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The Effect of Cake Waste Addition to Alfalfa Silage on Silage Quality and Fermentation Properties

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Abstract

In this study, it was aimed to determine the effects of addition of cake waste, which is a food industry waste, to alfalfa silage as a readily soluble carbohydrate source on silage quality, fermentation properties, *in vitro* organic matter digestion and *in vitro* CH_4 values. In the study, while silage group without additives constituted the control group, silages with 1%, 2%, 4% and 6% cake waste additions constituted the test groups. Statistically significant differences were found in crude protein (CP), crude ash (CA), dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), *in vitro* organic matter digestion (IVOMD), metabolizable energy (ME) and *in vitro* CH_4 values of silages (P<0.05). When IVOMD, ME and *in vitro* CH_4 values of silages were examined, increases were observed in all groups with additive compared to the control group. When the pH, ammonia nitrogen (NH_3-N/TN), carbon dioxide (CO_2), lactic acid (LA) and acetic acid (AA) values of the silages were examined, while the highest values were found in the control group, the lowest values were found in the silage group to which 6% cake waste was added (P<0.05). While propionic acid (PA) was detected in the control group, it was not detected due to the addition of cake waste. While butyric acid (BA) values were controlled and found due to 1% cake waste addition, it was not found in the other test groups. As compared to the control group, the silage's yeast values declined as cake waste increased. While mold values of the silages were controlled and found in the control group with 1% cake waste addition, it was not found in the other test groups. As a result, it was determined that the addition of 6% cake waste as a readily soluble carbohydrate source had a positive effect on silage quality and fermentation properties.

Key Words: Alfalfa, fermentation, silage additives, waste

Yonca Silajına Pasta Atığı İlavesinin Silaj Kalitesi ve Fermantasyon Özellikleri Üzerine Etkisi

Öz

Bu çalışmada gıda sanayi atığı olan pasta atığının kolay eriyebilir karbonhidrat kaynağı olarak yonca silajına ilavesinin silaj kalitesi, fermantasyon özellikleri, *in vitro* organik madde sindirimi ve *in vitro* CH_4 değerleri üzerine etkilerinin belirlenmesi amaçlanmıştır. Çalışmada katkısız silaj grubu kontrol grubunu oluştururken, %1, %2, %4 ve %6 pasta atığı ilaveli silajlar deneme gruplarını oluşturmuştur. Silajların kuru madde (DM), ham kül (CA), ham protein (CP), asit deterjan fiber (ADF), nötral deterjan fiber (NDF), *in vitro* organik madde sindirimi (IVOMS), metabolize olabilir enerji (ME) ve *in vitro* CH_4 değerlerinde gruplar arasında istatistiki olarak önemli farklılıklar bulunmuştur (P<0.05). Silajların IVOMD, ME ve *in vitro* CH_4 değerleri incelendiğinde kontrol grubuna kıyasla tüm katkılı gruplarda artışlar gözlemlenmiştir (P<0.05). Silajların pH, amonyak azotu (NH_3-N/TN), karbondioksit (CO_2), lactic asit (LA) ve asetik asit (AA) değerleri incelendiğinde, en yüksek değerler kontrol grubunda tespit edilirken, en düşük %6 pasta atığı ilave edilen silaj grubunda tespit edilmiştir(P<0.05). Silajların propiyonik asit (PA) kontrol grubunda tespit edilirken pasta atığı ilavesine bağlı olarak tespit edilmemiştir. Bütirik asit (BA) değerleri kontrol ve % 1 pasta atığı ilavesine bağlı olarak tespit edilirken diğer deneme gruplarında tespit edilmemiştir. Silajların maya değerleri kontrol grubuna kıyasla pasta atığı artışına bağlı olarak düşüş gözlemlenmiştir. Silajların küf değerleri ise kontrol ve %1 pasta atığı ilaveli grupta tespit edilirken diğer deneme gruplarında tespit edilmemiştir. Sonuç olarak kolay eriyebilir karbonhidrat kaynağı olarak % 6 oranında pasta atığı ilavesinin yonca silaj kalitesi ve fermantasyon özellikleri üzerine olumlu etkisinin olduğu belirlenmiştir.

