

## The effect of anise seed (*Pimpinella anisum* L.) on some quality parameters of alfalfa silages

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### Abstract

In current study, the effects of different doses of anise seed (*Pimpinella anisum* L.) on the chemical composition, fermentation quality and physical characteristics of alfalfa silages were investigated. Chemical composition, fermentation quality and physical characteristics of experiment silages were performed on the samples opened on the 75<sup>th</sup> day of ensiling. As a result, the differences in alfalfa silage in terms of dry matter, crude ash and ADF contents were not found statistically significant ( $P>0.05$ ). Silages treated with anise seed had higher NDF contents than control group and 0.5 % anise seed ( $P<0.05$ ). Crude protein, digestible dry matter, dry matter intake and relative feed value in control and 0.5 % anise seed treated silages had lower than other groups ( $P<0.05$ ). When alfalfa silage was evaluated in terms of its physical properties, no significant difference was observed in terms of smell and colour ( $P>0.05$ ), but its structures increased significantly in the 0.5%, 1.0 and 1.5 anise seeds compared to the control group ( $P<0.05$ ). At the end of the study, it was concluded that the quality of silage improved with the treatment of alfalfa silage with 0.5% anise seed.

**Key words:** Anise seed, alfalfa, fermentation, silage, volatile fatty acids

### Introduction

Silage additives should be used to increase the quality of silages, which are composed of some hard-to-ensiled forages, to ensure good fermentation and thus to increase the feed value. Due to the nutritional quality of alfalfa, it is used as an important legume forage plant for silage production worldwide. But due to the high buffering capacity of the alfalfa, low dry matter and low water-soluble carbohydrate content, it is a hard to ensiled. In recent years, there has been an increase in the use of medicinal and aromatic plants as silage additives in order to obtain good quality silage, improve silage quality and provide better protection of silage. Therefore, one of the alternative medicinal and aromatic plants is thought to be anise seed.

Anise, which has an important place among medicinal and aromatic plants, is a taxonomically one-year

aromatic plant belonging to the Apiaceae family. Anise is a plant with 30-50 cm high white flowers and small green yellow seeds. Anise is grown mainly for its berries (anise) (Madaus, 1979; Mirheydar, 2001; Rahmanoğlu, 2007). Its seeds contain 1-6 % essential oil. 75-80% of this oil is composed of trans-anetol, which gives this oil its unique scent (Ceylan, 1996; Kaya, 1990; Akgül and Ayar, 1993). In addition, it has been reported that it has antimicrobial, antifungal and antibacterial activity as well as degassing and appetizing properties (Shukla and Tripathi, 1987; Embong, et al., 1997; Rodrigues et al., 2003; Oray et al., 2008; Yazdani, et al., 2009).

In this study, it was aimed to investigate the effects of anise seed on alfalfa silage used as silage additive at different levels on the chemical composition, fermentation quality and physical properties of silages.

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## Material and Methods

### Ensiling and experimental design

A sixth regrowth of alfalfa was cut with a mower at early bloom stage. Alfalfa chopped with a chopper to a length of 2 -3 cm. The chopped alfalfa was ensiled in anaerob, 1-L, laboratory plastic jars. The anise seeds used as a supplement in the current study were obtained from a commercial enterprise. After removing stems and impurities inside, they were made ready to ensiling with alfalfa. The silage treatments were designed as follows (four replicates): untreated control, 0.5 % anise seed, 1.0 % anise seed, 1.5 % anise seed and 2.0 % anise seed. The silos were kept at 25°C and analysed after 75 days.

### Chemical composition

Dry matter, crude protein and crude ash of anise seed (Table 1) and silage samples were determined following the AOAC (1999).

**Table 1.** Dry matter, crude ash and crude protein contents of anise seed

	Dry matter, %	Crude protein, % DM	Crude Ash, % DM
Anise Seed	94.42	18.28	4.42

Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analysed according to methods described by Van Soest (1982). Ammonia nitrogen was

determined using the method of Broderick and Kang (1980) and pH was determined using the method of Polan et al., (1998). The lactic acid and volatile fatty acids (acetic acid, propionic acid and butyric acid) were analysed by HPLC (Canale et al., 1984). HPLC: Column: C18, 5 µm, 4.6 x 250-mm; Mobile Phase: Isocratic; 25-mM K-phosphate buffer; pH 2.4; Flow rate: 1.5 mL/min.; Column temperature: 30 °C; UV Sensor: Wavelength: 210 nm; Injection volume: 20 µL) Dry matter intake, digestible dry matter and relative feed value of the experiment silages were calculated by using the equations reported by Horrocks and Vallentine (1999).

### Physical properties

The physical properties of experiment silages were determined according to the scale specified by DLG (1987). Physical evaluation was assayed through the average of scores given by the six observers.

### Statistical analyses

The data were analysed for their statistical significance via a One-way ANOVA, with their significance reported at a 0.05 probability level (SPSS, 2007).

