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Farklı Seviyelerde Mısır Flake İlavesinin Yonca Silaj Kalitesine Etkilerinin Belirlenmesi

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Uçucu yağ asitleri
pH

ÖZ

Mevcut çalışma, farklı seviyelerde (%0, %5 ve %10) mısır flake ilavesi ile silolanan yonca silajlarında mısır flakenin yonca silajının kimyasal kompozisyon, sindirilebilirlik ve enerji değerleri ile fermentasyon özelliklerine etkisini belirlemek için yürütülmüştür. Çalışma kapsamında, 1-3 cm boyutlarında parçalanan yoncalar anaerob plastik kaplara, kontrol grubuna herhangi bir katkı eklenmeden, muamele gruplarına ise %5 ve %10 seviyelerinde mısır flake ilave edilerek silolanmıştır. Araştırma sonucunda kuru madde (KM), ham protein (HP), kül, asit deterjan fiber (ADF), nötral deterjan fiber (NDF), asit deterjan lignin (ADL) ve asit deterjan çözünmez ham protein (ADHP) parametrelerinin kontrol grubuna göre muamele gruplarında önemli ölçüde düştüğü saptanmıştır (P<0,01). Ham yağ (HY) %5 seviyesinde mısır flake ve kontrol gruplarında benzer bulunmuş fakat %10 seviyesinde önemli ölçüde artmıştır (P<0,01). Nişasta seviyesi de muamele gruplarında kontrole göre artmış en yüksek değer %10 mısır flake ilave edilen grupta tespit edilmiştir (P<0,01). Nötral deterjan çözünmez ham proteini (NDHP) %5 mısır flake ile önemli ölçüde düşerken %10 mısır flake ilave edilen grup ve kontrol grubunda benzer olmuştur (P<0,05). Metabolik enerji (ME) muamelelerden etkilenmemiş (P>0,05), fiber olmayan karbonhidrat, total sindirilebilir protein ve laktasyon net enerji değerleri mısır flake ilavesi ile önemli ölçüde artmıştır (P<0,01). Laktik ve bütirik asit mısır flake ilavesi ile önemli seviyede artmış, pH ise %10 mısır flake ilaveli grupta kontrol ve diğer muamele grubuna kıyasla önemli ölçüde azalmıştır (P<0,01). Ayrıca, asetik asit ve propiyonik asit muamele gruplarında kontrol grubuna göre önemli düzeyde yüksek olmuştur (P<0,01). Ancak artan mısır flake seviyesi (%10) ile asetik asit ve propiyonik asit seviyesi azalmıştır (P<0,01). Sonuç olarak mısır flake ilavesinin silaj için önemli parametreler olan özellikle laktik asit ve pH parametreleri üzerinde olumlu etkileri dikkate alındığı ve diğer parametreler üzerindeki net etkileri de incelendiğinde yonca silajı yapımında mısır flake kullanımının olumlu etkileri olacağı söylenebilir. Ancak ileriki çalışmalarda %10 ve üzerindeki seviyelerin değerlendirilmesine ihtiyaç vardır.

Determination of Alfalfa Silage Quality with Different Levels of Corn Flakes Addition

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ABSTRACT

The present research was carried out to determine the effects of corn flakes on the chemical composition, digestibility and energy values, and fermentation properties of alfalfa silages ensiled with different levels (0%, 5%, and 10%) of corn flakes addition. Within the scope of the study, alfalfa shredded to 1-3 cm sizes were ensiled in anaerobic plastic containers by

Alfalfa silage
Ensiling
Corn flakes
Fermentation
Volatile fatty acids
pH

adding 5% and 10% flaked corn to the treatment groups. As a result of the research, it was determined that the parameters of dry matter (DM), crude protein (CP), ash, acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL) and acid detergent insoluble crude protein (ADICP) decreased significantly in the treatment groups compared to the control group (P<0,01). Ether extract (EE) was found to be similar in the 5% corn flakes added group and the control group but increased significantly at the 10% corn flakes level (P<0,01). Starch also increased in the treatment groups compared to the control, and the highest mean was determined in the group to which 10% corn flakes were added (P<0,01). Neutral detergent insoluble crude protein (NDICP) decreased considerably with 5% corn flakes and was similar in the 10% corn flakes added group and the control group (P<0,05). Metabolizable energy (ME) was not affected by the treatments (P>0,05) but non-fiber carbohydrate, total digestible nutrients and lactation net energy values were significantly increased by the addition of corn flakes to alfalfa silage (P<0,01). Lactic and butyric acids significantly increased with the addition of corn flakes, and pH decreased significantly in the 10% corn flakes added group compared to the control and other treatment group (P<0,01). Additionally, acetic acid and propionic acid were significantly higher in the treatment groups than in the control group (P<0,01). However, acetic acid and propionic acid levels decreased with increasing corn flake level (10%) (P<0,01). As a result, considering the positive effects of corn flakes addition on important parameters for silage, especially lactic acid and pH, and examining the net effects on other parameters, it can be said that the use of corn flakes in alfalfa silage production would have positive effects. However, levels of 10% and further need to be evaluated in future studies.

