

Effects of Lavender (*Lavandula angustifolia*) augmentation of alfalfa silages

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Abstract

The aim of this study was to determine the chemical, fermentation, and microbiological properties of alfalfa silages that were augmented with lavender (*Lavandula angustifolia*) and to appraise their *in vitro* organic matter digestibility (IVOMD), metabolizable energy (ME), and net energy for lactation (NEL) contents. Lavender flowers were added to alfalfa silages at 0.5, 1.0, 1.5, and 2.0% of the weight of alfalfa. An unaugmented alfalfa silage was also evaluated. After 75 day of ensiling, pH, dry matter, NDF and ADF contents of alfalfa silages had decreased and OMD had increased with the addition of lavender. No significant differences in crude protein, ash, ether extract, lactic acid, acetic acid, propionic acid, lactic acid bacteria number, ME and NEL contents were detected. No butyric acid, *Enterobacteriaceae*, *Listeria* spp, sulphide reducing anaerobes, and yeasts were found in the alfalfa silages. Mould content decreased with the addition of lavender. Thus, the addition of lavender flowers to alfalfa silages may improve their quality.

Keywords: essential oil, fermentation, nutritional value, supplement

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Introduction

Lavender is an important medicinal plant grown in the Mediterranean region and an essential oil is obtained from its flowers (Guenther, 1952; Parejo *et al.*, 2002; Popescu *et al.*, 2015). Lavender, is tolerant of variation in soil types and grows best in calcareous soils. It is resistant to drought, heat and cold. Lavender can be propagated either vegetatively or from seed. The yield varies between 100 and 500 kg per acre depending on species, variety, climate and soil conditions (Aslançan & Sarıbaş, 2011).

Feed costs account for 65-70 % of total costs in ruminant livestock production. Alternative feed additives may reduce these costs and therefore improve economic production efficiency. Use of antibiotics used to in feeding animals that produce products for human consumption is prohibited in many countries due to concern over antibiotic resistance that could ultimately have negative affects on human health. A potential alternative to antibiotic use in animal feeding is the use of essential oils (Yadeghari *et al.*, 2013). Essential oils obtained from lavender reportedly reduce production of methane gas in the rumen (Djabri *et al.*, 2016). Thus, the aim of this study was to evaluate the use of lavender flower for feeding ruminant livestock through its incorporation into the ration by augmenting alfalfa silage.

Materials and Methods

Alfalfa was obtained from the growers operating in the region. Lavender was obtained from the Department of Crops Science of the Faculty of Agriculture and Natural Sciences of Uşak University. Lavender flowers were thoroughly mixed with alfalfa at 0.5, 1.0, 1.5, and 2.0% of the total mass and an unaugmented alfalfa silage was also evaluated. The fresh material were ensiled in 1 L plastic anaerob jars were kept at room temperature for 75 days. There were four replicates of each of the treatments. Essential oil components of the lavender were: 41.6% Linalol, 12.6% 3-Cyclohexen-1-ol Terpinene-4-ol, 10.5% Borneol, 10.0% Camphor, 2.3% Beta-Pinene, 2.2% 1-8 Cineole, 2.2% Lavandulol, 1.6% Malonic acid methyl ester 3,7 dimethyl 1-6 octadien 3 yl ester, and 1.4% Beta Farnesene.

After 75 days of ensiling, the jars were opened, and the contents were sampled. Determination of dry matter (DM), ash, fat and crude protein followed AOAC (1990) protocols. Briefly, the samples were weighed and then dried in an air-circulating oven at 65 °C for 48 hours after which time the samples were weighed

again to determine DM. The dried samples were ground to a diameter of 1 mm and ash content was determined by burning the finely ground sample in a furnace for 4-6 hours at 550 °C. Crude fat (CF) content was determined through ether extraction. Crude protein (CP) content was determined from nitrogen released by the Kjeldahl method multiplied by 6.25. Results of these analyses were expressed as a percentage of dry matter. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents of silage samples were determined using Fiber Analyzer (Ankom Technology Corp. Fairport, NY, USA) device following Van Soest *et al.* (1991). The pH was measured with a digital pH meter by thoroughly mixing 100 ml of distilled water and a 25 g sample of the silage and recording the pH of the slurry (Polan *et al.*, 1998). In the same way, 40 g of silage samples were mixed with 360 ml of distilled water, the mixture shaken for a minimum of 5 minutes, after which time it was poured through Whatman no: 1 paper, and 100 ml of the filtered solution was to determine ammoniacal nitrogen (NH₃-N) using the Kjeldahl distillation method (Broderick and Kang, 1980). An additional 10 ml of this filtered solution centrifuged for 30 minutes at 14,000 revolutions per minute and acetic acid (AA), propionic acid (PA) and butyric acid (BA) and lactic acid concentrations were determined using high performance liquid chromatography (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).

