Yuzuncu Yil University
Journal of Agricultural Sciences
(Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi)
https://dergipark.org.tr/en/pub/yyutbd

ISSN: 1308-7576 e-ISSN: 1308-7584

Research Article

The Effect of Oregano (*Origanum onites*) Pulp to Quality Parameters of Meadow Silage

Şirin Duygu YEŞİL[1], Mehtap GÜNEY*[2]

[1]Van Yuzuncu Yil University, Institute of Health Sciences, Animal Science, Van, Türkiye
[2]Van Yuzuncu Yil University Faculty, Agriculture Department, Animal Science, Van, Türkiye

[1]https://orcid.org/0000-0002-9384-7416, [2]https://orcid.org/0000-0002-0613-3600

*Corresponding author e-mail: mguney@yyu.edu.tr

Article Info

Received: 19.01.2023
Accepted: 11.06.2023
Online published: 15.09.2023
DOI: 10.29133/yyutbd.1235890

Keywords

Aerobic stability,
Meadow silage,
Oregano pulp,
Silage quality parameters

Abstract: It was aimed to determine the effect of ensiling by adding Oregano pulp at different rates to the meadow grass on the physical quality properties of silages, silage fermentation and aerobic stability. In the study, dry oregano pulp at the rate of 3% (OP3) to 5% (OP5) by weight basis and without additives (control) was added to the first harvested of meadow grass in 2021 and it was ensiled as 3 groups. A total of 18 silage samples, 6 for each group, were left for fermentation in 1 liter glass jars for 60 days. Addition of oregano pulp in two different ratios decreased the DM content of silages compared to the control group ($p<0.05$). Control and silage groups with added oregano pulp were obtained in 2nd and 3rd roughage quality class, and their relative feed values were between 101.06 and 106.55. As a result of the physical properties of the silages, silages without additives were obtained as "satisfactory", while "good" quality silages were obtained from the groups with 3% and 5% oregano pulp. pH levels of silages were obtained between 4.37-4.89. The LA concentrates of the silage decreased significantly with the addition of oregano pulp, while the AA and BA levels of the silages were also decreased ($p<0.001$). As the ratio of oregano pulp increased from 3% to 5%, it was observed that the aerobic stability of the silages increased compared to the control group. As a result, it was concluded that oregano pulp can be evaluated as a silage additive.

To Cite: Yeşil, Ş D, Güney, M, 2023. The Effect of Oregano (*Origanum onites*) Pulp to Quality Parameters of Meadow Silage. *Yuzuncu Yil University Journal of Agricultural Sciences*, 33(3): 408-419. DOI: https:/doi.org/10.29133/yyutbd.1235890

Footnote: This study is derived from first author master thesis and presented as an oral presentation at Van Yüzüncü Yil Üniversitesi 1. Uluslararasi Sağlik Bilimleri Kongresi on 27-30 October. 2022.

1. Introduction

Meadow and pasture forages are the most important roughage sources in our country. However, there are still significant quality problems in these areas due to problems such as harvesting and grazing (Özkan and Demirbağ, 2016). Making silage of meadow grass causes less nutrient loss compared to drying it. For this reason, it is reported that silages obtained from meadow grass provide significant savings in terms of both reducing labor and preventing nutrient loss (Akyıldız, 1986; Kaya et al., 2009).

The increase in animal production has led to the search for suitable feed additives as well as quality and cheap roughage sources from past to present. From additives, aromatic plants and/or essential oils obtained from these have important antimicrobial properties due to the active components

they contain, and are used as feed additives in both poultry and ruminant rations (Gladine et al., 2007; Cobellis et al., 2015). In recent studies, it is seen that natural aromatic plants or essential oils are used as silage additives (Kung et al. 2008; Hodjatpanah-Montazeri, 2016). As it is known, the purpose of using silage additives is to improve silage fermentation quality and allow ruminant animals to benefit at the maximum level (Filya 2000; Slottner and Bertilsson, 2006). It has been stated that the active ingredients of oregano herb have antimicrobial effects. It is due to the polyphenols in the antimicrobial in the plant, and the major active compounds are carvacrol and thymol (Mellencamp et al., 2011; Üstü and Uğurlu, 2018). Oil of oregano herb is also as important export source in Türkiye (Bozdemir, 2019). After oil is obtained from oregano herbs, the remaining pulp remains as a product processing waste (Abdollahzadeh et al., 2010). In addition, waste pulp, which can cause environmental pollution cannot be evaluated in terms of rich nutrient content. Oregano pulp, which is valuable in terms of its rich nutritional content and phenolic substances in its composition, is considered to be used as a silage additive in animal nutrition. The use of aromatic plant pulp as silage additives in animal nutrition is a new and current issue. There was limited research on the use of oregano itself and its essential oil as a silage additive, but no study was found, except for a single study on its pulp.

It has been reported that the pH levels of ensiled by adding thyme pulp to alfalfa and meadow grass changed between 4.34 and 4.62 and thyme pulp did not change the pH of the silages. In addition, It is reported that crude protein levels decreased, NDF and ADF levels increased, and thyme pulp changed silage microbiota population and their fermentation metabolites, and had many biologically active compounds such as polyphenols that could act as silage fermentation inhibitors. The researchers reported that the thyme pulp additive caused a certain increase in the acetic acid level in grass silage and the silage fermentation profile formed a heterofermentative fermentation as a result of the interaction between the phenolic compound and lactic acid bacteria (Aksu et al., 2017). In a another study using essential oil, oregano, cinnamon and their mixture were added to feed peas (400 mg kg^{-1}) and subjected to 60 days of fermentation. It was stated that the aerobic stability of silages was significantly improved on the 7th day of ensiling (Soycan-Önenç et al., 2015). The essential oils of mint, thymol, oregano and cinnamon were ensiled by adding 120 and 240 mg kg^{-1} DM to the corn forage. The chemical composition and aerobic stability of the silages after ensiling were determined. Accordingly, the contribution of oregano, cinnamon essential oils significantly decreased the NDF content of the silage compared to the control and increased the chemical composition and aerobic stability of the corn silage (Hodjatpanah-Montazeri, 2016).