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1. Introduction

Ensiling forage is a common technique worldwide because it gives ruminants a steady, regular, and dependable supply of nutrients (Nascimento Agarussi et al., 2019). Alfalfa (*Medicago sativa* L.) is the oldest species cultivated as a forage crop and is widely known as the "father of all forages" (al-fal-fa) owing to its high protein content and profusion of several bioactive compounds (Bora and Sharma, 2011). Additionally, alfalfa is an indispensable perennial legume in animal feeding and is a remarkable feed ingredient in that it can be harvested 6-7 times a year, is delicious, has good digestibility, and can be made into both dry grass and silage (Ünal et al., 2024). Alfalfa, one of the most important feed ingredients in the world through its high nutritional quality, efficiency and adaptability, is generally used as hay in animal nutrition. However, since alfalfa is rich in leaves and has a high grass yield, significant nutrient losses occur during the period from drying to feeding to animals due to the thin and weak leaf stalks, or mold and microbial spoilage due to incomplete drying in regions with high rainfall (Ergün et al., 2011; Orak and Gökkaya, 2014).

Ensiling is a conventional technique for preserving fresh feed that relies on lactic acid bacteria's anaerobic fermentation. Following closure, aerobic microbial respiration consumes the trapped oxygen, resulting in an anaerobic environment that stops respiration (Li et al., 2020). Alfalfa are one of the materials that is difficult to make silage with its high buffering capacity, low dry matter (DM), water-soluble carbohydrate content (Gao et al., 2021; Kang et al., 2021; Boğa and Ayaşan, 2022) and tubular hollow stem structure that prevents complete removal of air during ensiling. Therefore, during the silage of forage plants such as alfalfa, which are rich in protein and minerals but poor in carbohydrates, the use of additives to ensure fermentation is sometimes necessary (Kamalak et al., 2009). For this purpose, additives that are aimed at closing the carbohydrate gap that is insufficient in the environment are mostly used. The commonly used carbohydrate sources are molasses, grape pulp, apple pulp, and sugar (McDonald et al., 2002; Karabulut and Filya, 2007; Canbolat et al., 2013; Ünal et al., 2024). On the other hand, as an alternative to these, fruits with high sugar content and cheap prices also come to the forefront for this purpose (Kamalak et al., 2009; Canbolat et al., 2013). Information on the use of variously processed cereal grains in alfalfa silage production is limited.

The high steam and pressure used in corn flakes production increases the digestibility of starch in the grain. In addition, with this production technology, harmful microorganisms in the product are also removed. On the other hand, increasing the soluble carbohydrate content has made corn flakes a suitable raw material for use in alfalfa silage production. However, no evidence was found in the literature regarding the use of corn flakes in alfalfa silage production. The hypothesis of this study was that positive effects will be determined on some important indicators of alfalfa silage made by adding different levels of corn flakes. Therefore, this study was conducted to investigate the effects of corn flakes addition on the chemical composition, digestibility, energy values, and fermentation properties of alfalfa silage.

2. Material and Method

2.1. Preparation of Silage Samples

The alfalfa used in the experiment was harvested in May 2023 from the Aksaray Eskil Region (between latitude 38,401752 and longitude "33,412792" coordinates). Fresh alfalfa (*Medicago sativa* L.) samples harvested during the flowering period and at a DM value of approximately 20% were withered in the laboratory for one day and shredded into 1-3 cm sizes with a chopping machine. Corn flakes were supplied from a commercial company (KON-ÇİNİ Tarım Ürünleri & Yem) and added to the shredded alfalfa in 3 parallel at levels of 0, 5 or 10%. By providing uniform distribution, it was thoroughly compressed in 1 kg plastic lidded anaerobic drums and silage was carried out by providing an oxygen-free environment. The tightly capped samples were fermented for 60 days. At the end of the silage period, the following analyses were performed.

Table 1. Nutrients of corn and corn flake (%).