### Results and Discussion

The effects of anise seed on dry matter, crude ash, crude protein, neutral detergent fiber (NDF), acid detergent fiber contents (ADF) of alfalfa silage are presented in Table 2.

**Table 2.** The effects of anise seed on chemical composition of alfalfa silage

Parameters	Anise Seed Levels, %					P
	Control	0.5	1.0	1.5	2.0	
DM, %	24.11	25.11	24.11	24.22	24.53	0.38
CA, % DM	8.63	7.85	8.35	8.71	8.92	0.23
CP, % DM	28.30 <sup>a</sup>	28.20 <sup>a</sup>	25.56 <sup>b</sup>	26.00 <sup>b</sup>	24.91 <sup>b</sup>	0.02
NDF, % DM	24.03 <sup>b</sup>	25.25 <sup>b</sup>	27.83 <sup>a</sup>	27.68 <sup>a</sup>	28.15 <sup>a</sup>	0.01
ADF, % DM	18.85	19.46	20.88	20.56	20.67	0.32

<sup>a-b</sup>: The groups in the same line labelled different letters are statistically significant (P<0.01).

DM: Dry Matter; CA: Crude Ash; CP: Crude Protein; NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber.

It was concluded that the differences observed in terms of dry matter, crude ash and ADF contents of alfalfa silage with the supplementation of anise seeds were not statistically significant (P>0.05). ADF content increased numerically with anise seed treatment. However, NDF content increased significantly in groups containing 1.0, 1.5 and 2.0 % anise seeds (P<0.01). Also, with the addition of anise seed, the crude protein content of alfalfa silages decreased (P<0.05). This decrease can be said to be caused by the low protein content of anise seed. (Table 1). Şakalar and Kamalak (2016) indicated that the crude protein content of alfalfa silage added to dried molasses sugar beet pulp decreased. One of the main purposes of ensiling to reduce dry matter losses in the fresh material

to be ensiled as much as possible (Kızılımşek et al., 2016). In this study, it was aimed to stop the produce of undesired microorganisms due to the anise seed, which used antimicrobial properties. Considering the chemical composition of the experiment silages, it was observed that the anise seed did not have a negative effect on silage fermentation. Arslan Duru (2019a) found that lavender (flower + stem), which he treated due to its antimicrobial properties, had no negative effect on the chemical composition of alfalfa silages. Also, Aksu et al. (2017) reported that dry thyme pulp increased the NDF content of alfalfa silage.

The effects of anise seed on pH, ammonia nitrogen, lactic acid, acetic acid, propionic acid and butyric acid contents of alfalfa silage are shown in Table 3.

**Table 3.** The effects of anise seed on some fermentation characteristics of alfalfa silage

Parameters	Anise Seed Levels, %					P
	Control	0.5	1.0	1.5	2.0	
pH	4.71 <sup>a</sup>	4.31 <sup>b</sup>	4.39 <sup>b</sup>	4.44 <sup>b</sup>	4.46 <sup>b</sup>	0.03
NH <sub>3</sub> -N	4.80	3.46	4.72	4.20	4.91	0.36
Lactic Acid, %	2.26	2.18	2.62	2.59	2.84	0.67
Acetic Acid, %	0.35	0.33	0.44	0.37	0.40	0.60
Propionic Acid, %	0.14	0.10	0.14	0.14	0.09	0.74
Butyric Acid, %	0.17	0.10	0.06	0.03	0.04	0.46

<sup>a-b</sup>: The groups in the same line labelled different letters are statistically significant (P<0.05).

NH<sub>3</sub>-N: Ammonia nitrogen; LA: Lactic acid; AA: Acetic acid; PA: Propionic acid; BA: Butyric acid.

No statistically significant difference was observed in the alfalfa silage in terms of ammonia nitrogen, lactic acid, acetic acid, propionic acid and butyric acid contents by anise seed treatment (P>0.05). Lactic acid should be the primary acid in good quality silage. This acid is stronger than other silage organic acids (acetic, propionic and butyric) and is generally directly responsible for the decrease in silage pH. In silage treatments, the lactic acid content anise seed supplement decreased by 0.5% in the group, while in other treatment groups it increased numerically. The fact that the pH of silages with anise seed additives does not decrease below 4 explains this phenomenon. Another situation that can be evaluated positively in terms of silage fermentation quality is the decrease in the butyric acid content numerically with the addition of anise seeds. Also, ammonia nitrogen concentration in silages is an important criterion that shows the level of protein degradability by butyric acid bacteria during fermentation. It is reported that ammonia nitrogen should be lower than 80 g / kg total nitrogen in a quality silage (Pettersen, 1988). The fact

that butyric acid is very low in experiment silages and the ammonia nitrogen content is within the specified limits indicates that anise seed has a positive effect on alfalfa silage. Arslan Duru (2019b) concluded that the alfalfa ensiled with lavender (flower) does not change the lactic acid, acetic acid, propionic acid content and does not contain butyric acid content.