Anahtar Kelimeler: Atık, fermantasyon, silaj katkıları, yonca

INTRODUCTION

Roughage supply is an important problem in ruminant feeding (1,2). Especially in winter months, silage is produced in order to meet the water-rich green feed requirement of ruminant animals (3-5). Silage, which is used extensively in countries with advanced animal husbandry, is a storage method that minimizes nutrient losses in feeds (6,7). Alfalfa is one of the most widely grown forage crops in the world. One of the most important features of alfalfa is its high nutritional quality as animal feed. Alfalfa is a perennial and multi-forming plant among leguminous feed crops, and is a delicious feed plant rich in nutrients, especially protein (8,9). Since alfalfa does not contain enough water-soluble carbohydrates as in other legume green feeds, lactic acid production is insufficient because lactic acid bacteria (LAB) do not multiply sufficiently during ensiling (10). Since it is the lactic acid that causes the pH to decrease in silage and in this case a rapid decrease in pH cannot be achieved, when the pH desired for a good silage cannot be reached, undesirable reactions occur in the material and silage quality decreases (11). Alfalfa, which is rich in leaves and has a very high grass yield, has significant nutrient losses during the period from drying to feeding to animals due to the thin and weak leaf stalks or due to incomplete drying in areas with high rainfall, significant nutrient losses occur due to mold and microbial spoilage (8,9,11). In order to prevent these physical and microbiological losses, ensiling alfalfa grass with a material with high water-soluble carbohydrate content can be a solution. In order to ensure the safe ensiling of feed plants with low water-soluble carbohydrate content and high buffering capacity such as alfalfa, it is necessary to add external additives while making silage (12). For this purpose, additives such as cereal grains, molasses, grape pulp, sugar as well as fruit and fruit pulp can be used to compensate for the lack of soluble carbohydrates, which are insufficient in the environment (1,7,13).

This study was carried out to determine the effects of the addition of cake waste, one of the food industry wastes, to alfalfa silage as an easily soluble carbohydrate source on silage quality and fermentation properties.

MATERIAL AND METHODS

Ethical Statement

This study is not subject to HADYEK permission in accordance with Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees" with the decision of Harran University Animal Experiments Local Ethics Committee (HRÜ-HADYEK) numbered 161607 dated 07/09/2022.

Study Design and Silage Preparation

The alfalfa (*Medicago sativa*) plant was used in this study as the silage material. The method reported by Playne and McDonald (14) was used in the analysis of the buffering capacity of the fresh alfalfa plant. The control group in the study consisted of alfalfa without any additives, while the experimental groups included alfalfa with 1% cake waste (1% CW), 2% cake waste (2% CW), 4% cake waste (4% CW), and 6% cake waste (6% CW) waste added. Each silage group was prepared with six replications and was compressed and ensiled in 1.5 L glass jars equipped with a lid that enables gas release only by hand compressing to a final density of 600 g/L. For 60 days, silages were kept in a dark location at room temperature (22–24 ºC).

Fermantation Profile Analysis

The upper 3-5 cm of the silages in the opened jars was discarded. One hundred millilitres of distilled water were added to 25 g of the silage sample taken homogeneously from each jar, which was then crushed. The pH value of the filtrate obtained after the silage was crushed was quickly measured with a pH meter (Hanna HI991002, pH/ORP meter, Romania) and recorded. In addition, the obtained filtrates were placed into 10 ml centrifuge tubes for ammonia nitrogen and volatile fatty acid analysis. While 0.1 ml of 1M HCl was added to the tubes prepared for the determination of ammonia nitrogen, 0.25 ml of 25% metaphosphoric acid was added to the tubes prepared for the analysis of lactic and volatile fatty acids and kept in the deep freezer (-20ºC) until the analysis was done. Ammonia nitrogen analyses of the silage samples were made according to the method reported by Broderick and Kang (15), and the lactic and volatile fatty acids (acetic, butyric, and propionic acid) analyses were conducted using the technique described by Suzuki and Lund (16). To analyze lactic acid (LA) and essential fatty acids, an HPLC device (with a Shimadzu L.C-20 AD HPLC pump, Icsep Coregel (87H3 column), a Shimadzu SIL-20 ADHT Autosampler, a Shimadzu cto-20ac Colum oven, and a Shimadzu SPD M20A Detector (DAD)) was used. The opened silages were exposed to oxygen for five days to measure aerobic stability (determination of CO_2 production values) (17). Dry matter (DM) (method 950.01), crude ash (CA) (method 942.05) and crude protein (CP) (method 945.01) analyses of the silages obtained in the study were calculated according to AOAC (18). NDF and ADF analyses were performed by Van Soest et al. (19). For all analyses, fresh silage materials and the silages were dried by air drying method, ground in a laboratory mill and passed through a 1 mm sieve to be prepared for analysis. The methane (CH_4) content of the silages was determined using the method described by Menke and Steingass (20), and the IVOMD and metabolizable energy (ME) values of the silages were determined using the method described by Menke et al. (21). The total amount of yeast and mold analyses of the silages followed the reports of Filya et al. (22).