Materials	Dry matter	Crude protein	Ash	Crude fiber	Ether extract	Starch	Neutral detergent fiber	Acid detergent fiber	Acid detergent lignin
Corn flake	85.83	6.74	1.3	1.41	3.41	64.57	6.77	2.51	0.35
Alfalfa	15.94	30.58	-	-	-	4.02	35.56	28.20	-

2.2. Chemical Analysis

At the end of the ensiling period, opened samples were prepared for chemical analysis. Silage samples taken to represent the mass were dried in an air circulating oven at 60ºC for 48 hours to determine their DM contents. After DM analysis, the samples were ground in a laboratory mill (IKA MF.10) to pass through a 1 mm sieve diameter. Crude protein (CP) analysis was performed using the Dumas method, ash by burning in a muffle furnace at 550°C for 3-5 hours, ether extract (EE) and crude fiber (CF) analysis were performed using the petroleum ether extraction method according to the methods specified in AOAC (1999). Neutral detergent fiber (NDF) and acid detergent fiber (ADF), acid detergent insoluble lignin (ADL), acid detergent insoluble crude protein (ADICP) and neutral detergent insoluble crude protein (NDICP) analyses were performed on the Ankom Fiber Analyzer according to the method reported by Van Soest (1982). Starch contents of research silages were determined by polarimetric method.

2.3. Digestibility and Energy Calculations

Digestibility and energy values were calculated according to the chemical composition analysis of the silage samples of the research. In this context, total digestible nutrients (TDN_{1X}), metabolizable energy (ME), and net energy lactation (NE_L) values were calculated with the formulas in the NRC (2001) report; non-fiber carbohydrate (NFC) was calculated with the equation NFC = 100 – (CP+EE+ash+NDF).

2.4. Fermentation Properties

At the end of 60 days of fermentation; pH, acetic, propionic, butyric and lactic acid levels of the samples were determined. For this purpose, 40 g of silage sample was mixed in a blender by adding 360 mL of pure water and completed to 400 mL. This liquid was filtered again through filter paper (Whatman 54) and centrifuged. Samples were stored at -20°C until analyzed. Lepper's abbreviated method was used for lactic acid analysis (Akyıldız, 1984). Acetic acid, propionic acid, and butyric acid analyses were determined by gas chromatography (Model, Agilent, 7890, USA).

2.5. Statistical Analysis

At the end of the research, the one-way analysis (ANOVA) of variance was applied to all variables obtained from the experimental groups, and the differences between the means of the groups were determined by using the Duncan multiple comparison test. Differences were considered significant at $P < 0.01$ and $P < 0.05$.

3. Results and Discussion

Silage is a very complex fermentation matrix that differs in its chemical makeup, water-soluble carbohydrates, local microbiota, and nitrogenous substances that microorganisms can use (Saarisalo et al., 2007). The quantity and kind of microorganisms in the plant, as well as the feed's DM content, buffering ability, and water-soluble carbohydrate content, all influence the occurrence of the intended silage fermentation. In other words, the properties of the silage material directly affect the fermentation properties of the silage (Guan et al., 2018). Alfalfa are one of the forages that are difficult to ensile due to both its DM content and water-soluble carbohydrate level. Therefore, additives are needed to increase the amount of soluble carbohydrates when making silage. For this purpose, in this study, corn flakes, a highly digestible raw material, was added to alfalfa and ensiled.

As a result of the study, the chemical composition of alfalfa silage made with different levels of corn flakes addition (5% or 10%) was shown in Table 1. Of these parameters, DM, CP, CA, ADF, NDF, ADL, and ADICP decreased significantly in the treatment groups compared to the control group ($P<0.01$). Ether extract was similar in the 5% corn flakes supplemented group and the control group but increased significantly at the 10% level ($P<0,01$). Starch levels also increased in the treatment groups compared to the control, with the highest value found in the group with 10% corn flakes added ($P<0.01$). Neutral detergent insoluble crude protein decreased significantly with 5% corn flakes ($P<0.05$) and was similar in the 10% corn flakes group and the control group. As is known, NDF and ADF are important factors affecting feed intake and feed's digestibility (Gül, 2023) and can be fermented by microorganisms to produce water-soluble carbohydrates or organic acids (Peng et al., 2024). The notable decrease in ADF and NDF in the current study supports this opinion. In addition, the decrease in CP can be attributed to the decrease in CP density due to the addition of carbohydrate sources. There is no evidence in the literature regarding alfalfa silage with corn flakes added. However, Ünlü et al. (2015) reported that the addition of 5, 10 or 15% cracked corn reduced the CP, CF, ADF, and NDF in alfalfa silage. In addition, Acar and Bostan (2016) stated that CP, ADF and NDF levels decreased in alfalfa silage to which 50 or 100 g/kg barley mash was added. These results were partially consistent with the current study. Although not related to the addition of corn flakes or grain, Doğan Daş (2024) stated that DM, ADF and NDF contents were not affected in alfalfa silage to which pasta was added at levels of 0,00, 0,75, 1,50, and 3,00% as a carbohydrate source, while CP decreased at levels of 1.50 and 3.00% compared to the control. Ünal et al. (2024) clarified that DM decreased in 10% level in silage with 5 or 10% apple pomace addition, and CP, EE, CA, starch, NDF, ADF, ADL, ADICP, and NDICP were not affected. Canbolat et al. (2010) found that grape pomace was added to alfalfa silage at different levels (40, 80, 120, 160 or 200 g/kg) and that as the amount of grape pomace increased, CP, CA, NDF and ADF levels decreased, while water-soluble carbohydrates, EE and polyphenol levels increased. Doğan (2019) and Erişçi et al. (2022) reported that the addition of apple (5 or 10%) or apple pomace (5, 10 or 15%) to alfalfa silage did not affect the chemical composition. Although these differences among studies may be related to the type and level of additives, the ensiling period and the harvest time of the alfalfa and its nutrients are also important factors.