Sulfide reducing anaerobes in the silages were determined according to the method of Stanley *et al.* (1971). The numbers of lactic acid bacteria, Enterobacteriaceae, Listeria spp., yeast and moulds were determined following Harrigan (1998).

To determine IVOMD and ME levels of silages, the *in vitro* gas production technique proposed by Menke and Steingass (1988) was used. Net energy for lactation was calculated using equations found in Blümmel and Ørskov (1993).

The data analyzed with SPSS version 16.0 (SPSS, Inc. Chicago, Illinois, USA). The significance of treatment effects was determined by one-way analysis of variance and mean separation was done with Duncan's multiple range procedure. Analyses of the lactic acid bacteria and moulds were conducted with the Frequency procedure.

Results and Discussion

The effect of augmenting alfalfa silage with lavender on the proximate analysis of the silages is shown Table 1. The dry matter content of alfalfa silages was increased with the addition of lavender ($P < 0.01$). No significant differences in crude ash, crude protein and ether extract contents were detected among treatments ($P > 0.05$). Nadeau *et al.* (2000a) observed similar results for alfalfa silages treated with cellulase, inoculant, and formic acid. Similar results were also obtained for alfalfa silage augmented with pre-fermented juices of barley, wheat and grass (Denek *et al.*, 2012). Similarly, relative to the control alfalfa silage, the NDF and ADF contents of the silages decreased with the addition of lavender. However, the amount of lavender augmentation had no effect on either NDF or ADF content of the silages. The observed reductions in fibre levels in the augmented silages are also supported by numerous reports in the literature (Goering *et al.*, 1991; Sheperd *et al.*, 1995; Nadeau *et al.*, 1996; Nadeau *et al.*, 2000b; Kung *et al.*, 2003; Ce *et al.*, 2016).

Table 1 Nutrition composition of alfalfa silages augmented with lavender

Treatment	DM, %	Ash, % DM	NDF, % DM	ADF, % DM	CP, % DM	EE, % DM
Control	24.81 ± 0.98 ^c	13.49 ± 0.77	37.13 ± 1.56 ^a	28.47 ± 0.66 ^a	18.83 ± 0.91	3.08 ± 0.96
0.5 %	26.20 ± 0.24 ^b	13.80 ± 0.84	33.25 ± 0.89 ^b	26.54 ± 0.59 ^b	18.98 ± 0.86	2.90 ± 0.59
1.0 %	26.70 ± 0.24 ^{ab}	13.98 ± 0.31	33.12 ± 1.70 ^b	26.16 ± 1.05 ^b	19.03 ± 0.70	3.03 ± 0.71
1.5 %	27.34 ± 0.79 ^a	13.92 ± 0.61	33.38 ± 1.20 ^b	26.13 ± 1.22 ^b	18.77 ± 0.52	2.97 ± 0.47
2.0 %	27.45 ± 0.63 ^a	13.86 ± 0.28	33.75 ± 2.20 ^b	26.53 ± 1.26 ^b	18.16 ± 0.67	3.07 ± 0.22

^{a,b,c} Within a column, means sharing a common superscript are not different ($P < 0.05$)

DM: Dry matter; NDF: Neutral detergent fibre; ADF: Acid detergent fibre; CP: Crude protein; EE: Ether extract