pH decreased with anise seed supplement compared to the control group (P<0.05). In particular, 0.5 % anise seed significantly reduced the pH of alfalfa silages compared to the control group. Acceleration of the decrease in the pH of the silage is necessary for the formation of a good quality fermentation. pH is 3.5-4.0 in a good quality silage, but pH values of 4.0 and above are very common in legume silages (Weissbach, 1996). As expected, the pH of alfalfa silage was observed to be 4.71 and approached 4.0 by anise seed treatment. It can be said that this phenomenon is caused by the antimicrobial effect of anise seed. There are similar studies indicating that application of some different additives reduced the pH content of alfalfa silage (Guo et al., 2008; Canbolat et al., 2013; Koç et al. 2020).

**Table 4.** The effects of anise seed on physical characteristics of alfalfa silage

Parameters	Anise Seed Levels, %					P
	Control	0.5	1.0	1.5	2.0	
Smell	8.50	11.75	13.00	12.08	11.17	0.08
Colour	1.83	2.00	2.00	2.00	2.00	0.08
Structure	2.83 <sup>b</sup>	3.92 <sup>a</sup>	4.00 <sup>a</sup>	3.75 <sup>a</sup>	3.25 <sup>ab</sup>	0.02
	Satisfactory	Excellent	Excellent	Excellent	Excellent	

<sup>a-b</sup>: The groups in the same line labelled different letters are statistically significant (P<0.05)

When silages were compared in terms of their physical properties, it was understood that there were improvements in structure with the addition of anise seed (P <0.05). While differences in smell and colour were not statistically significant (P> 0.05), numerical increases were observed with anise seed. Physical analysis, which is an inexpensive and simple way used to determine the quality of silages and to show that the

structural activities proceed smoothly, is an evaluation with the sense organs. According to the fresh material used in ensiling, different colour tones are seen from light green to black. Shades from dark green to black mean that there is protein and cellulose degradability in silage. In the silage quality determination using the silage assessment scale recommended by the DLG, it is described as "Excellent" between 16-20 points and

"Satisfactory" between 10-15 points. When the research silages were evaluated in terms of their physical properties (smell colour, structure), it was observed that silages did not have a negative feature, visual mould occurring and similar deterioration was almost not occurred, but it was determined that the

quality of silage increased with anise seed supplement. Similar to the current study, Turan and Soycan Öneç (2018) stated that the physical properties of alfalfa silage improved with the addition of cumin essential oil.

**Table 5.** The effects of anise seed on dry matter intake, digestible dry matter and relative feed value of alfalfa silage

Parameters	Anise Seed Levels, %					P
	Control	0.5	1.0	1.5	2.0	
DMI, %	4.99 <sup>a</sup>	4.76 <sup>a</sup>	4.32 <sup>b</sup>	4.35 <sup>b</sup>	4.27 <sup>b</sup>	0.01
DDM, %	74.86 <sup>a</sup>	74.35 <sup>a</sup>	72.64 <sup>b</sup>	72.89 <sup>b</sup>	72.80 <sup>b</sup>	0.04
RFV	289.73 <sup>a</sup>	273.98 <sup>a</sup>	242.91 <sup>b</sup>	245.54 <sup>b</sup>	240.72 <sup>b</sup>	0.01

<sup>a-b</sup>: The groups in the same line labelled different letters are statistically significant (P<0.01).

<sup>a-b</sup>: The groups in the same line labelled different letters are statistically significant (P<0.05).

DMI: Dry matter intake; DDM: Digestible Dry Matter; RFV: Relative Feed Value.

It was concluded that silages with dry matter intake, relative feed value (P<0.01), digestible dry matter (P<0.05) values, control and 0.5 % anise seed were higher than other groups. Dry matter intake, digestible dry matter and relative feed value decreased with increasing ADF and NDF content of silage treated with anise seed. Canbolat et al. (2010) reported that alfalfa silage varied between grape pulp treatment between 60.62-63.17% of dry matter, 2.27-2.56% of digestible dry matter and 106.91-125.62 of relative feed value. This difference between the findings may have resulted from the different harvest periods of alfalfa. Because the ADF and NDF values in the study were found to be quite higher than the current study.

### Conclusion

At the end of the research, it was observed that the fermentation quality of alfalfa silages increased with anise seed supplement. Considering its fermentation properties, the pH value of alfalfa silages decreased especially with the treatment of 0.5% anise seeds, and the ammonia nitrogen content decreased numerically. Its physical properties also supported that good fermentation occurred. When all data are reviewed, it is concluded that the addition of 0.5% anise seed will increase the fermentation quality of alfalfa silage and may have a positive effect on long-term preservation of these silages.

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### Conflict of Interest

The authors declared that there is no conflict of interest.

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