (1%, 2% and 4%) are given in Table 2.

In this study, when Table 2 was analyzed, it was found that the differences between the groups were statistically significant (P<0.05) in DM, CA, CP and NDF values of silages.

When the DM contents of the silages prepared by adding different ratios of biscuit to alfalfa plants were analyzed, an increase in the DM levels was observed in parallel with the increase in biscuit addition compared to the control group. This increase in DM level suggested that this increase was due to the high level of biscuit DM (90.12%). Similarly, Aydın (2023) investigated the effect of the addition of wafer waste as a readily soluble carbohydrate source to alfalfa silage on silage quality, fermentation characteristics, *in vitro* organic matter digestion and CH4 values and reported that there was a parallel increase in DM in parallel with the increase in the addition of wafer waste and that this increase was realized depending on the DM content (98.75%) of the wafer waste. Canbolat *et al*. (2013) reported that the addition of gladichia fruit, which was used as a carbohydrate source in alfalfa silage, increased the DM values of alfalfa silages, and this increase was due to the differences in DM between fresh alfalfa and gladichia fruit (88.60%) in the study they conducted to determine the effects of gladichia fruit on fermentation, aerobic stability, *in vitro* gas production and microbiological properties of silages made under laboratory conditions.

When the ash values were analyzed, a decrease was observed due to the addition of biscuit. This decrease was realized due to the low ash level of biscuit (2.18% DM). Şerbetçi (2020) conducted a study to determine the effects of waste wafer addition on fermentation, aerobic stability and *in vitro* digestibility of alfalfa silages and observed that the ash content decreased with the increase in the wafer level added to alfalfa. He stated that the decrease in ash content was due to the low ash content of the wafer used as an additive.

When the CP values of the silages obtained were analyzed, there was a decrease due to the addition of biscuit compared to the control group (P<0.05). This decrease was realized due to the low CP (3.2% DM) content of biscuit. Similarly, it was reported that the addition of wafer waste decreased the CP values, and this decrease was due to the low CP content of wafer waste (Aydın, 2023).

The difference between the ADF values of the silages was found statistically insignificant (P>0.05). When NDF values were analyzed, biscuit addition decreased NDF values (P<0.05). The NDF values obtained in this study are in accordance with the report that adding carbohydrate source to silages accelerates the lactic acid bacteria activities in the environment, causing the breakdown of cell wall components and

Table 1. Crude nutrient analysis results of alfalfa and biscuit.

Silage material	DM	Ash	CP	ADF	NDF
Alfalfa	19.94	12.03	14.72	44.28	55.85
Additive					
Biscuit	90.12	2.18	3.2	3.14	7.48

* DM: Dry matter, %; CP: Crude protein, % DM; ADF: acid detergent fiber, % DM; NDF: Neutral detergent fiber, % DM.

Table 2: Nutrient composition of silage groups prepared in the study.

GROUPS	DM	Ash	CP	ADF	NDF
Control	20.405^{b}	13.213^{a}	14.648^{a}	44.355	56.630^{a}
1% Biscuit	20.295^{b}	12.274^{b}	14.583^{ab}	44.327	56.587^{a}
2% Biscuit	20.016^{b}	12.033^{bc}	14.483^{b}	43.852	53.049^{b}
4% Biscuit	22.337^{a}	11.763^{c}	14.250^{c}	43.450	52.500^{b}
SEM	0.287	0.169	0.048	0.227	0.623
P	**<0.001**	**<0.001**	**<0.001**	0.495	**<0.001**

a,b,c: Values with different letters in the same column were found different (P<0.05); **DM:** Dry matter, %; **CP:** Crude protein, DM%; **ADF:** Acid detergent fiber, %DM; **NDF:** Neutral detergent fiber, %DM**; SEM:** Standard Error of Mean.

Table 3: The effect on pH, CO_2 values and fermentation properties of silages prepared by adding biscuit to alfalfa plant.