This study was carried out to investigate silage fermentation, physical quality criteria of silages and especially aerobic endurance by adding oregano pulp, which is a waste material, to meadow grass in regions where oregano oil is grown in our country.

2. Material and Methods

2.1. Preparation of additive

Dry oregano pulp (*Origanum onites*) used as an additive was purchased from the producer company located in Antalya (İnan Tarım Ecodab). Then, the pulps were ground in a mill with a sieve diameter of 1 mm, and 3% and 5% on a weight basis was added meadow grass.

2.2. Preparation of silages

The feed material of the study was the first harvest of meadow forage grown in the meadow-pasture area in Edremit district of Van province. It is obtained from the natural meadow area where meadow grass, leguminous and grass grasses are grown mixed. After the meadow grass was harvested, it was cut into 2-4 cm sizes in Van Yüzüncü Yıl University Research and Application Farm and made ready for silage production. The meadow grass brought to the laboratory were made on nylon tarpaulin laid on a flat and clean surface, and was ensiled in 3 groups by adding 3% and 5% dry oregano pulp, no additives added to the control group. The silage samples were left to ferment for 60 days after being compressed in 1 liter glass jars with lids, 6 in each group, 18 in total, in accordance with the average weight standard of 950 g. Chemical composition of Oregano pulp and meadow grass are given in Table

Table 1. Nutrient composition of meadow grass and Oregano pulp before ensiling, DM%

Nutrient composition	Meadow grass	Oregano pulp
DM	25.70	93.91
Ash	11.13	7.99
CP	8.89	8.57
CF	1.82	1.89
NDF	50.27	41.23
ADF	36.19	31.89

DM: Dry matter; CP: Crude protein; CF: Crude fat; NDF: Neutral detergent fiber; ADF: Acid detergent fiber.

2.3. Physical analysis of silages

The physical properties such as color, odor and structure of the silages opened on the 60th day were determined by using the evaluation key recommended by the German Agriculture Organization (Alçiçek and Özkan, 1997). Flieg scores of silages were made according to the evaluation key reported by Kılıç (1986).

2.4. Chemical analysis of silages

At the end of fermentation, 25 g sample of each sample taken from the silages opened was mixed with 100 ml of distilled water and pH measurement was immediately made. Ammonia analysis of silages were determined by Kjeldahl distillation method immediately after pH measurement in silage samples (Markham, 1942). Then, the silage samples taken from each jar were dried in an oven at 65 °C for 48 hours to determine the dry matter content. Dried samples were ground in a mill with a sieve diameter of 1 mm and prepared for chemical analysis. Dry matter (DM), crude protein (CP) and ash contents of silages were determined according to the Weende analysis method (AOAC, 2000). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were analysed as described by Van Soest et al. (1991), and the crude fat (CF) content was measured according to ANKOM (2008) using an ANKOM XT15 device.

Phenolic compounds in dry oregano pulp were made according to the method specified by Singleton and Rossi (1965) and read in a gas chromatography device. Condensed tannin amount was determined spectrophotometrically according to the method reported by Makkar et al. (1995).

The relative feed value (RFV) index was used to determine the forage quality of the ensiled meadow grass by adding oregano pulp at two different rates. The RFV index, which is used as an important tool in the evaluation process of forage, was calculated according to Rivera and Parish (2010). In order to determine of RFV, first of all, the estimated digestible dry matter (DDM) amount was calculated over the ADF values of the silages. Depending on the body weight of the animal, dry matter intake percentage (DMI) was calculated based on the NDF value and the relative feed value was determined by replacing these values in the formula.

$$DDM\% = 88.9 - (0.779 x ADF\%) \quad (1)$$

$$DMI\% = \frac{120}{NDF}\% \quad (2)$$

$$RFV = (DDM\%) * \frac{DMI\%}{1.29} \quad (3)$$

For volatile fatty acid (VFA) and lactic (LA) analyzes in silage liquids, the samples taken from the deep freezer were centrifuged at 3500 g for 15 minutes and then placed in the automatic sampler compartment of the high pressure liquid chromatography (HPLC) device to measure acetic, propionic, butyric and lactic acid measurements, and samples were made Van Yüzüncü Yıl University Science Research and Application Center (Leventini et al., 1990).

The aerobic stability test on silage samples was carried out according to Ashbell et al. (1991). The silages opened after the 60th day of ensiling were subjected to a 10-day aerobic stability test. For this, 250-300 g of fresh silage samples, 2 from each group, were taken and kept at room temperature for 10 days in a simple aerobic unit prepared. During this period, the aerobic endurance of the silages was

calculated with the help of the CO_2 amount measured after the titration with the precipitation of CO_2 gas.

2.5. Statistical Analyses

Statistical analysis of the data was carried out according to a completely random experiment design and was based on the following formula.

