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Evaluation of the Usage of Wafer Waste as an Easily Soluble Carbohydrate Source in Alfalfa Silage

Sadık Serkan AYDIN[1,a,*]

[1]Harran University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Şanlıurfa, Turkey.

[a]ORCID: 0000-0002-3252-3944

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***Correspondence:** Sadık Serkan AYDIN
Harran University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Şanlıurfa, Turkey.
e-mail: sadik.aydin@harran.edu.tr

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Abstract: This study examined the addition of wafer waste as a readily soluble carbohydrate source to alfalfa silage for its impact on silage quality, fermentation characteristics, *in vitro* organic matter digestion, and *in vitro* CH_4 values. Fresh alfalfa was ensiled with 0% wafer waste (Control), 1% wafer waste, 2% wafer waste, 4% wafer waste, and 6% wafer waste for 60 days. The differences between the groups in dry matter (DM), crude protein (CP), crude ash (CA), neutral detergent fiber (NDF), acid detergent fiber (ADF), metabolizable energy (ME), *in vitro* organic matter digestion (IVOMD), and *in vitro* CH_4 values of silages were found to be statistically significant. In comparison to the control group, increases in IVOMD and ME values were seen in all additive-containing groups. Depending on the quantity of wafer waste, the pH, ammonia nitrogen (NH_3-N/TN), and carbon dioxide (CO_2) values of the silages declined. In the study, the highest amount of lactic acid occurred in the group with 6% wafer waste added. Propionic acid (PA) was detected only in the control group. Butyric acid (BA) values tended to decrease depending on the addition of wafer waste and were not found in the groups to which 4% and 6% wafer waste addition. Yeast and mold values of the silages decreased in parallel with the increase in the wafer waste rate. As a result, it was determined that adding wafer waste as an easily soluble carbohydrate source positively affected alfalfa silage quality and fermentation characteristics.
Keywords: *Alfalfa, Fermentation, Silage.*

Yonca Silajında Kolay Çözünebilen Karbonhidrat Kaynağı Olarak Gofret Atıklarının Kullanımının Değerlendirilmesi

Özet: Bu çalışmada, yonca silajına kolay çözünür bir karbonhidrat kaynağı olarak gofret atığının ilavesinin silaj kalitesi, fermantasyon özellikleri, *in vitro* organik madde sindirimi ve *in vitro* CH_4 değerleri üzerindeki etkisi incelenmiştir. Taze yonca bitkisi %0 gofret atığı (Kontrol), %1 gofret atığı, %2 gofret atığı, %4 gofret atığı ve %6 gofret atığı ile 60 gün süreyle silolanmıştır. Silajların kuru madde (KM), ham kül (HK), ham protein (HP), 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 farklılıklar istatistiki olarak önemli bulunmuştur. Silajların IVOMS ve ME değerleri incelendiğinde kontrol grubuna kıyasla tüm katkılı gruplarda artışlar gözlemlenmiştir. Gofret atıklarının miktarına bağlı olarak silajların pH, amonyak nitrojeni (NH_3-N/TN) ve karbondioksit (CO_2) değerlerinde düşüş gözlenmiştir. Çalışmada en yüksek laktik asit miktarı %6 gofret atığı eklenen grupta oluşmuştur. Propiyonik asit (PA) sadece kontrol grubunda tespit edilmiştir. Bütirik asit (BA) değerleri ise gofret atığı ilavesine bağlı olarak azalma eğilimi göstermiş ve %4 ve %6 gofret atığı ilave edilen gruplarda tespit edilememiştir. Silajların maya ve küf değerleri gofret fire oranındaki artışa paralel olarak azalmıştır. Sonuç olarak kolay eriyebilir karbonhidrat kaynağı olarak gofret atığı ilavesinin yonca silaj kalitesi ve fermantasyon özellikleri üzerine olumlu etkisinin olduğu belirlenmiştir.
Anahtar Kelimeler: *Fermentasyon, Silaj, Yonca.*

Introduction

Alfalfa, the so-called "queen of forages", is one of the world's most widely cultivated forage crops. Alfalfa is consumed by variousanimals, including ruminants (cattle, sheep, and goats) and non-ruminants (mainly horses). One of the essential characteristics of alfalfa is its high nutritional quality as an animal forage. It is used in animal forage as fresh green forage or silage, pellets, or cubed products. The most crucial feature of alfalfa is its high energy, protein content, and digestibility (Karabulut and Filya, 2007). Preserving forage crops as silage in humid regions can improve dry matter utilization and forage quality by reducing space and storage losses compared to dry grass. Successful ensiling of legume green forages without additives is quite tricky (König, 2020). It is reported that this situation, which is especially valid for alfalfa, arises mainly from 3 reasons (McDonalds et al., 1991). These are the high buffer capacity of legumes, low water-soluble carbohydrate content, and low dry matter content it has. In recent years, studies have been carried out using various silage additives that enable the production of high-quality legume green grass silages by eliminating these negativities, and high-quality alfalfa silage has been obtained. These additives are especially 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).