Statistical Analysis

In the study, One-Way Analysis of Variance (One-Way Anova) was used to determine whether the data obtained from the groups were widely different. Duncan's multiple comparison tests were used to control the significance of the difference between the groups and $p<0.05$ considered as significant. For this purpose, the SPSS (23) software program was used.

RESULTS

The nutrient analysis results of alfalfa plant used as silage material and pastry waste used as additive in the study are presented in Table 1.

Tablo 1. Nutrient content of the alfalfa used as silage material and the cake waste used as the additive in the study

	BC	DM	CA	CP	ADF	NDF	IVOMD	ME	CH_4
Alfalfa	454	23.60	10.86	16.64	32.95	58.30	52.82	7.54	7.80
CW	-	98.00	0.96	7.50	-	-	78.80	11.05	10.30

CW: Cake Waste; BC:Buffering capacity meq/kg DM; DM: Dry matter, %; CA: Crude ash DM%; CP: Crude protein, DM%; ADF: Acid detergent insoluble fiber, %DM; NDF: Neutral detergent insoluble fiber, %DM; IVOMD: *In Vitro* organic matter digestibility %, ME: Metabolizable energy, CH_4: *In Vitro* methane gas (%).

The nutrient contents of the silages prepared by adding different levels (1%, 2%, 4% and 6%) of cake waste (CW) to alfalfa plant and their IVOMD, ME and *in vitro* CH_4 values are given in Table 2.

Table 2. Nutrient content and IVOMD, ME and *in vitro* CH_4 values of the alfalfa silages prepared by adding cake waste at different rates

Groups	DM	CA	CP	ADF	NDF	IVOMD	ME	CH_4
Control	20.58^{d}	12.50^{a}	17.13^{a}	35.21^{a}	47.67^{a}	46.93^{c}	6.70^{b}	7.07^{c}
%1 CW	21.62^{d}	10.75^{b}	17.00^{a}	34.09^{a}	44.63^{b}	54.51^{b}	8.19^{a}	7.54^{c}
%2 CW	23.53^{c}	10.21^{c}	16.90^{a}	31.55^{b}	43.78^{bc}	56.90^{a}	8.47^{a}	7.83^{bc}
%4 CW	25.30^{b}	10.18^{c}	16.90^{a}	31.58^{b}	43.01^{c}	57.12^{a}	8.60^{a}	8.58^{ab}
%6 CW	27.00^{a}	9.76^{c}	16.25^{b}	31.90^{b}	40.10^{d}	58.18^{a}	8.63^{a}	8.94^{a}
SEM	0.58	0.23	0.08	0.41	0.59	0.99	0.19	0.19
P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.001

$^{a-d}$ Values with different letters in the same column were different ($p<0.05$); CW: Cake Waste; DM: Dry matter, %; CA: Crude ash, DM%; CP: Crude protein, DM%; ADF: Acid detergent insoluble fiber, DM%; NDF: Neutral detergent insoluble fiber, DM%; IVOMD: *In vitro* organic matter digestion; ME: Metabolizable energy MJ/kg DM; CH_4: *In vitro* methane, %.

In the present study, when Table 2 was examined, the differences between the groups in DM, CA, CP, ADF, NDF, IVOMD, ME and *in vitro* CH_4 values of the silages were found to be statistically significant ($p<0.05$).

Within the scope of this study, the fermentation properties of silages prepared by adding cake waste to alfalfa plants and the correlation results of the analyzes are given in Table 3 and Table 4.

Table 3. Fermentation properties of the silages prepared by adding cake waste at different rates

Groups	pH	NH_3-N	LA	AA	PA	BA	CO_2	Yeast	Mold
Control	5.49^{a}	26.23^{a}	2.66^{e}	6.87^{a}	0.75^{a}	9.40^{a}	2.55^{a}	3.12^{a}	5.70^{a}
%1 CW	5.27^{a}	10.99^{b}	4.66^{d}	5.30^{b}	0.00^{b}	6.48^{b}	2.40^{b}	2.89^{b}	1.30^{b}
%2 CW	4.37^{b}	8.66^{c}	10.27^{c}	4.49^{c}	0.00^{b}	0.00^{c}	1.80^{c}	2.80^{c}	0.00^{c}
%4 CW	4.22^{b}	7.98^{cd}	10.88^{b}	4.11^{d}	0.00^{b}	0.00^{c}	1.57^{d}	2.41^{d}	0.00^{c}
%6 CW	4.09^{b}	7.31^{d}	20.50^{a}	3.08^{e}	0.00^{b}	0.00^{c}	1.54^{e}	2.33^{e}	0.00^{c}
SEM	5.49	1.64	1.43	0.29	0.07	0.92	0.10	0.068	0.507
P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