Analysis of variance was used to determine the relationships between the Oregano pulp additive and the physical, chemical and aerobic stability properties of silages, and Duncan's multiple comparison test was used SAS 9.4 to determine the differences (SAS, 2014).

$$Yij = \mu + ai + eij \quad (4)$$

$$\mu = overall\ average \quad (5)$$

$$Yij = i.level\ of\ Oregano\ pulp \quad (6)$$

$$ai = i.effect\ of\ level \quad (7)$$

$$eij = random\ error \quad (8)$$

3. Results and Discussions

The average phenolic compounds of oregano pulp used in the study were determined as 41.070 g kg^{-1}. Condensed tannin content in the samples was found to be 8.409 g kg^{-1} on average. The effect of ensiling the meadow grass with the addition of oregano pulp on the chemical composition is given in Table 2. Crude protein, CF, NDF and ADF contents of silages are not affected by oregano pulp additive, but differences between DM and ash contents were significant ($p<0.05$). The DM content of the silages decreased with the addition of oregano pulp at 2 different rates compared to the control group. It was observed that the DM content, which was 26.71% in the control, decreased to 23.94% with the contribution of OP3 and to 23.32% with the contribution of OP5. The ash content of the silages was observed to be higher in OP5 (15.80%) than the control (14.02%) and similar to OP3 (14.76%) on a DM% basis (Table 2; $p<0.05$).

Table 2. The effect of oregano pulp on the nutrient composition of meadow grass silage, DM%

	Groups of silages			
	Control	**OP3**	**OP5**	**p-value**
Item	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	
DM	26.71±0.53^{a}	23.94±0.66^{b}	23.32±0.45^{b}	0.0014
Ash	14.02±0.66^{b}	14.76±0.37^{ab}	15.80±0.10^{a}	0.039
CP	8.47±0.30	9.18±0.26	9.27±0.18	0.085
CF	1.58±0.32	1.87±0.15	1.65±0.17	0.650
NDF	52.16±0.88	53.05±0.75	50.66±0.83	0.148
ADF	40.19±0.50	40.24±0.53	39.76±0.31	0.718

There is a difference between the means in the same row and with different letters ($p<0.05$), OP3: meadow silage+dry oregano pulp of 3%; OP5: meadow silage+dry oregano pulp of 5%; DM: Dry matter, CP: Crude protein, CF: Crude fat, NDF: Neutral detergent fiber, ADF: Asit detergent fiber.

Soycan-Önenç and Turgud (2019) found that the DM contents of the silages did not change with the additive as a result of the addition of oregano essential oil to alfalfa silage, and the addition of lavender essential oil to alfalfa silage (Duru, 2019). In the study, although the silage material was ensiled and well preserved in the DM range recommended for silage feeds, DM loss occurred in the groups to which additives were added. This situation is thought to be related to the insufficient content of water-soluble carbohydrates in oregano pulp. It has been reported that feed materials are generally ensiled between 200-500 g kg^{-1} (Muck, 2010), and the higher the water-soluble carbohydrate (WSC) content of the silage material, the lower the dry matter loss that may occur during fermentation (Basmacıoğlu and Ergül, 2002). In terms of ash content, differences were found in the control and oregano pulp added groups ($p<0.039$). The use of tannin-rich gladia fruit in meadow silage was decreased the ash content

with the additive (Guven and Kalamak, 2021). It has been reported that the ash content of silages increased with the contribution of the gladia fruit to the sugar beet pulp (Özkan, 2012). In the current study, it was observed that 5% oregano pulp additive increased the ash content of silages compared to the control silage. It is thought that during the silage making of OP5 group, it is highly likely that sand, stone and especially dry branch pieces were mixed in the oregano pulp in large quantities. Örün and Erdoğan (2021) reported that the ash content may be an indicator of excessive amounts of mineral substances in the structure of the feed material or foreign substances (sand, stones, dry branches, etc.) mixed with the feed. In the study, CP, CY, NDF and ADF contents of the silages were not affected by the oregano pulp additive (Table 2).

In the study, the relative feed values of meadow silage did not change with the oregano pulp additive. In terms of forage quality class of silages, it is seen that 2nd quality silages from the OP5 group were obtained (Table 3). In the study, the relative feed values of meadow silage ensiled by adding oregano pulp varied between 101.06% and 106.55%. Ertekin et al. (2022) reported that the relative feed values in silages made with *Lolium multiflorum* L. varied between 78.1-88.2%. This result is quite lower than the study, and it was determined that the roughage quality class was at a good level in all silage groups in the our study. It is thought that is due to the fact that the plant composition of the meadow grass used in the study has a richer content in terms of legume grasses. When the results of the relative feed values are compared with the RFV of full bloom alfaalfa has a value of 100 (Rohweder et al., 1978), it is seen that the forage quality class is "2 and 3 quality". Indeed, Redfearn et al. (2006) reported that as the relative feed value falls below 100, the quality of the feed decreases, and if it is above 100, the feed value increases. All relative feed values obtained in the study were found above 100 (Table 3).

Table 3. Forage quality class and relative forage values of silages

Silage groups	N	DDM%	DMI%	RFV%	Forage class
		$\bar{x}$ ± SE	$\bar{x}$ ± SE	$\bar{x}$ ± SE	
Control	6	57.59±0.39	2.30±0.04	102.84±1.65	3rd quality
OP3	6	57.55±0.42	2.26±0.03	101.06±2.05	3rd quality
OP5	6	57.93±0.24	2.37±0.04	106.55±2.07	2 nd quality
p-values		0.72	0.15	0.16	

There is a difference between the means in the same row and with different letters ($p<0.05$), OP3: meadow silage+dry oregano pulp of 3%; OP5: meadow silage+dry oregano pulp of 5%; DDM: digestible dry matter; DMI: dry matter intake; RFV: relative feed value.