This study was conducted to determine the effects of addingwafer waste, one of the food industry wastes causing environmental pollution, to alfalfa silage as a readily soluble carbohydrate source on silage quality, fermentation characteristics, and *in vitro* organic matter digestion and *in vitro* CH_4 values.

Material and Method

In this study, the alfalfa (*Medicago sativa*) plant was used as silage raw material. The buffer capacity of fresh alfalfa used in the study was determined according to the method reported by Playne and McDonald (1966). In the study, while additive-free alfalfa plants constituted the control group, the groups with 1%, 2%, 4%, and 6% wafer waste additions constituted the test groups. The control and each test group were ensiled in 1.5-liter glass jars with four replicates. Before the silages were opened, they were kept in the dark place for 60 days to complete the fermentation. After removing the top 3–5 cm of the jars, 25 g of homogeneously sampled silage was shredded in a blender for 2 minutes with 100 ml of pure water. The pH of the resulting liquid was then promptly measured with a pH meter and recorded. The silage liquid from the blender was filtered, transferred to 10 ml tubes, and treated with 0.1 ml of 1M HCl to evaluate ammonia nitrogen and 0.25 ml of 25% metaphosphoric acid to analyze volatile fatty acids and lactic acid before being placed in a deep freezer (-20 °C) until analysis. NH_3-N/TN analyses of silage samples were carried out according to the method reported by Broderick and Kang (1980). The method described by Suzuki and Lund was used to assess the concentrations of volatile fatty acids (butyric, acetic, and propionic acid) and lactic acid. (1980). High-performance liquid chromatography (HPLC) (Shimadzu L.C-20 AD HPLC pump, Shimadzu SPD M20A Detector (DAD), Shimadzu SIL-20 ADHT Autosampler, Icsep Coregel (87H3 colon), Shimadzu CTO-20AC Columun oven) apparatus was employed for this. The silages obtained in the study were subjected to an aerobic stability test (determination of CO_2 production values) for five days (Ashbell et al., 1991).

The silages prepared for this investigation were subjected to dry matter (DM), crude ash (C), and crude protein (CP) analysis by AOAC guidelines (2005). As per Van Soest et al. (1991), NDF and ADF analyses were carried out. Fresh alfalfa and silage samples were dried in an oven at 65 °C for 48 hours, and then samples were ground through a 1 mm sieve with a laboratory mill for composition analysis. The silages obtained in the study had their metabolizable energy (ME), *in vitro* organic matter digestibility (IVOMD), and *in vitro* methane (CH_4) content assessed using the method described by Menke and Steingass (1988). Yeast and mold analysis of the silages were determined according to the method reported by Filya et al. (2000).

All data of chemical composition and fermentation parameters were analyzed by one-way analysis of variance (ANOVA) using SPSS statistics (IBM, Armonk, NY, USA). Duncan's test was employed for multiple comparisons. $p< 0.05$ and $p< 0.01$ indicated significant and highly significant, respectively.

This study is not subject to HADYEK permission by Article 8 (k) of the "Regulation on Working Procedures and Principles of Animal Experiments Ethics Committees".

Results

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

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

Within the scope of this study, the fermentation characteristics of the silages prepared by adding different ratios of wafer waste as an easily soluble carbohydrate source to alfalfa plant and the correlation results of the performed analyzes are given in Table 3 and Table 4.

Discussion and Conclusion

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

When the DM contents of the silages prepared by adding different ratios of wafer waste to alfalfa plants were examined, an increase was observed in DM levels in parallel with the increase in wafer waste addition compared to the control group. It was concluded that this increase in DM level was due to the high DM level (98.75%) of wafer waste. A decrease was observed when CA values were examined due to adding wafer waste. The low CA level (0.85%) of wafer waste caused this decrease.

Table 1. Crude nutrient analysis results of alfalfa plant used as silage material and wafer waste used as additive in the study.

	BC	DM	CA	CP	ADF	NDF	IVOMD	ME	CH_4
Alfalfa	452	23.55	10.90	16.68	32.99	58.34	52.75	7.52	7.85
Wafer Waste	-	98.75	0.85	6.2	-	-	76.89	11.65	10.25

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 digestion %, **ME:** Metabolizable energy, **CH_4:** *In Vitro* methane gas (%).