$^{a-e}$ Values with different letters in the same column were found to be different ($p<0.05$); CW: Cake Waste; NH_3-N/TN: Ammonia nitrogen, LA: Lactic acid g/kg DM, AA: Acetic acid g/kg DM, PA: Propionic acid g/kg DM; BA: Butyric acid g/kg DM; CO_2: Carbondioxide g/kg DM.

Table 4. Correlation relationship between fermentation characteristics and yeast values of the alfalfa silages prepared by adding cake waste at different rates

		NH_3-N	pH	LA	AA	PA	BA	Yeast	Mold	CO_2	IVOMD	ME	CH_4
NH_3-N	PC	1	,758**	-,688**	,904**	,981**	,867**	,783**	,993**	,797**	-,946**	-,891**	-,644**
	P		,0001	,001	,0001	,0001	,0001	,0001	,0001	,0001	,0001	,0001	,002
pH	PC		1	-,831**	,874**	,652**	,926**	,842**	,788**	,935**	-,782**	-,760**	-,734**
	P			,0001	,0001	,002	,0001	,0001	,0001	,0001	,0001	,0001	,0001
LA	PC			1	-,928**	-,573**	-,807**	-,888**	-,690**	-,873**	,729**	,632**	,776**
	P				,0001	,008	,0001	,0001	,001	,0001	,0001	,003	,0001
AA	PC				1	,827**	,913**	,936**	,903**	,925**	-,893**	-,812**	-,791**
	P					,0001	,0001	,0001	,0001	,0001	,0001	,0001	,0001
PA	PC					1	,777**	,684**	,973**	,683**	-,903**	-,861**	-,547*
	P						,0001	,001	,0001	,001	,0001	,0001	,013
BA	PC						1	,840**	,901**	,970**	-,871**	-,793**	-,712**
	P							,0001	,0001	,0001	,0001	,0001	,0001
Yeast	PC							1	,778**	,923**	-,773**	-,713**	-,828**
	P								,0001	,0001	,0001	,0001	,0001
Mold	PC								1	,824**	-,944**	-,890**	-,649**
	P									,0001	,0001	,0001	,002
CO_2	PC									1	-,815**	-,735**	-,765**
	P										,0001	,0001	,0001
IVOMD	PC										1	,958**	,640**
	P											,0001	,002
ME	PC											1	,614**
	P												,004
CH_4	PC												1

PC: Pearson correlation; *: Correlation is significant at the 0.05 level; **: Correlation is significant at the 0.01 level; P: Significance degree, NH_3-N/TN: Ammonia nitrogen, CO_2: Carbondioxide 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, IVOMD: *In Vitro* organic matter digestibility %, ME: Metabolizable energy MJ/kg DM, CH_4: *In Vitro* methane %.

DISCUSSION AND CONCLUSION

When the DM contents of the silages prepared by adding different ratios of cake waste to alfalfa plants were examined, an increase in the DM levels was observed in parallel with the increase in the addition of cake waste compared to the control group. It was concluded that this increase in DM level occurred due to the high DM level (98.00%) of cake waste. When CA values were examined, a decrease was observed due to the addition of cake waste. This decrease was realized due to the low CA level of cake waste. When the CP values of the silages were examined, a decrease was observed due to the addition of cake waste compared to the control group. This decrease was due to the low CP content of the cake waste. When ADF and NDF values of the silages were examined, a decrease was observed in parallel with the addition of cake waste. In this study, the reason for the difference in ADF and NDF values between the control and test groups is thought to be due to the low ADF and NDF content of cake waste. In the present study, it was determined that the addition of increased cake waste significantly decreased the ADF content of alfalfa silage. The digestibility of feeds with low ADF content is also high (24,25). In the study conducted by Canbolat et al. (13), in which grape pulp was added to alfalfa silage at different ratios; it was reported that NDF and ADF levels decreased, and water-soluble carbohydrate increased as the amount of grape pulp added to the silage increased. When IVOMD, ME and *in vitro* CH_4 values of the silages were examined, although increases were observed in all test groups compared to the control group, the highest IVOMD value (58.18%), ME value (8.63) and *in vitro* CH_4 value (8.94%) occurred due to the addition of 6% CW. It is thought that LA is the main fermentation product in silages and LA is fermented in rumen and utilized by ruminants and accordingly increases IVOMD and ME values (26). When Table 4 is examined, the positive correlation observed between LA and IVOMD (R: 0.729) as well as between LA and ME (R: 0.632) supports this statement. When *in vitro* CH_4 values of silages were examined, an increase was observed in all test groups compared to the control group, but the highest value was reached with the addition of 6% CW. *İn vitro* CH_4 values increased in parallel with the increase in IVOMD level. When Table 4 is examined, the positive correlation observed between IVOMD and *in vitro* CH_4 (R:0,614) supports this statement. In a study in which waste jam mixture was used as an alfalfa silage additive, Yayla (27) reported that while the additive used generally decreased the CP, NDF, ADF content of silage, it increased the digestible dry matter and dry matter consumption.

