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Possibilities of using dried mulberry pulp as an additive in alfalfa silage

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Summary

This study was conducted to determine the effects of dried mulberry (*Morus alba*) pulp with high water-soluble carbohydrate (WSC) content on the nutrient substance composition, pH and silage acids of alfalfa silage. The alfalfa used in the study was harvested in the 10% flowering period and ensiled by mixing with dried mulberry pulp at different ratios (0%, 2.5%, 5%, 7.5%, 10%, 12.5% and 15%). The alfalfa that was ensiled for three months was analyzed at the end of this period, and the values measured for the control and treatment groups were compared. As a result, it was determined that the addition of dried mulberry pulp at different ratios into alfalfa silage was effective on some properties of the silage. In the case of adding mulberry pulp at 5-7.5% into the silage, it was observed that the silage pH decreased, and the lactic acid ratio increased. This positive result shows that in alfalfa silage mulberry pulp may be used as an additive. Hence, it may be stated that it would be suitable to add 5-7.5% mulberry pulp to increase the quality in alfalfa silage.

Keywords: alfalfa silage, mulberry pulp, nutrient composition, pH

Alfalfa, which can grow in different climates and is a quality legume feed plant, has a significant place in feeding especially ruminant animals with its high protein, vitamin and mineral contents (8, 15, 18). While the alfalfa plant may be provided fresh for animal consumption, it may also be given dried (12). However, mechanical losses that occur during drying of alfalfa (especially in regions with humid climates) and some significant problems that are encountered make storage of alfalfa by ensilage more important (12, 14, 15). It is known that silage production is one of the best preservation methods in terms of protection of the nutrient substances in a plant (18). Although the alfalfa plant has an excellent nutrient composition in terms of silage especially in its flowering period, its low dry matter content, its water-soluble carbohydrate content and high buffer capacity make ensilage difficult (1, 11, 15, 19). Lactic acid bacteria prevent rapid drops in pH and reproduction of unwanted microorganisms by using easily soluble carbohydrates during ensilage (5). For this reason, legume feed crops and especially alfalfa are usually ensiled using additive materials (9). Different researchers have reported that ensilage procedures carried out by using lactic acid bacteria, saccharose and fermented fruit juices increase the quality of alfalfa silage (9).

While a part of mulberry, which is prevalently grown in many regions of the world (including Asia, Africa, Americas, Europe and India), is freshly consumed, a part of it is processed into various foods (16). Since the mulberry pulp that results out of processing mulberry does not have an economic value, it is not sufficiently utilized. However, opportunities of drying mulberry pulp and using it in more areas are being studied. For this purpose, utilization the potential of mulberry pulp which is rich in carbohydrates as an alfalfa silage additive are a topic of interest.

This study was conducted to determine opportunities of using dried mulberry pulp as an additive to improve the fermentation properties of alfalfa silage.

Material and methods

The alfalfa material that was used in the study was grown at the Research and Application farm of the Research and

Tab. 1. Chemical composition of dried mulberry pulp before ensiling (%)

DM	CA	OM	EE	CP	Sugar	ADF	NDF	C	HC	WSC (g/kg DM)
95.1	7.3	87.8	8.2	9.5	45.34	17.9	20.6	13.56	2.84	179.68

Explanations: DM – dry matter, CA – crude ash, OM – organic matter, EE – ether extract, CP – crude protein, ADF – acid detergent fiber, NDF – neutral detergent fiber, C – cellulose, HC – hemicellulose, WSC – water soluble carbohydrates

Training Center of the School of Agriculture at Bingöl University. The alfalfa for ensilage that was harvested in the flowering period was left to wither for half a day, and afterwards, it was cut and made ready for ensilage. The mulberry pulp that was used as a silage additive was obtained by drying the waste that remained after fresh mulberry was used in molasses production. The dried mulberry pulp was made ready for use by grinding in a grinding mill with the help of a 2.5-mm sieve. The nutrient contents of the mulberry pulp used as a silage additive are given in Table 1.