GROUPS	pH	CO_2	IVOMD	ME	CH_4
Control	5.82^a	8.877^a	53.736^b	7.986^b	15.187^b
1% Biscuit	5.53^b	7.398^b	53.609^b	7.943^b	16.533^a
2% Biscuit	5.51^b	6.943^b	54.103^b	7.979^b	16.800^a
4% Biscuit	5.12^c	4.301^c	56.223^a	8.420^a	17.133^a
SEM	0.075	0.512	0.343	0.062	0.241
p	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

^{a,b,c}: Values with different letters in the same column were found different (P<0.05); **CO_2** : Carbon dioxide formation g/kg DM, **IVOMD:** *In vitro* organic matter digestibility %**, ME:** Metabolizable energy MJ/kg DM, **CH_4 :** *In vitro* methane gas (%)**, SEM**: Standard Error of Mean.

activating the proliferation of some anaerobic bacteria, increasing the breakdown of NDF, ADF and hemicellulose in silage (Bolsen *et al*., 1996).

When the pH values of the silages were analyzed, a decrease was observed in all experimental groups compared to the control group, but the lowest pH value (5.12) was obtained from the 4% biscuit supplemented group (P<0.05). With the addition of biscuit to alfalfa silage, there was an increase in DM and a tendency to decrease in silage pH. As the easily fermentable carbohydrate content increases in the ensiled material, the ideal acidic environment required for a good silage is formed. Therefore, the decrease in silage pH with the addition of biscuit to alfalfa silage is a necessary condition (Kılıç, 1986). After seventy days of fermentation, the silages were subjected to a five-day aerobic stability test and CO2 formation was examined. Biscuit supplementation increased lactic acid, pH decreased when the environment became acidic and accordingly CO2 output was less. Dry matter losses are directly related to increased CO2 production as a result of respiration (Kurtoğlu, 2011). The high DM in the same group supports this situation.

When IVOMD, ME value and CH4 values of silages were analyzed, IVOMD, ME value and CH4 values increased in all experimental groups compared to the control group (P<0.05). The highest values were in the group with 4% biscuit supplementation. The decrease in plant cell wall components of forages such as ADF and NDF, which are difficult to dissolve in rumen, parallel to the increase in biscuit supplementation increased the IVOMD and *in vitro* gas production of silages. The ME contents of alfalfa silages varied between 7.986 and 8.420 MJ/kg DM and the highest ME value was determined in alfalfa silage with 4% biscuit supplementation. The increase in the ME content of silages and the increase in IVOMD and CH4 gas production due to the increase in the level of biscuit added to alfalfa can be attributed to the decrease in NDF and ADF levels (Canbolat *et al*., 2010). ME contents of alfalfa silages were found to be compatible with the results of Getachew *et al*. (2002).

CONCLUSION

In this study, it was concluded that biscuits past their expiration date can be used as a silage additive in the ensiling of alfalfa. It was determined that it was appropriate to add 4% of expired biscuit waste in the ensiling of alfalfa when ensiling alfalfa. In future studies, it can be supported by both *in vitro* and *in vivo* digestion experiments and studies on biscuit additive levels.

Conflict of Interest

The authors declare there is no conflict of interest.

References

Acar Z, Bostan M (2016): Değişik doğal katkı maddelerinin yonca silajının kalitesine etkilerinin belirlenmesi. *Anadolu Tarım Bilimleri Dergisi*, 31(3), 433-440.

Aksu T, Denek N, Aydın S S, Doğan D B, Savrunlu M, Özkaya S (2017): Kuru kekik posasının çayır ve yonca silajının fermantasyon kalitesi ve *in vitro* madde sindirilebilirliğine etkisi. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 23(2): 211-217.

Aksu T, Yakışır BÖ (2019): Farklı seviyelerde melaslı kuru şeker pancarı posası ilavesinin yonca silajı kalitesi üzerine etkisi. *Van Veterinary Journal*, 30(2): 71-76.

Ashbell G, Weinberg ZG, Azrieli A, Hen Y, Horev B (1991): A simple system to study the aerobic determination of silages. *Canadian Agricultural Engineering*, 34: 171-175.

AOAC (2005): *Official Methods of Analysis. 18th Publishing, Association of Official Analytical Chemists*, Arlington, USA.

Aydın SS (2023): Evaluation of the usage of wafer waste as an easily soluble carbohydrate source in alfalfa silage. *Harran Üniversitesi Veteriner Fakültesi Dergisi*, 12(1): 41-46.