Table 2. The nutrient contents and IVOMD, ME and *in vitro* CH_4 values of alfalfa silages prepared by adding different ratios of wafer waste.

Groups	DM	CA	CP	ADF	NDF	IVOMD	ME	CH_4
Control	20,71^{d}	13,14^{a}	17,68^{a}	36,21^{a}	48,67^{a}	45,91^{c}	6,70^{c}	6,68^{b}
1%wafer waste	22,02^{cd}	11,20^{b}	17,21^{b}	35,08^{a}	45,63^{b}	49,77^{c}	7,31^{c}	7,53^{b}
2% wafer waste	22,77^{c}	10,35^{c}	17,19^{b}	32,90^{b}	44,78^{bc}	56,24^{b}	8,31^{b}	9,87^{a}
4% wafer waste	24,71^{b}	9,53^{d}	16,98^{b}	32,58^{b}	44,01^{c}	63,24^{a}	9,41^{a}	10,23^{a}
6% wafer waste	26,79^{a}	9,28^{d}	15,94^{c}	32,55^{b}	41,10^{d}	64,30^{a}	9,56^{a}	10,71^{a}
SEM	0,528	0,335	0,138	0,407	0,593	1,806	0,280	0,431
P	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**

a-c: Values with different letters in the same column were found to be different ($P<0.05$), **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, **CH_4:** *In Vitro* methane gas (%).

Table 3. Fermentation characteristics of alfalfa silages prepared by adding different ratios of wafer waste.

Groups	pH	NH_3-N/TN	LA	AA	PA	BA	Yeast	Mold	CO_2
Control	5,46^{a}	28,33^{a}	3,33^{e}	8,46^{a}	0,72^{a}	6,46^{a}	3,15^{a}	5,70^{a}	2,51^{a}
1%wafer waste	5,25^{a}	16,83^{b}	4,59^{d}	5,75^{b}	0,00^{b}	3,95^{b}	1,90^{b}	1,48^{b}	2,41^{b}
2% wafer waste	4,43^{b}	12,68^{c}	8,67^{c}	4,89^{c}	0,00^{b}	1,42^{c}	1,70^{c}	1,48^{b}	2,05^{c}
4% wafer waste	4,16^{bc}	11,38^{cd}	13,20^{b}	4,15^{d}	0,00^{b}	0,00^{d}	1,01^{d}	1,01^{c}	1,66^{d}
6% wafer waste	3,95^{c}	9,14^{d}	13,59^{a}	4,04^{e}	0,00^{b}	0,00^{d}	1,01^{d}	0,00^{d}	1,45^{e}
SEM	,147	1,593	,973	,372	,066	,575	,180	,450	,094
P	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**

a,b,c,d,e : Values with different letters in the same column were found to be different ($P<0.05$), **NH_3-N/TN:** Ammonia nitrogen, **CO_2:** Carbon dioxide g/kg DM, **LA:** Lactic acid g/kg DM, **AA:** Acetic acid g/kg DM, **PA:** Probiotic acid g/kg DM, **BA:** Butyric acid g/kg DM, **Yeast:**log10/cfu/gr, **Mold**:log10/cfu/gr.

Table 4. Correlation between yeast and mold values and fermentation characteristics of alfalfa silages prepared by adding different ratios of wafer waste.

		NH_3-N	pH	LA	AA	PA	BA	Yeast	Mold	CO_2	IVOMD	ME	CH_4
NH_3-N	**PC**	1	,855**	-,844**	,976**	,910**	,944**	,957**	,957**	,823**	-,798**	-,801**	-,779**
	P		,000	,000	,000	,000	,000	,000	,000	,000	,000	,000	,000
pH	**PC**		1	-,917**	,843**	,633**	,910**	,845**	,750**	,909**	-,865**	-,868**	-,857**
	P			,000	,000	,003	,000	,000	,000	,000	,000	,000	,000
LA	**PC**			1	-,875**	-,629**	-,954**	-,906**	-,769**	-,988**	,916**	,919**	,821**
	P				,000	,003	,000	,000	,000	,000	,000	,000	,000
AA	**PC**				1	,926**	,972**	,989**	,969**	,849**	-,835**	-,838**	-,783**
	P					,000	,000	,000	,000	,000	,000	,000	,000
PA	**PC**					1	,817**	,889**	,961**	,596**	-,631**	-,634**	-,613**
	P						,000	,000	,000	,006	,003	,003	,004
BA	**PC**						1	,969**	,893**	,928**	-,895**	-,898**	-,836**
	P							,000	,000	,000	,000	,000	,000
Yeast	**PC**							1	,955**	,883**	-,862**	-,866**	-,773**
	P								,000	,000	,000	,000	,000
Mold	**PC**								1	,763**	-,740**	-,743**	-,703**
	P									,000	,000	,000	,001
CO_2	**PC**									1	-,892**	-,895**	-,803**
	P										,000	,000	,000
IVOMD	**PC**										1	1,000**	,914**
	P											,000	,000
ME	**PC**											1	,913**
	P												,000
CH_4	**PC**												1