The physical properties of the silages in terms of color, odor and stricture were examined. The total score of 14.67 in control group was 16.00 in the OP3 group and 16.83 in the OP5 group, and the highest total score was obtained from the OP5 silage. When the silages are examined in terms of quality classes, it is seen that satisfactory and good silages are obtained with the control and 2 different ratios of oregano pulp (Table 4). It was reported that the physical quality properties of silages prepared with the contribution to meadow grass of forage locust flakes increased (Atalay, 2015). It is stated that the silage quality criteria of lavender oil contribution to alfalfa forage were determined as satisfactory (Duru, 2019). Özturk et al. (2020) is reported that mixtures in hops with corn and forage soybean were obtained as "good" and "very good" silage quality classes. Our study is similar to these results.

According to the flieg scoring system were determined quality criteria of silages, and the highest flieg score was obtained from the control silage (83.75), while the lowest flieg score was obtained from the OP3 (57.42) silage. It is seen that the flieg scores of silages with 3% and 5% oregano pulp additive decreased compared to the control group (Table 5; $p<0.05$). As is known, the flieg score is calculated based on the pH and dry matter content of silages. In the study, it is thought that the decrease in the flieg score in the oregano pulp added meadow is related to the increase in pH compared to the control group and the decrease in the DM content in these groups. Gladicia fruit used as a silage additive did not cause a significant change in the pH of the wet sugar beet pulp, while the fleig score was slightly increased compared to the control (Özkan, 2012). Flieg scores of silages ensiled by adding thyme oil additive to sugar beet pulp did not change, and their flieg scores ranged between 90.81-98.33. Researchers have also reported that thyme oil can be added to silage as an additive if it balances the cost against losses due to mold and spoilage (Çayıroğlu et al., 2020).

Table 4. Scoring of the physical properties of oregano pulp added silages according to the method recommended by the German Agriculture Organization

	N	Smell	Stricture	Colour	Total point	Quality Class
Control	1	8	4	1	13	middle
	2	14	4	2	20	very well
	3	8	4	2	14	good
	4	8	4	1	13	middle
	5	8	4	2	14	good
	6	8	4	2	14	good
	Avg.	9	4	1.67	14.67	satisfactory
OP3	1	14	4	2	20	very well
	2	8	4	2	14	good
	3	8	4	2	14	good
	4	14	4	2	20	very well
	5	8	4	2	14	good
	6	8	4	2	14	good
	Avg.	10	4	2	16.00	very well
OP5	1	8	4	2	14	good
	2	14	4	2	20	good
	3	14	4	1	19	very well
	4	14	4	2	20	very well
	5	8	4	2	14	very well
	6	8	4	2	14	good
	Avg.	11	4	1.83	16.83	very well

OP3: meadow silage+dry oregano pulp of 3%; OP5: meadow silage+dry oregano pulp of 5%.

Table 5. Quality criteria of silage groups according to the flieg scoring system

	DM	pH	Flieg score
	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$
Control	26.71 ± 0.53^{a}	4.37 ± 0.18^{b}	83.75 ± 7.19^{a}
OP3	23.94 ± 0.66^{b}	4.89 ± 0.03^{a}	57.42 ± 2.20^{b}
OP5	23.32 ± 0.45^{b}	4.61 ± 0.13^{ab}	67.17 ± 5.70^{b}
p-value	0.0014	0.0393	0.0124

DM: Dry matter; OP3: Meadow silage+3% oregano pulp; OP5: Meadow silage+5% oregano pulp.

The successful completion of the fermentation process of the silage material is possible by following the silage making principles at the beginning. Muck (2010) reported that it is difficult to control the biological activity in the silo, therefore the ensiling process should be managed well and undesirable microorganisms may develop in silages where fermentation is not well managed. The results of the fermentation quality of the silage material ensiled with the addition of oregano pulp to meadow grass are given in Table 6. The pH levels of the silages were higher in the OP3 silage group than in the control group. The pH of meadow silage, which was 4.37 in the control, increased to 4.89 in the OP3 silage group (Table 6; $p<0.05$). The pH of the silages increased slightly in the OP5 silage group compared to the control group, but the difference was not statistically significant. In the study, it was seen that the additive of oregano pulp increased the pH of the silages. It has been reported that the pH levels of silages vary between 4.34 and 4.62 as a result of the contribution of thyme pulp to alfalfa and meadow grass, and thyme pulp does not change the pH of silages (Aksu et al., 2017). In a different study, it was reported that the addition of 400 mg kg^{-1} oregano oil (*Origanum onites*) to fresh peas did not change the silage pH compared to the control group, but the addition of cinnamon at the same level increased the silage pH (Soycan-Önenç et al., 2015). In our study, pH values of silages varied between 4.37-4.89. Kaya (2005) reported that the pH value varies between 3.8 and 4.8 in quality silages, while they reported that the Enterobacteria group microorganisms that cause deterioration are effective in the pH environment between 6-7 and lose their effectiveness at pH values below 5 (Filya, 2001). Therefore, it is possible to say that the pH range obtained in the study is in a pH range that may prevent the growth of unwanted microorganisms in the silo, and even within the recommended ranges. The increased pH

levels in the groups to which oregano pulp was added in the study can be explained by the fact that the oregano pulp did not have sufficient lactic acid content or had a lower amount of water-soluble carbohydrates compared to the control group. It has been reported that the rate of decrease in pH of silages with low water-soluble carbohydrate content is also slow (Merry et al. 1993). In addition, it is thought that phenolic compounds in oregano pulp may have realized a higher buffering capacity during silage fermentation.