Table 2. Effect of corn flakes additive on chemical composition of alfalfa silage

Parameters	Corn Flakes Level (%)			*P value*
	0	**5**	**10**	
DM, %	31.38^{A}	26.19^{B}	20.61^{C}	0.000
CP, % DM	20.04^{A}	17.90^{B}	14.87^{C}	0.000
EE, % DM	2.60^{B}	2.82^{B}	3.92^{A}	0.002
CA, % DM	16.43^{A}	11.94^{B}	9.13^{C}	0.000
Starch, % DM	2.50^{C}	21.14^{B}	35.28^{A}	0.000
ADF, % DM	27.82^{A}	18.49^{B}	15.51^{C}	0.000
NDF, % DM	35.84^{A}	22.06^{B}	18.87^{C}	0.000
ADL, % DM	6.47^{A}	3.50^{B}	3.02^{B}	0.000
ADICP, % DM	3.15^{A}	1.19^{C}	1.56^{B}	0.000
NDICP, % DM	2.54^{a}	1.23^{b}	1.32^{ab}	0.038

ADF: Acid detergent fiber, **ADICP:** Acid detergent insoluble crude protein, **ADL:** Acid detergent lignin, **CA:** Crude ash, **CP:** Crude protein, **DM:** Dry matter, **EE:** Ether extract, **NDF:** Neutral detergent fiber, **NDICP:** Neutral detergent insoluble crude protein.

$^{A, B, C:}$ The difference between the means shown with different letters on the same line was statistically significant (P<0,01). $^{a, b:}$ The difference between the means shown with different letters on the same line was statistically significant (P<0,05).

The data in Table 2 listed the digestibility and energy values of alfalfa silage made by adding corn flakes at 0% (control), 5% or 10% levels. In the present study, ME was the only parameter among these parameters that was not significantly affected by the treatments (P>0,05). The NFC and TDN_{1X} increased considerably with the addition of corn flakes and statistically the highest values were detected in the 10% added group (P<0.01). The NE_L also increased significantly with treatments and was found to be higher in both treatment groups than the control group, and no difference was observed between treatments (P<0.01). The increase in NFC, TDN_{1X} and NE_L criteria in alfalfa silage with corn flakes is very important in terms of digestibility and bioavailability of the silage. So much so that the high NFC in the feed raw material indicates that it is of higher quality and can be easily digested by animals (Raffrenato et al., 2017; Bai et al., 2023). Although not related to corn flakes, Ünlü et al. (2015) reported that the addition of different levels of cracked corn (5, 10 or 15%) increased ME and nitrogen-free extract matter at 10 and 15% levels. Acar and Bostan (2016) noted that total digestible nutrients decreased in alfalfa silage to which crushed barley was added at 100 g/kg level. Ünal et al. (2024), on the other hand, stated that digestibility and energy values were not affected in silage with 5 or 10% apple pulp addition.

Table 3. Effect of corn flakes additive on digestibility and energy values of alfalfa silage

Parameters	**Corn Flakes Level (%)**			***P value***
	0	**5**	**10**	
NFC, %	25.02^{C}	45.07^{B}	53.03^{A}	0.000
ME, Mcal/kg	2.19	2.40	2.54	0.133
NE_L, Mcal/kg	1.17^{B}	1.56^{A}	1.61^{A}	0.000
TDN_{1X}, %	53.56^{C}	67.21^{B}	72.51^{A}	0.000

ME: Metabolizable energy, **NE_L:** Net energy lactation, **NFC:** Non-fiber carbohydrates, **TDN_{1X}:** Total digestible nutrients. $^{A, B, C:}$ The difference between the means shown with different letters on the same line was statistically significant (P<0,01).