PC: Pearson correlation, ***:** Correlation is significant at 0.05 level, ****:** Correlation is significant at 0.01 level, **NH_3-N/TN:** Ammonia nitrogen, **CO_2:** Carbon dioxide g/kg DM, **LA:** Lactic acid g/kg DM, **AA:** Acetic acid g/kg DM, **PA:** Probiotic acid g/kg DM, **BA:** Butyric acid g/kg DM, **IVOMD:** *In vitro* organic matter digestion %, **ME:** Metabolizable energy, **CH_4:** *In vitro* methane gas (%).

Compared to the control group, a decline in the CP values of the silages was seen when the wafer waste was added. This decrease was due to the low CP content of wafer waste. When the silages' NDF and ADF values were investigated, a decline in ADF and NDF values was seen that correlated with wafer waste's addition. In this study, the difference in ADF and NDF values between the control and test groups is thought to be due to wafer waste's low ADF and NDF content. ADF content is an important criterion that gives information about the degree of digestion of rough forages. In the present study, it was determined that the addition of wafer waste significantly decreased the ADF content of alfalfa silage. The digestibility of forages with low ADF content is also high. Increases were seen in all test groups as opposed to the control group when the silages' IVOMD and ME values were investigated, and the addition of 6% wafer waste resulted in the silages' highest IVOMD value (64.30) and ME value (9.56). It is thought that LA is the main fermentation product in silages, and LA is fermented in the rumen and utilized by ruminants and accordingly increases IVOMD and ME values (Okuyucu et al., 2018). When Table 4 is examined, it is seen that there is a positive correlation between LA and IVOMD (R: 0.916) and LA and ME (R: 0.919). A rise was seen in all trial groups compared to the control group when the silages' *in vitro* CH_4 values were tested, with the addition of a 6% wafer producing the highest result. With the addition of 6% wafer waste, an increase in *in vitro* CH_4 values was observed in parallel with the increase in IVOMD degree. When Table 4 is examined, the observation of (R:0,914) between IVOMD and *in vitro* CH_4 supports this statement. In the research by Acar and Bostan (2016) in which sugar beet molasses, barley paste, and whey powder (0, 50mg/kg, and 100mg/kg DM) were used as alfalfa silage additives, it was found that while the additives generally decreased the CP, NDF, ADF content of silage, they increased the digestible dry matter and dry matter consumption. It was found that while the amounts of acetic acid and butyric acid decreased in silages, the amount of lactic acid increased. Similarly, Şakalar and Kamalak (2016) reported that while molassed dry sugar beet pulp increased the IVOMD and ME value of alfalfa silage, it decreased the CA, CP, ADF, ammonia amount and pH of alfalfa silage in a study in which they added molassed dry sugar beet pulp at the ratios of 0%, 1.5, 3.0, 4.5 and 6.0% to alfalfa harvested during the flowering period.

In this study, when the fermentation characteristics (pH, NH_3-N, LA, AA, PA, BA, yeast, mold, CO_2) of alfalfa silages prepared by adding wafer waste at different ratios were examined, the differences between the groups were found to be significant (P<0.05). When the pH levels of the silages were measured, the control group had the highest pH value (5.46), while the group that had added 6% wafer waste had the lowest pH value (3.95) (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, the silage pH is expected to decrease with the addition of wafer waste to alfalfa silage. The lower pH values in the groups to which wafer waste was added compared to the control group were attributed to the tolerance of the low water-soluble carbohydrate content of alfalfa plant with wafer waste and the increase in lactic acid values due to the addition of wafer waste. When the correlation table (table 4) of the silages obtained in this study was examined, it was observed that there was a negative correlation (R: -,917) between pH and LA. Silage pH indicates silage acidity and, therefore, the degree of fermentation. Lower pH is preferred for quality silage. Yakışır (2018) reported that adding different levels of molasses dry sugar beet pulp to alfalfa silage decreased the pH value of alfalfa and increased the lactic acid value. This statement conforms with the present study.