The LA level, which was 14.51% in the control group, decreased to 1.35% in OP3 silage and 2.47% in OP5 silage (Table 6; $p<0.001$). As it is known, high LA level is desired in terms of silage fermentation quality. Alçiçek and Özkan (1997) reported that LA content should be over 2.0% in quality silages, Lorenzo and O'Kiely (2008) reported that LA levels should be 50-70 g kg^{-1} DM in quality silages. In the study, it is thought that the conversion of sugars to LA decreased in parallel with the insufficient content of water-soluble carbohydrates in the oregano pulp added groups. However, it can be thought that LA level was provided at a sufficient level to create a protective effect for the fermentation quality of the silages. It is seen that two different levels of oregano pulp additive significantly reduce the LA content of silages. With the realization of sufficient lactic acid production in silo feeds, the pH of the silage reaches the desired level. As a matter of fact, in the study, it was observed that the LA level decreased in the oregano pulp added groups, the pH levels of the silages increased. It has been reported that LA and enterobacteria levels of the silages did not change compared to the control, as a result of adding 0, 40 and 80 mg of essential oil to the feed per kg of wet weight on the quality of silage (Kung et al., 2008). In another study, it was reported that cumin (from *Cuminum cyminum* L.) stimulated the growth of Lactobacillus plantarum and lactic acid production (Kıvanç et al. 1991). Soycan-Önenç and Turan (2018) stated that the addition of 300 mg kg^{-1} cumin essential oil in the last harvest caused the cell membrane to break down by stimulating cell membrane-degrading enzymes. In the literature research, only one study was found on the use of thyme pulp additive as a silage additive. In this study by Aksu et al (2017), it was reported that dry thyme pulp used as a silage additive decreased the LA content in meadow silage group with 5% thyme pulp, and this result was found to be similar to the study conducted.

It was seen that the AA levels of the silages were decreased OP3 and OP5 compared to the control group (Table 6; $p<0.025$). It is observed that besides the production of lactic acid bacteria in the control group, some species belonging to the enterobacteria family also ferment water-soluble carbohydrates in the silo and cause acetic acid production, and so the addition of oregano pulp suppresses both LA and AA production. It is thought that this situation is caused by the antimicrobial effects of phenolic compounds in oregano pulp. Contrary to this situation, Aksu et al. (2017) reported that the addition of thyme pulp caused a certain increase in the acetic acid level in meadow silage and that the silage fermentation profile formed a heterofermentative fermentation as a result of the interaction between the phenolic compound and lactic acid bacteria.

In the study, the BA levels were decreased with the addition of oregano pulp compared to the control group. The BA level, which was 3.93% in the control group, decreased to 0.93% in the OP3 silage group and 2.22% in the OP5 silage group. In a similar study, it was stated that the fermentation quality of the silage varies according to the LA and BA content, and that good silage should contain high lactic acid content and low or no butyric acid content (Kiraz and Kutlu, 2016). Filya (2000) is stated that the use of water-soluble carbohydrates in silage by aerobic microorganisms initiates clostridial activity. It is known that high level of ammonia is formed by clostrida bacteria in the silo and thus is an important factor in the deterioration of silage quality. As a matter of fact, the NH_3-N level did not change in the study. In other words, it was observed that proteolysis did not occur in silages prepared by adding oregano pulp to meadow grass. It is thought that the decreased butyric acid level in the study is due to the phenolic substances in the oregano pulp. It has been reported that butyric acid bacteria are the most important competitors of acetic acid bacteria during silage fermentation, and butyric acid production causes significant loss of nutrients. It is stated that butyric acid bacteria either reduce or completely consume the nutrients they need by using the carbohydrates used by acetic acid bacteria, so butyric acid is not required in silages (Alçiçek and Özkan, 1997). When the fermentation properties of silages are evaluated in general, it is possible to say that the phenolic substances in the oregano pulp suppress the growth of desired microorganisms as well as unwanted microorganisms in the silage.

Table 6. Effect of oregano pulp additive on fermentation parameters of meadow silage

	Silage groups			
Fermentation parameter	**Control**	**OP3**	**OP5**	**p-value**
	$\bar{x} \pm SE$	$\bar{x} \pm SE$	$\bar{x} \pm SE$	
pH	4.37±0.18^{b}	4.89±0.03^{a}	4.61±0.13^{ab}	0.040
LA, DM%	14.51±1.14^{a}	1.35±0.09^{b}	2.47±0.30^{b}	0.001
AA, DM%	5.76±0.44^{a}	4.30±0.47^{b}	4.26±0.20^{b}	0.025
PA, DM%	5.04±0.42	4.52±0.34	4.36±0.31	0.391
BA, DM%	3.93±0.38^{a}	0.93±0.24^{c}	2.22”±0.38^{b}	0.001
NH_3-N, mg/100ml	0.20±0.02	0.29±0.03	0.30±0.06	0.208

There is a difference between the means with different letters in the same column ($p<0.001$); OP3: Meadow silage+3% oregano pulp; OP5: Meadow silage+5% oregano pulp; LA: Lactic acid; AA: Acetic acid; PA: Propionic acid; BA: Butyric acid; NH_3-N: Ammonia.