Lactic and butyric acid concentrations of alfalfa silage with corn flakes addition were significantly increased in both treatment groups (5 and 10%) compared to the control group (0%) (P<0,01; Table 3). Additionally, acetic and propionic acids also increased significantly with the addition of corn flakes compared to the control, and the highest values for both parameters were obtained in the 5% corn flakes added group (P<0.01). Although there was no difference between the alfalfa silage with 5% corn flakes added and the control group for pH, a significant decrease was found in the 10% added group compared to the other two groups (P<0.01). Due to the low water-soluble carbohydrate content of legumes, it is necessary to increase the carbohydrate concentration of their silages (Borreani et al., 2018). Additives promote rapid and efficient utilization of water-soluble carbohydrate derivatives, resulting in a rapid decrease in pH with intensive lactic acid production (Weinberg and Muck, 1996; Ennahar et al., 2003). Nascimento Agarussi et al. (2019) reported that the pH considered sufficient for legume silages was generally between 4.500 and 4.929. In a good quality silage, the pH is desired to be between 3.5-4.0 but pH values of 4,0 and above are very common in legume silages (Filya, 2001). The main reason for the decrease of pH value in silage is the production of organic acids. Among these, lactic acid is the ideal fermentation product in silage, while acetic acid, propionic acid and butyric acid are less preferred (Besharati et al., 2022). In fact, it can be said that the reason why corn flake cannot provide the easily fermentable carbohydrates required for lactic acid bacteria fermentation is because the proteins in the medium turn into ammonia during fermentation and the pH levels of alfalfa silages remain high (Filya, 2001). Few carbohydrate sources were tested to improve the fermentation quality of alfalfa silage, and their effects were unclear (Gao et al., 2021). Oude et al. (2001) reported that the lactic acid count decreased with increasing pH. Ünlü et al. (2015) reported that the addition of different levels of cracked corn (5, 10 or 15%) significantly increased lactic and butyric acids in alfalfa silage and did not affect acetic acid and pH. According to Acar and Bostan (2016), the addition of 50 and 100 g/kg crushed barley increased the lactic acid level. Doğan Daş (2024) stated that the pH decreased in alfalfa silage to which pasta was added at levels of 0.75, 1.50, and 3.00%. In the studies of Öztürk et al. (2006) in which corn was added to alfalfa silage and Hashemzadeh-Cigari et al. (2011) in which molasses was added, the lactic acid level increased significantly, supporting the results of the present study. In one of the studies conducted with apple pomace, Ünal et al. (2024) stated that apple pomace at different levels (5 or 10%) increased lactic acid, decreased butyric acid, and pH was not affected. In another study, Canbolat et al. (2010) reported that in alfalfa silages with 40, 80, 120, 160 and 200 g/kg grape pomace addition, lactic acid and propionic acid increased, and acetic acid decreased at levels above 40 g/kg, butyric acid decreased in 120, 160 and 200 g/kg, and pH decreased at levels of 160 and 200 g/kg.

Table 4. Effect of corn flakes additive on some fermentation characteristics of alfalfa silage

Parameters	**Corn Flakes Level (%)**			***P value***
	0	**5**	**10**	
Lactic acid (g/kg)	1.55^{B}	2.16^{A}	2.62^{A}	0.003
Acetic acid (g/kg)	4.54^{C}	16.26^{A}	6.17^{B}	0.000
Propionic acid (g/kg)	0.22^{C}	0.64^{A}	0.55^{B}	0.000
Butyric acid (g/kg)	0.10^{B}	0.43^{A}	0.36^{A}	0.000
pH	6.67^{A}	6.64^{A}	5.93^{B}	0.000

$^{A, B, C:}$ The difference between the means shown with different letters on the same line was statistically significant ($P<0.01$).

4. Conclusions

As a result, considering the positive effects of corn flakes addition on important parameters for silage, especially lactic acid and pH, and examining the net effects on other parameters, it can be said that the use of corn flakes in alfalfa silage production would have positive effects. However, in future studies, levels of 10% and further levels need to be studied to evaluate their effects on lowering pH and increasing the number of lactic acid bacteria.

Author’s Contribution

The contributions of the authors are equal.

Conflict of Interest

The authors have declared there is no conflict of interest.

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