When the NH_3-N/TN values of the silages made in this study were compared, the control group had the greatest NH_3-N/TN value (28.33%) and the group with 6% wafer waste addition had the lowest NH_3-N/TN value (9.14%), with a decrease being shown because of the addition of wafer waste (P<0.05). In poorly preserved silages, the protein fraction is largely degraded; therefore, high ammonia-N (% of total nitrogen) indicates poor fermentation. Levels <10% of total nitrogen indicate good fermentation (Yakışır, 2018). This decrease in silage NH_3-N/TN values is thought to be because easily soluble carbohydrate sources have a good impact on silage fermentation and reduce proteolysis (Bingöl et al., 2009; de Oliveira et al., 2021). Chen et al. (2020) and Yakışır and Aksu (2019) reported that the usage of additives with easily soluble carbohydrate content as additives in making silage from alfalfa plant creates a good fermentation environment for lactic acid bacteria and that microorganisms can multiply rapidly and minimize protein degradation by lowering the pH of the environment. When the correlation table was examined, the negative correlation between LA and NH_3-N/TN (R: - 0,844) and the positive correlation between pH and NH_3-N/TN (R: 0,855) supported this statement.

When the LA values of the silages obtained from this study were examined, an increase was observed in all test groups in comparison to the control group, and the highest LA value (13.59 g/kg DM) was determined in the group with 6% wafer waste addition (P<0.05). Gao et al. (2021) reported an increase in LA values due to the addition of molasses and fructose to alfalfa silage, and this statement is in conformity with the present study. In a study (Canbolat et al., 2013), in which Galician fruit rich in tannin and carbohydrate content was used as a silage additive, it was reported that the LA amount of alfalfa silage increased. When the silages' AA values were tested, all trial groups showed a decline compared to the control group, and the lowest AA value (4.04 g/kg DM) occurred due to the addition of 6% wafer waste. The decrease in acetic acid in silages can be considered an advantage in increasing silage consumption in ruminants fed mainly on silage (Kamalak et al., 2012). While PA in silages was detected in the control group, it was not found due to the addition of wafer waste (P<0.05). BA values showed a decreasing trend depending on the addition of wafer waste, but it was not detected in the groups to which 4% and 6% wafer waste was added (P<0.05). Acetic acid and butyric acid are undesirable because they deteriorate the quality of silage (Cheng et al., 2022; Wang et al., 2021). Acar and Bostan (2016) reported that the usage of sugar beet molasses, barley paste, and whey powder as alfalfa silage additives decreased the amount of acetic acid as well as butyric acid and increased the amount of lactic acid in silages, and this statement is in conformity with the present study. In this study, when the yeast and mold values (Table 3) of the silages prepared with the addition of wafer waste at different ratios to alfalfa plants were examined, a decrease was observed because of the rise in wafer waste in comparison to the control group. It was reported that silage pH values decreased due to LA conversion of easily soluble carbohydrate sources and prevented yeast and mold growth in silage. In the study, on the 5th day of aerobic stability, the CO_2 production amounts of the silage groups varied between (1.45-2.52 g/kg DM), and a decrease was observed in all test groups due to the increase in wafer waste compared to the control group (P<0.05). It was determined that the lowest yeast mold amount and CO_2 ratio level and the highest LA value in the groups with 6% wafer waste addition decreased the pH value in the silages in the groups with additive and accordingly prevented the growth and activity of yeast and molds and had an improving effect on aerobic stability values. When the correlation table was examined, the negative correlation between LA and CO_2 (R: -,988), negative correlation between LA and yeast (R: -,906), and negative correlation between LA and mold (R: -,769) support this statement.

When the results obtained in this study were evaluated, it was concluded that alfalfa silages prepared with the addition of wafer waste had improved the quality of the silage, fermentation properties, and *in vitro* organic matter digestion, could be used as silage additives and the best results were obtained with the addition of wafer waste at 6% level.

Conflict of Interest

The authors stated that they did not have any real, potential or perceived conflict of interest.

Ethical Approval

This study (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 (HRU-HADYEK) 2022/006/11 dated 07/09/2022 and numbered 161607) not subject to permission.

Similarity Rate

We declare that the similarity rate of the article is 13% as stated in the report uploaded to the system.

Author Contributions

Motivation / Concept: SSA
Design: SSA
Control/Supervision: SSA
Data Collection and / or Processing: SSA
Analysis and / or Interpretation: SSA
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