Aerobic stability in silage feeds indicates the ability of oxygen-exposed silages to resist microbial growth (McDonald et al. 1991). Aerobic stability is one of the desired features in the silo, and it is reported that each silage has its own aerobic stability, and that this parameter is generally low in quality silages (Filya, 2004). In fact, after the silage is opened, it is inevitable that aerobic deterioration will occur in the silage (Çayıroğlu et al., 2016). However, the short duration of this period is very important for the animals to benefit from the silo feed to the maximum extent. In the study, the aerobic stability of meadow silage without oregano pulp was 1.75% in DM, and it was determined as 0.75% in the OP3 silage group and 0.35% in the OP5 silage group. As the ratio of oregano pulp increased from 3% to 5%, it was observed that the aerobic stability value decreased, that is, the durability of the silage increased (Figure 1). Kung et al. (2008) is stated that the effect of adding essential oil mixtures to corn silage on aerobic deterioration did not have an effect on the fermentation process of silages and aerobic deterioration. On the other hand, it has been reported that the contribution of cinnamon leaf, thyme and sweet orange essential oils in different amounts has a positive effect on 7 day aerobic stability in silage feeds (Chaves et al., 2012). Aksu et al. (2017) reported that in silages opened at the end of the 60 th day, the dry thyme pulp additive has a preventive effect on undesirable microorganisms such as enterobacter, yeast and mold without causing a decrease in the lactobacilli count of the silages compared to the control, and it can provide a very important advantage in terms of improving the aerobic stability of the silages. In a different study, it was stated that gladia fruit rich in phenolic compounds improves the aerobic stability of silos by reducing CO_2 production in silages (Canbolat et al., 2013). Filya et al. (2000) reported that the dry matter content of the ensiled material is an important factor affecting the aerobic stability and the aerobic stability of the silages made with the material with low dry matter content decreased. However, in this study, it was observed that aerobic deterioration was low in silages, despite a decrease in LA and AA levels of silages compared to the control group, and a decrease in dry matter content, especially in the OP5 silage group, in the groups to which oregano pulp was added. In the study, yeast and mold content was not detected. Therefore, it is very difficult to comment on this issue. However, in order to maximize the aerobic stability of the silage, it was thought that the risk of contamination with yeast and mold should be minimized during the harvest of the material to be ensiled. As a matter of fact, it can be predicted that the addition of oregano pulp at a level that will increase the durability of the oregano pulp in the silo may reduce the growth of yeast and mold.

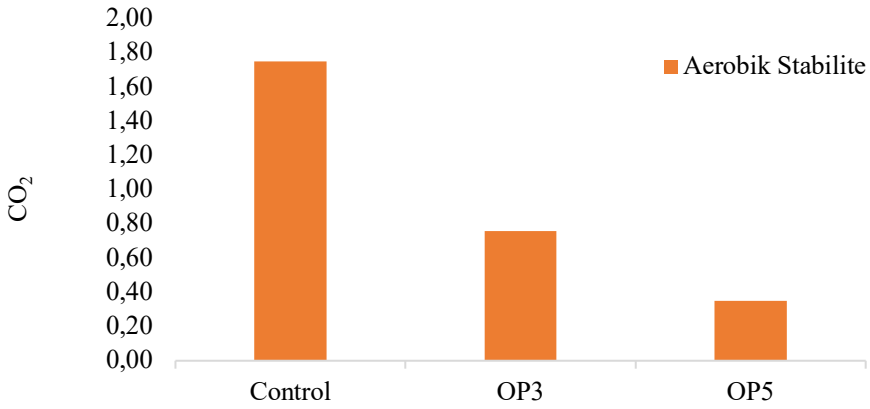

Figure 1. Effect of oregano pulp additive on aerobic stability of meadow silage (CO_2, g kg^{-1} DM).

Conclusion

As a result, DM content of silages ensiled by adding oregano pulp to meadow grass decreased compared to the control group, but in terms of forage quality class, 2nd quality feed value was obtained in the group in which 5% oregano pulp was added. It has been observed that good quality silages in terms of physical properties are obtained from silages that are ensiled with the addition of oregano pulp to the meadow grass. The NH_3-N level of the oregano pulp additive did not change and did not cause proteolysis in meadow silage. However, it decreased the LA levels of silages by suppressing the microbial fermentation in the silo in general. On the other hand, it was concluded that the incorporation of the pulp obtained after the distillation of oregano essential oils rich in phenolic compounds, which may accumulate and be waste into the meadow silage improves the sensory properties of the silages and increases aerobic endurance up to 5%. It is thought that waste pulp can be used as a silage additive in regions where oregano oil is obtained, especially in terms of increasing the aerobic quality of silages. In addition, it is thought that the use of oregano pulp as a silage additive together with water-soluble carbohydrate sources is important in terms of silage fermentation and aerobic stability and will contribute to future in vivo studies.

References

AOAC. (2000). Association of Official Analytical Chemistry Official methods of analysis of AOAC International (18th Ed.). Association of Official Analytical Chemists, Washington DC, USA.

Abdollahzadeh, F., Pirmohammadi, R., Fatehi, F., & Bernousi, I. (2010). Effect of feeding ensiled mixed tomato and apple pomace on performance of holstein dairy cows. *Slovak Journal Animal Sciences*, 43(1), 31-35. https://doi.org/10.4081/ijas-2010.e41.

Aksu, T., Denek, N., Aydin, S. S., Das, B. D., Savrunlu, M., & Ozkaya, S. (2017). Effect of dried thyme pulp *(Tymbra Spicata* L. *spicata)* on fermantation quality and i*n vitro* organic matter digestibility of meadow grass and alfalfa silages. *Kafkas Üniversitesi Veteriner Fakultesi Dergisi,* 23(2), 211-217. 10.9775/kvfd.2016.16266.

Akyıldız, A. R. (1986). *Yemler Bilgisi ve Teknolojisi.* Ankara, Turkiye: Ankara University Faculty of Agricultural Publications.

Alçiçek, A., & Özkan, K. (1997). *Determination of silage quality by physical and chemical methods in silo feeds* Turkey I. Silage Congress, Bursa, Turkiye.

Ashbell, G., Weinberg, Z. G., Azrieli, A., Hen, Y., & Horev, B. (1991). A simple system to study the aerobic deterioaration of silages. *Canadian Agricultural Engineering Journal,* 33, 391-393.