Preparation of silages. The alfalfa used in the trial was harvested in its 10% flowering period, withered in the shade for half a day and cut into 1.5-2-cm pieces. The control group did not have any mulberry pulp addition (0%), mulberry pulp was homogenously mixed into the other groups by 2.5%, 5%, 7.5%, 10%, 12.5% and 15%, and the mixtures were filled into silage containers with 3 replications. The silages that were stored at room temperature for 60 days were then opened and analyzed. In the obtained silages, dry matter, crude ash, organic matter, ether extract, crude protein, ADF, NDF, pH, cellulose, hemicellulose, lactic acid, acetic acid, propionic acid, butyric acid and yeast analyses were conducted.

Analysis of silages. The prepared silages were dried in a stove at 70°C until they reached a constant weight, and dry matter was calculated. In determination of crude ash, the specimens were combusted in a 550°C muffle furnace, and their crude ash contents were calculated. The protein levels of the silage specimens were determined with the Kjeldahl method. The crude protein levels were determined by multiplying the nitrogen ratio with 6.25. Ether extract analysis was conducted using the method specified in K. Helrich (1990).

ADF and NDF levels were determined based on the method reported by Van Soest and Robertson (1985) and by using an ANKOM 200 Fiber Analyzer device (ANKOM Technology Crop., Fairport, NY, USA). Using the measured ADF and NDF values, cellulose and hemicellulose values were calculated. Lactic acid analysis was conducted colorimetrically with gas chromatography according to the method described by (3). Acetic acid, propionic acid and butyric acid were measured with a gas chromatograph (Shimadzu GC-2010+, Kyoto, Japan) with a capillary column (30 m × 0.25 mm × 0.25 µm) (Restek, Bellefonte, PA, USA) and a flame ionization detector (FID) over a temperature range of 45-230°C (7). Yeast and mold counts were made according to the method by (11), and WSC content was analyzed according to the method by (6).

Statistical analysis. The data obtained from the study were analyzed based on the random blocks trial design using the SAS statistical software, and LSD test was used to determine the differences between the mean values.

Result and discussion

Nutrient analyses of the silages in the control and treatment groups were conducted, and the obtained results are given in Table 2. In the study it was determined that the mulberry pulp that was used significantly increased the dry matter levels of the silages ($P < 0.01$). The dry matter content of the silages showed an increase by addition of the mulberry pulp which had a higher dry matter content (95.1%). While the lowest dry matter ratio was in the control group, the highest one was in the group where 15% mulberry pulp was added. The dry matter ratios that were obtained showed differences to those in the findings of (5). The differences between the control and treatment groups were significant in terms of the crude ash ratios ($P < 0.01$). While the control group and the group where 2.5% mulberry pulp was added were similar, the other groups had lower crude ash ratios. This situation may be explained by that the crude ash content of the mulberry pulp (7.3%) was lower than that of the control (9.61%).

In the analyzed silages, there was an increase in the organic matter content in connection to the increase in the mulberry addition ratio. The groups where 12.5% and 15% mulberry pulp was added had significantly higher ($P < 0.01$) organic matter ratios. It was determined that addition of the mulberry pulp with a high organic matter content (87.8%) led to an increase in the organic matter content of the silages.

By addition of different ratios of mulberry pulp into alfalfa silage, the protein

Tab. 2. Chemical composition of alfalfa silages (%)

Level (%)	DM	CA	OM	EE	CP	ADF	NDF	pH	HC	C
0	25.77^{d}	9.61^{a}	16.16^{c}	3.96	17.79^{a}	29.82^{ab}	39.70	4.54^{a}	9.87	23.43
2.5	26.30^{d}	9.20^{ab}	17.11^{c}	4.48	17.60^{ab}	28.55^{bc}	40.67	4.29^{b}	12.12	22.79
5	28.63^{c}	8.72^{bc}	19.93^{b}	4.27	17.21^{bc}	28.12^{c}	41.21	4.07^{bcd}	13.09	22.42
7.5	29.13^{bc}	8.66^{bc}	20.48^{b}	5.36	16.69^{cd}	29.63^{abc}	41.08	4.00^{d}	11.45	23.31
10	30.63^{b}	9.02^{b}	21.65^{b}	4.69	16.67^{cd}	28.73^{bc}	40.47	4.03^{cd}	11.74	22.17
12.5	34.73^{a}	8.34^{c}	26.38^{a}	4.63	16.43^{de}	30.85^{a}	40.87	4.04^{cd}	10.02	23.22
15	35.90^{a}	8.73^{bc}	27.18^{a}	5.06	16.11^{e}	29.36^{abc}	40.77	4.24^{cd}	11.41	21.72
P	**	**	**	NS	**	*	NS	**	NS	NS