When the pH values of the silages obtained were examined, while the highest pH value (5.49) was obtained from the control group, the lowest pH value (4.09) was determined in the group with 6% CW addition (p<0.05). As the water-soluble carbohydrate content of the silage material increases, the ideal acidic environment required for obtaining quality silage is formed. Therefore, it is expected that the pH of silage decreases with the addition of cake waste to alfalfa silage. In the study, the low pH values in the groups with additives compared to the control group were due to the low amount of water-soluble carbohydrates in alfalfa plant. The pH values of silages are influenced by many factors such as the WSC content, dry matter level, buffering capacity of the plant (28). When the correlation table (table 4) of the silages obtained in this study was examined, it was observed that there was a negative correlation between pH and LA (R: -,831).

When the NH_3-N/TN values of the silages prepared in this study were compared, the highest NH_3-N/TN value (26.23%) was determined in the control group and the lowest NH_3-N/TN value (7.31%) was determined in the group with 6% cake waste addition (p<0.05). Total nitrogen levels <10% indicate good fermentation (29). Yakışır and Aksu (12) reported that the use of additives with easily soluble carbohydrate content as additives in making silage from alfalfa plants creates a good fermentation environment for lactic acid bacteria, microorganisms can multiply rapidly and minimize protein destruction by lowering the pH of the environment. When the correlation table was examined, the negative correlation between LA and NH_3-N/TN (R: - 0,688) and the positive correlation between pH and NH_3-N/TN (R: 0,758) supported this statement. When the LA values of the silages obtained from this study were examined, an increase was observed in all test groups compared to the control group, but the highest LA value (20.50 g/kg DM) was determined in the group with 6% CW addition (p<0.05). When the AA values of the silages were examined, a decrease was observed in all test groups compared to the control group, but the lowest AA value (3.08 g/kg KM) occurred due to the addition of 6% CW. While PA was detected in the control group, it was not detected with the addition of cake waste (p<0.05). While BA values were controlled and found in the groups with 1% CW addition, it was not found in the groups with 2%, 4% and 6% CW addition (p<0.05). In this study, the yeast values of alfalfa silages made with cake waste added reduced as a result of the increased cake waste in comparison to the control group. Mold values of the silages were controlled and found due to 1% CW addition; it was not found in the other test groups. It was reported that silage pH values decreased due to LA conversion of easily soluble carbohydrate sources and prevented yeast as well as mold growth in silage. In the study conducted by Canpolat et al. (1), it was reported that the addition of gladiolus fruit as a readily soluble carbohydrate source to alfalfa silage increased the LAB value and decreased the number of molds, which supports the current study. In the study, the CO_2 production amounts of the silage groups varied between (1.54-2.55 g/kg DM), but a decrease was observed in all experimental groups due to the increase in cake waste (p<0.05). Canpolat et al. (13) reported that the addition of grape pulp as a readily soluble carbohydrate source to alfalfa silage improved aerobic stability. It was determined that the lowest yeast, mold and CO_2 levels and the highest LA value in the groups with 6% CW addition decreased the pH value in the silages in the groups with additives and accordingly prevented the growth and activity of yeasts as well as molds and had an improving effect on aerobic stability values. When the correlation table is examined, negative correlation between LA and CO_2 (R:-,873), negative correlation between LA and yeast (R:-,888) and negative correlation between LA and mold (R:-,690) support this statement.

In this study, the addition of cake, which is a food industry waste, as an alternative easily soluble carbohydrate source to alfalfa, which is one of the difficult to ensiled plants, positively affected the chemical and microbiological properties of silages. When examined in terms of all parameters, it was concluded that 6% of cake waste should be added.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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