Atalay, A. İ. (2015). *Yemlik Keçiboynuzu Kırığının Çayır Silajında Kullanımı* (phD), Kahramanmaras Sutçu Imam University, Institute of Science, Kahramanmaras, Türkiye.

Basmacıoğlu, H., & Ergül M. (2002). Silaj Mikrobiyolojisi. *Hayvansal Üretim Dergisi,* 43(1), 12-24.

Bozdemir, Ç. (2019). Türkiye'de yetişen kekik türleri, ekonomik önemi ve kullanım alanları. *Yuzuncu Yil University Journal of Agricultural Sciences,* 29(3), 583-594. https://doi.org/10.29133/yyutbd.511777.

Canbolat, O., Kalkan, H., & Filya, I. (2013). Yonca silajlarında katkı maddesi olarak gladiçya meyvelerinin (Gleditsia Triacanthos) kullanılma olanakları. *Kafkas Universitesi Veteriner Fakultesi Dergisi,* 19(2), 291-297. 10.9775/kvfd.2012.7710.

Chaves, A. V., Baah, J., Wang, Y., McAllister, T. A., & Benchaar C. (2012). Effects of cinnamon leaf, oregano and sweet orange essential oils on fermentation and aerobic stability of barley silage. *Journal of Science Food Agriculture*, 92, 906-915. 10.1002/jsfa.4669.

Cobellis, G., Acuti, G., Forte, C., Menghini, L., Vincenzi, SDe., Orru, M., Valiani, A., Pacetti, D., & Trabalza-Marinucci, M. (2015). Use of *Rosmarinus officinalis* in sheep diet formulations: Effects on ruminal fermentation, microbial numbers and in situ degradability. *Small Ruminant Research,* 126, 10-18. https://doi.org/10.1016/j.smallrumres.2015.01.018.

Çayıroğlu, H., Coşkun, İ., & Sahin., A. (2016). Silajın aerobik stabilitesini etkileyen faktörler ve iyileştirme stratejileri. *Alınteri Zirai Bilimler Dergisi*, 31(2), 91-97.

Duru, A. A. (2019). Lavanta (*Lavandula angustifolia*) katkısının yonca silajlarının kalitesi üzerine etkisi. *Uluslararası Mühendislik Araştırma ve Geliştirme Dergisi,* 11(2), 426-431. https://doi.org/10.29137/umagd.486921.

Ertekin, I., Atis, I., Aygun, Y. Z., Yilmaz, S., & Kizilsimsek, M. (2022). Effects of different nitrogen doses and cultivars on fermentation quality and nutritive value of Italian ryegrass (*Lolium multiflorum* Lam.) silages. *Animal Bioscience*, 35(1), 39-46. 10.5713/ab.21.0113.

Filya, I., Ashbell, G., Hen, Y., & Weinberg, Z. G. (2000). The effect of bacterial inoculants on the fermentation and aerobic stability of whole crop wheat silage. *Animal Feed Science and Technology,* 88, 39-46. https://doi.org/10.1016/S0377-8401(00)00214-5.

Filya, I. (2000). Bazı silaj katkı maddelerinin ruminantların performansları üzerindeki etkileri. *Hayvansal Üretim Dergisi,* 41, 76-83.

Filya, I. (2001). *Silaj Teknolojisi*. Izmir, Turkiye: Hakan Ofset.

Filya, I. (2004). Nutritive value and aerobic stability of whole crop maize silage harvested at four stages of maturity. *Animal Feed Science and Technology,* 116, 141-150. https://doi.org/10.1016/j.anifeedsci.2004.06.003.

Gladine, C., Rock, E., Morand, C., Bauchart, D., & Durand, D. (2007). Bioavailability and antioxidant capacity of plant extracts rich in polyphenols, given as a single acute dose, in sheep made highly susceptible to lipoperoxidation. *British Journal Nutrition,* 98, 691-701. 10.1017/S0007114507742666.

Guven, I., & Kalamak, A. (2021). Gladiçya meyvesinin çayırotu silajında kullanımı. *Black Sea Journal of Engineering and Science,* 4(1), 22-28. https://doi.org/10.34248/bsengineering.836056.

Hodjatpanah-Montazeri, A., Danesh Mesgaran, M., Vakili, A., & Tahmasebi, A. M. (2016). Effect of essential oils of various plants as microbial modifier to alter corn silage fermentation and in vitro methane production. *Iranian Journal of Applied Animal Science,* 6(2), 269-276.

Kaya, Ö. (2005). *Tekirdağ İli Koşullarında Yetiştirilen Birinci ve İkinci Ürün Mısır Çeşitlerinin Silaj Kalitesi ve Aerobik Dayanıklılıkları Üzerine Etkileri*. (Master Thesis), Trakya University, Instutu of Science, Tekirdağ, Türkiye.

Kaya, I., Unal, Y., & Elmalı, D. A. (2009). Effects of different additives on the quality of grass silage and rumen degradability and rumen parameters of the grass silage in rams. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi,* 15(1), 19-24. 10.9775/kvfd.2008.44-A.

Kılıç, A. (1986). *Silo Yemi (Öğretim, Öğrenim ve Uygulama Önerileri).* Izmir, Türkiye: Bilgehan Bazımevi.

Kiraz, A. B., & Kutlu, H. R. (2016). Bakteriyel İnokulant Kullanımının Silajlarda Fermantasyon Özellikleri Üzerine Etkileri. *Harran Tarım ve Gıda Bilimleri Dergisi,* 20(3), 230-238. https://doi.org/10.29050/harranziraat.259118.