Lavender significantly ($P < 0.01$) reduced the pH values of alfalfa silages, especially when augmented with 1.5 % and 2.0 % lavender flowers (Table 2). However, no significant difference was detected in the content of lactic, acetic acid and propionic acid of silages as a result of addition of lavender to alfalfa silages ($P > 0.05$). The quantity of lactic acid bacteria in the alfalfa silages in this study was found to be less than

reported by Filya *et al.* (2001), Ke *et al.* (2015), and Wen *et al.* (2017) but similar to the report of Canbolat *et al.* (2013). The reason for using essential oils to augment alfalfa is to prevent the development of harmful microorganisms in the silage and improve its fermentation characteristics. In the present study, the concentration (cfu/kg) of desirable lactic acid bacteria was increased by augmentation with lavender (control = 4.39 ± 0.20 ; augmented silages = 4.68 ± 0.05). The presence of Enterobacteriaceae, *Listeria* spp, sulphite reducing anaerobes, and yeast were not detected and moulds were only found in a fraction of the samples. The lack of these undesirable microorganisms indicates proper fermentation with a rapid reduction in pH after ensiling. Tengerdy *et al.* (1991), Çiftçi *et al.* (2005), Zhang *et al.* (2009), and Canbolat *et al.* (2011) have reported similar results in alfalfa silages that were augmented with either lactic acid bacteria or additional carbohydrate-containing juices.

Table 2 pH and organic acid contents of alfalfa silages augmented with lavender flowers

Treatment	pH	Lactic acid, %	Acetic acid, %	Propionic acid, %	Butyric acid, %
Control	5.09 ± 0.04^a	2.50 ± 0.07	0.74 ± 0.07	0.16 ± 0.01	0.00
0.5 %	4.98 ± 0.03^{bc}	2.09 ± 1.56	0.93 ± 0.08	0.23 ± 0.05	0.00
1.0 %	5.01 ± 0.03^b	2.88 ± 1.42	0.80 ± 0.06	0.13 ± 0.02	0.00
1.5 %	4.92 ± 0.06^c	2.26 ± 0.50	1.10 ± 0.45	0.16 ± 0.07	0.00
2.0 %	4.92 ± 0.08^c	2.41 ± 0.53	0.66 ± 0.17	0.18 ± 0.09	0.00

^{a,b,c} Within a column, means sharing a common superscript are not different ($P < 0.05$)

As a result of the addition of lavender to alfalfa silages, IVOMD increased significantly ($P < 0.05$; Table 3). Non-significant numerical increases in ME and NEL were also observed ($P > 0.05$). The reduction of the plant cell wall components, resulting from the augmentation of the alfalfa with lavender and manifest as NDF and ADF may have been responsible for the increased IVOMD. These findings for IVOMD are similar to those reported by Contreras-Govea *et al.* (2011), Canbolat *et al.* (2013), and Koc *et al.* (2008) for augmented alfalfa silages.

Table 3 Nutritional value of alfalfa silages augmented with lavender flowers as predicted from *in vitro* gas production

Treatment	IVOMD, %	ME, MJ/kg DM	NEL, MJ/kg DM
Control	55.35 ± 0.91^b	7.47 ± 1.12	4.44 ± 0.82
0.5 %	67.97 ± 3.95^a	8.21 ± 0.97	4.97 ± 0.72
1.0 %	66.94 ± 5.68^a	8.54 ± 0.90	5.24 ± 0.73
1.5 %	66.76 ± 1.79^a	8.51 ± 0.22	5.21 ± 0.14
2.0 %	69.14 ± 2.71^a	8.88 ± 0.43	5.50 ± 0.34

^{a,b} Within a column, means sharing a common superscript are not different ($P < 0.05$)

IVOMD: *in vitro* organic matter digestibility, ME: Metabolizable energy, NEL: Net energy for lactation

Alfalfa is a difficult crop to ensile because conditions required for the fermentation required to produce good quality silage may not be obtained (Bolsen *et al.*, 1996). Improper fermentation can result in proliferation of undesirable microorganisms and loss of DM. What determines the quality of silages is pH and the amount of lactic acid formed during fermentation. Butyric acid bacteria are the most important competitor of acetic acid bacteria during the fermentation of silages. Formation of butyric acid in silage can result in a marked loss of nutrients. Therefore, butyric acid is not desired in silages. Hashemzadeh-Cigari *et al.* (2014) stated that lactic acid content did not change with the addition of additives to alfalfa silages. Tabacco *et al.* (2006) reported that no butyric acid content occurs as a result of adding additive to alfalfa silages.

Conclusion

The addition of lavender flower at different levels to alfalfa silage improved its fermentation properties without decreasing its nutritional value. Thus, better silages were obtained and it may be concluded that lavender has potential to be used as a preservative in alfalfa silages.

Conflict of Interest Declaration

The author has no conflict of interest that would compromise the conduct of this work or interpretation of the results.

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