Explanations: a-e – means with a different letter in the same column are significantly different; NS – non significant; * – $P < 0.05$, ** – $P < 0.01$; DM – dry matter, CA – crude ash, OM – organic matter, EE – ether extract, CP – crude protein, ADF – acid detergent fiber, NDF – neutral detergent fiber, HC – hemicellulose, C – cellulose ratio varied in the range of 16.11-17.79%, and the differences among the groups were significant ($P < 0.01$). Addition of the mulberry pulp into the silage led to a decrease in the crude protein ratio starting with the addition ratio of 5%. The lowest crude protein ratios were in the groups with 12.5% and 15% mulberry pulp addition. Lowering of the protection ratio by addition of mulberry pulp into silages may be explained by the fact that the mulberry pulp included a lower ratio of crude protein (9.5%) in comparison to the alfalfa. While the protein ratios of the silage groups differed in comparison to those reported by (5) and (10), they were similar to those found by (17) and (2).

The ether extract ratios of the trial groups varied in the range of 3.96-5.36%, and the differences among the mean values were found to be insignificant. The addition of the mulberry pulp with a higher ether extract ratio (8.2%) in comparison to the alfalfa only led to a numerical increase in the ether extract contents of the silages. The pH of a silage is one of the main indicators that show the silage and fermentation quality (17). While the lowest pH in the study was observed in the group containing 7.5% mulberry pulp, it was determined that addition of the mulberry pulp significantly ($P < 0.01$) reduced the silage pH. It is known that the pH of alfalfa does not sufficiently drop as a result of ensiling it without additives, the reason for this is that its low water-soluble carbohydrate content affects lactic acid production negatively (17). Microorganisms transform WSCs into various organic acids, especially lactic acid, and as a result of this, the silage pH decreases fast (5). The obtained results showed that the mulberry pulp with high WSC contents positively affected the pH of the silage. The results obtained in relation to the silage pH values were similar to those reported by (10).

(5) stated that addition of additives with high WSC content into alfalfa silage reduces ADF and NDF ratios as a result of disintegration of the cells of microorganisms with acidic hydrolysis or fibrinolytic enzyme production. This finding of theirs was different to the finding in this study. The values regarding the NDF ratios of the groups showed a variation in the range of 39.7--41.21%, and the differences among the mean values were found to be statistically insignificant. The findings were similar to those found by (17) and different to those stated by (10). In terms of the ADF ratios, while the difference between the control group and the 5% mulberry pulp addition group was significant ($P < 0.05$), the differences among the other groups were insignificant. The ADF ratios of the silages were highly similar to the findings of (17) but different to those of (10).

In general, increasing the WSC content of feed crops before ensilage leads to a drop in the pH and increase in the quality of silage fermentation (13). While the mulberry pulp added into the alfalfa increased the lactic acid and acetic acid ratios, it significantly reduced the butyric acid ratio ($P < 0.01$) (Tab. 3). It may be stated that addition of the mulberry pulp affected silage fermentation positively. The highest value of the lactic acid ratio was in the group containing 5% mulberry pulp, while the highest value of the acetic acid ratio was in the group containing 7.5% mulberry pulp. While adding mulberry pulp into the silage up to 5% increased the lactic acid content, higher addition ratios showed a lowering effect on lactic acid. It was reported that WSC addition over the critical value reduces the fermentation speed and efficiency (13). It may be stated that the result obtained in the study was caused by this situation. The results on the LA and AA were similar to those reported by a previous study (5) where sea buckthorn pomace was added to the alfalfa silage to 5%.