Kivanc, M., Akgul, A. & Dogan, A. (1991). Inhibitory and stimulatory effects of cumin, oregano and their essential oils on growth and acid production of *Lactobacillus plantarum* and *Leuconostoc mesenteroides. International Journal of Food Microbiology*, 13, 81–86. 10.1016/0168-1605(91)90140-k.

Kung, L. Jr., Williams, P., Schmidt, R. J., & Hu, W. (2008). A blend of essential plant oils used as an additive to alter silage fermentation or used as a feed additive for lactating dairy cows. *Journal of Dairy Science,* 91, 4793-4800. https://doi.org/10.3168/jds.2008-1402.

Leventini, M. W., Hunt, C., Roffler, R. E., & Casebolt, D. G. (1990). Effect of dietary level of barley-based supplements and ruminal buffer on digestion and growth by beef cattle. *Journal of Animal Science,* 68, 4334-44. 10.2527/1990.68124334x.

Makkar, H. P. S., Blummel, M., & Becker, K. (1995). Formation of complete between polyvinyl pyrolidones or polyethylene glycol and tannins and their implication in gas production and true digestibility in in vitro technique. *British Journal Nutrition,* 73, 897-913. https://doi.org/10.1079/BJN19950095.

Markham, P. (1942). A steam distilation apparatus suitable for micro-kjeldahl analyses. *Journal Biochemistry,* 36, 790-791. 10.1042/bj0360790.

Mcdonald, P., Henderson, A. R., & Heron, S. J. E. (1991). *The biochemistry of silage* (pp. 1-1340). Marlow, UK.

Mellencamp, M. A., Koppien, J., Lamb, R., & Dvorak, R. (2011). *Antibacterial and antioxidant activity of oregano essential oil*. Proceeding-Posters, USA.

Merry, R. J., Cussen-Mackenna, R. F., & Jones, R. F. (1993). Biological silage additives. *Ciencia e Investication Agraria,* 20, 372-401.

Muck, R. E. (2010). Silage microbiology and its control though additives. *Revista Brasileira de Zootecnia,* 39, 183-191. https://doi.org/10.1590/S1516-35982010001300021.

Orun, M., & Erdoğan, S. (2021). *Alternatif Kaba Yem Kaynağı Olarak Kullanılan Kerkol, Heliz ve Geven Bitkisi ile Meşe Yapraklarının İnvitro Gerçek Sindirilebilirlik ve Nispi Yem Değerlerinin Belirlenmesi*, (Master Thesis), Van Yuzuncu Yıl University, Institute of Science, Van, Türkiye.

Özkan, U., & Sahin Demirbag, N. (2016). Türkiye'de kaliteli kaba yem kaynaklarını mevcut durumu. *Türk Bilimsel Derlemeler Dergisi*, 9(1), 23-7.

Özkan, C. O. (2012). *Gladiçya (Gleditsia triacanthos) meyvesi, şeker pancarı posası silajında kullanımı*. (PhD), Kahramanmaras Sutçu Imam University, Institute of Science, Kahramanmaras, Türkiye.

Özturk, Y. E., Gulumser, E., Mut, H., Başaran, U., Copur Dogrusoz, M. (2020). Şerbetçi otunun mısır ve yemlik soya ile karışımlarının silaj kalitesinin belirlenmesi. *Harran Tarım ve Gıda Bilimleri Dergisi,* 24(4), 440-446. 10.29050/harranziraat.726427.

Redfearn, D., Zhang, H., & Caddel, J. (2006). *Forage quality interpretations*. http://pods.dasnr.okstate.edu/docushare/dsweb/Get/Document-2557/F-2117web.pdf. Acess date: 03. 03. 2022.

Rivera, D., & Parish, J. (2010). *Interpreting forage and feed analysis report.* 2620. Mississippi State University.

Rohweder, D.A., Barnes, R.F. & Jorgensen, N. (1978). Proposed hay grading standards based on laboratory analyses for evaluating quality. *Journal Animal Science, 47,* 747-759. https://doi.org/10.2527/jas1978.473747x.

SAS. (2014). SAS/STAT. Statistical Analysis System for Windows. Relase 9.4. SAS Institute Inc.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphotungustic acid reagents. *American Journal Enology Viticulture,* 16, 144-158.

Slottner, D., & Bertilsson, J. (2006). Effect of ensiling The technology on protein degradation during ensilage. *Animal Feed Science and Technology,* 27, 101-111. https://doi.org/10.1016/j.anifeedsci.2004.10.007.

Soycan-Onenc, S., Koc, F., Coskuntuna, L., Ozduven, M. L., & Gumuş, T. (2015). The effect of oregano and cinnamon essential oils on fermentation quality and aerobic stability of field pea silages. *Asian Australas Journal of Animal Science,* 28, 1281-1287. 10.5713/ajas.15.0122.

Soycan-Onenc, S., & Turan, A. (2018). Effect of cumin essential oil usage on fermentation quality, aerobic stability and in vitro digestibility of alfalfa silage. *Asian-Australas Journal of Animal Science*, 31(8), 1252-1258. 10.5713/ajas.17.0834.

Soycan-Onenc, S., & Turgud, F. K. (2019). Effect of oregano, umin and cinnamon essential oils on fermentation quality in alfalfa silages. *Ege Üniversitesi Ziraat Fakültesi Dergisi,* 56(3), 367-373. https://doi.org/10.20289/zfdergi.516234.

Üstü, Y., & Uğurlu, M. (2018). Kekiğin tıbbi kullanımı. *Ankara Medical Journal,* 2, 242-245. https://doi.org/10.17098/amj.435291.

Van Soest, P.J., Robertson, J.B. & Levis, B.A. (1991). Method for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal Dairy Science*, 74, 3583-3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2.