Bolsen KK, Ashbell G, Weinberg ZG (1996): Silage fermentation and silage additives. Asian-Australasian *Journal of Animal Science*, 9(5): 483-493.

Canbolat Ö, Yıldırım HK, Karaman Ş, Filya İ (2010): Üzüm posasının yonca silajlarında karbonhidrat kaynağı olarak kullanılma olanakları. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 16(2): 269-276.

Canbolat Ö, Yıldırım HK, Filya İ (2013): Yonca silajlarında katkı maddesi olarak Gladiçya meyvelerinin (Gleditsia Triacanthos) kullanılma olanakları. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 19(2): 291-297.

Çetiner H, Polat T (2022). Şanlıurfa Koşullarında Arpa Hat ve Çeşitlerin Ot Verimi İle İlgili Bazı Tarımsal Karakterlerin Belirlenmesi. *MAS Journal of Applied Sciences*, 7(2), 495-505.

Çotuk GM, Önenç SS (2016): Yonca silajına kepek ve puding ilavesinin silaj fermantasyonu, aerobik stabilite ve *in vitro* sindirilebilirlik üzerine etkileri. *Hayvansal Üretim*, 58(1): 13-19.

Denek N, Can A, Avci M, Aksu T, Durmaz H (2011): The effect of molasses-based pre-fermented juice on the fermentation quality of first-cut lucerne silage. *Grass and Forage Science*, 66: 243-250.

Getachew G, Crovetto GM, Fondevila M, Krishnamoorthy U, Singh B Spanghero M, Steingass H, Robinson PH, Kailas MM (2002): Laboratory variation of 24 h *in vitro* gas production and estimated metabolizable energy values of ruminant feeds. *Animal Feed Science and Technology*, 102,169-180.

Ke WC, Ding WR, Xu DM, Ding LM, Zhang P, Li FD, Guo XS (2017): Effects of addition of malic or citric acids on fermentation quality and chemical characteristics of alfalfa silage. *Journal of Dairy Science*, 100(11): 8958–8966.

Kılıç A (1986): *Silo yemi*. Bilgehan Basımevi. Bornova, İzmir, s: 68-72.

Koç F, Aksoy ÖS, Okur AA, Çelikyurt G, Korucu D, Özduven ML (2017): Effect of pre-fermented juice, Lactobacillus plantarum and Lactobacillus buchneri on the fermentation characteristics and aerobic stability of high dry matter alfalfa bale silage. *The Journal of Animal and Plant Sciences*, 27(5): 1426-1431.

Korkmaz F, Önenç SS (2017): Raf ömrü dolan bazı gıdaların ruminant beslemede alternatif yem kaynağı olarak kullanımı. *Hayvansal Üretim*, 58(1): 28-32.

Kurtoğlu V (2011): *Silaj ve silaj katkıları*. Aybil Yayınevi, Konya.

Menke KH, Raab L, Salewski A, Steingass H, Fritz D, Schneider W (1979): The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor *in vitro*. *Journal of Agricultural Science,* 93(1): 217-222.

Menke KH (1988): Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research and Development*, 28: 7-55.

Soysal Mİ (1998). *Biyometrinin Prensipleri* (İstatistik I ve II Ders Notları), Tekirdağ Ziraat Fakültesi, Ders Kitabı No: 64, T.Ü. 331 s.

SPPS Inc, SPSS Statistics for Windows, Version 17.0. 2008. Chicago.

Şakalar B, Kamalak A (2016): Melaslı kuru şeker pancarı posasının yonca bitkisinin silolanmasında kullanılması. *Anadolu Tarım Bilimleri Dergisi*, 31: 157-164.

Şerbetçi Mİ (2020): *Yonca Silajlarına Gofret İlavesinin Fermantasyon Özellikleri, Aerobik Stabilite ve Yem Değeri Üzerine Etkileri*. Yüksek Lisans Tezi, Tekirdağ Namık Kemal Üniversitesi Fen Bilimleri Enstitüsü, Tekirdağ.

Van Soest PV, Robertson JB, Lewis BA (1991): Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74(10): 3583-3597.