While there was no significant difference between the control and treatment groups in terms of the propionic acid and butyric acid ratios, significant differences were found among the treatment groups ($P < 0.01$). The lowest propionic and butyric acid ratios were measured in the group that contained 15% mulberry pulp, and there was a decrease by half in terms of butyric acid and by 1.4-fold in terms of acetic acid in comparison to the control group. Results for PA are similar to those reported in a study with Sea Buckthorn pomace (4); however, BA values differed from those reported in another study with Honey Locust Pods added to alfalfa silage (5).

In terms of the yeast counts, while all groups were different in comparison to the control group, the

Tab. 3. Effects of mulberry pulp additives on fermentation characteristics and microbiological characteristics of alfalfa silage

Level (%)	LA		AA		PA		BA		Yeast (log10 g/kg DM)	Mold (log10 g/kg DM)
	g/kg DM	%	g/kg DM	%	g/kg DM	%	g/kg DM	%		
0	7.32^{f}	25.4	9.76^{c}	33.9	7.75^{ab}	26.9	3.98^{abcd}	13.8	2.77^{d}	0
2.5	8.79^{e}	20.4	19.38^{b}	44.9	9.46^{a}	21.9	5.51^{a}	12.8	4.89^{ab}	0
5	16.04^{a}	30.3	23.04^{b}	43.6	9.01^{a}	17.0	4.79^{ab}	9.1	5.34^{a}	0
7.5	15.25^{ab}	25.7	30.70^{a}	51.7	9.04^{a}	15.2	4.35^{abc}	7.3	4.54^{b}	0
10	14.66^{b}	27.7	28.32^{a}	53.5	7.23^{ab}	13.6	2.77^{bcd}	5.2	5.36^{a}	0
12.5	13.40^{c}	26.6	29.13^{a}	57.9	5.46^{b}	10.9	2.30^{cd}	4.6	3.90^{c}	0
15	12.51^{d}	24.9	30.51^{a}	60.6	5.37^{b}	10.7	1.95^{c}	3.9	4.54^{b}	0
P	**		**		**		**		**	NS

Explanations: a-f – means with different letter in the same column are significantly different; NS – non significant; ** – $P < 0.01$; LA – lactic acid, AA – acetic acid, PA – propionic acid, BA – butyric acid highest value was in the group with 10% mulberry pulp addition ($P < 0.01$). The mulberry pulp added to the alfalfa silages led to an increase in the yeast counts in all treatments. The increase in the number of yeasts may be explained in that the high WSC in the mulberry pulp served as a nutrient source for yeasts. The findings on the yeast counts were similar to those reported by (4). In the analysis, no mold was observed in either the control or the treatment groups.

In this study, it was observed that the use of dried mulberry pulp as an additive in making alfalfa silage affected the nutrient composition of the silage, increased the dry matter and organic matter contents, lowered the crude ash, crude protein ratios and pH value and did not significantly affect the ether extract, crude cellulose, hemicellulose and NDF ratios. Considering the effects of the mulberry pulp on the silage fermentation of alfalfa, it was determined that the treatments increased the lactic acid and acetic acid ratios and reduced the propionic acid and butyric acid ratios. The main difficulties encountered in ensiling alfalfa are lack of sufficient dry matter ratio and failing to reduce the pH value down to the desired level. Therefore, it may be stated that ensiling the alfalfa plant with mulberry pulp, which has a high WSC content, had a positive effect in terms of obtaining a higher-quality silage.

Consequently, it was observed that the use of 5-7.5% dried mulberry pulp in alfalfa silage created a positive effect on some properties of the silage to be obtained. It was determined that the mulberry pulp addition by the specified ratios brought the dry matter ratio and pH level in the alfalfa to the desired levels and increased the lactic acid ratio which is desired to be high. Therefore, while ensiling alfalfa, addition of mulberry pulp by 5-7.5% may be recommended.

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