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The effect of lemon seed essential oil on composition, chemical characteristics, and gas production parameters of alfalfa silage

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Abstract

Introduction: Alfalfa is one of the most important forage plants, which is used in livestock feeding as hay in Iran. However, significant amounts of alfalfa nutrients are lost due to mechanical treatments during drying and storage process. The production of alfalfa silage in recent years has attracted more attention for feeding ruminants. Alfalfa is one of the forages that is difficult to ensiling, because of its high protein level and buffering capacity and low water soluble carbohydrate content, which require silage additives during ensilage. Additives with protective properties (based on organic acids) have been used to prevent mold and yeast growth in silage and increase aerobic stability. Recently, the use of aromatic plants and their products has increased due to their antifungal, antibacterial, and antioxidant effects. **Aim:** This experiment was performed to investigate the effects of lemon seed essential oil (LSEO) on chemical properties and aerobic stability of alfalfa silage. **Materials and Methods:** Alfalfa were harvested at flowering stage and chopped with a forage harvester to a theoretical length of 3 to 5 cm and wilted in the laboratory at 24 h. After the wilting period, a large sample of harvested alfalfa was randomly divided into three subsamples. Experimental treatments were: alfalfa without additive (control), alfalfa with 60 mg LSEO/kg (LSEO60), and alfalfa with 120 mg LSEO/kg (LSEO120). At the end of 60 days ensiling, silos were opened and immediately pH and dry matter of samples were measured and the remaining samples were stored for measuring Ash, ADF, NDF, CP and in vitro gas tests at -20^{o} C. After opening the silos, gas production was measured by *in vitro* method with five replications at 2, 4, 6, 8, 12, 16, 24, 36, 48, 72, 96, and 120 hours. The data were analyzed based on a completely randomized design. **Results:** Results showed that adding the essential oil (at both levels) to alfalfa silage reduced the pH of the silage ($p<0.05$). Addition of LSEO to alfalfa silage (120 mg/kg) increased water soluble carbohydrate concentration (4.91%; $p<0.05$). Crude protein content was increased in treatments supplemented with LSEO120 (12.95%) compared with control ($P<0.05$). Aerobic stability was increased in LSEO treated groups compared with control($P<0.05$). Adding 60 mg LSEO/kg alfalfa reduced the gas production volume compared with control ($P<0.05$). Overall, the obtained data indicate a positive effect of LSEO on the quality of alfalfa silage and its fermentation properties. **Conclusion:** The results of this study showed that the addition of lemon seed essential oil to alfalfa silage increased aerobic stability of silage compared

with control. Lemon seed essential oil had a significant effect on ammonia nitrogen and crude protein. Different levels of lemon essential oil decreased the pH significantly. Addition of lemon seed essential oil at high level of 120 mg/kg increased the amount of gas production and fermentation parameters of gas production.

Keywords: Aerobic stability, Alfalfa silage, *In vitro* gas production, Lemon seed essential oil.

Introduction

Alfalfa is one of the most important forage plants, which is used in livestock feeding as hay in Iran. However, significant amounts of alfalfa nutrients are lost due to drying and storage process. The production of alfalfa silage in recent years has attracted more attention for the ruminant breeders (Chaves et al. 2012). Alfalfa is one of the forages that is difficult to ensiling because of its high protein level, buffering capacity, and low water soluble carbohydrate content; thus, silage additives must be used during ensiling. Exposure of silage to air at the time of feeding causes corrosion of the silage. Yeasts, being able to metabolize lactic acid, are the first cause of corruption, which cause a rise in pH. This rise in pH is also a stimulant for the growth of other harmful microorganisms in the silage, resulting a reduction in livestock production due to food depreciation or poisoning (Henderson 1993). Additives with protective properties (based on organic acids) have been used to prevent mold and yeast growth in silage, as well as increase aerobic stability (Henderson 1993). Recently, the use of aromatic plants and their products has been increased due to their antifungal, antibacterial, and antioxidant effects. The essential oil plants have an important usage among the medicinal plants. Because of antimicrobial effects, obtained essential oils from aromatic plants were shown to be useful in animal nutrition (Soycan-Önenç et al. 2017).

Regarding to the dry climate and economic situation in Iran, the use of by-products is important in ruminant nutrition (Teles et al. 1972). Methods have been devised for the separation and drying of the kernels and commercial production of citrus kernel oil (Eckey 1954). The composition of citrus kernel oil has been studied in other countries (Satlar 1987), and suitable methods have been proposed for drying citrus kernels for refining and bleaching these oils (Helmy 1990). Sour lemon (*Citrus Limon*) belongs to the citrus family (*Rutaceae*) and is an evergreen tree with up to 6 m height. In a study by Li et al. (2006), it was revealed that flavonoids in citrus skin have anti-cancer, antiviral, and anti-inflammatory activities (Poulose et al. 2006).

Sour lemons have antimicrobial activity due to the presence of biologically active substances such as flavonoids, terpenes, coumarines, and limonine. Due to the lack of sufficient knowledge of nutritional value, limitations, and proper use of citrus waste (especially sour lemon kernel essential oils), as well as the lack of appropriate research in this field in Iran, it seems necessary to conduct this study. Accordingly, this experiment was performed to investigate the effects of lemon seed essential oil on chemical properties, aerobic stability, and in vitro nutritional value of alfalfa silage.

Materials and methods

The collected lemon pomace from Tabriz markets were cut and the seeds were separated, washed and dried at room temperature for a few days. About 200 g of milled lemon seed were immersed by maceration using N-hexane solvent (Sayyah et al. 2005).

Alfalfa were harvested at flowering stage and chopped with a forage harvester to a theoretical length of 3 to 5 cm and wilted in the laboratory for 24 h. After the wilting period, a large sample of harvested alfalfa was randomly divided into three subsamples. Treatments included alfalfa without additives (control), alfalfa + 60 mg lemon seed essential oil per kg (LSEO60), alfalfa + 120 mg lemon seed essential oil per kg (LSEO120). Silo contents were packed immediately after being sprayed with ethanol solution to prevent evaporation. The mini-silos (10 cm diameter and 70 cm

height) were then sealed and stored at room temperature for 60 days. There were three replicate per treatment. At the end of 60 days ensiling, silos were opened and pH and the dry matter contents were immediately measured and the remaining samples were stored at -20^{o} C before measuring ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), CP, and *in vitro* gas tests (Van Soest et al. 1991; AOAC 2000). Water-soluble carbohydrates were determined according to Dubois et al. (1956). Concentration of VFA were measured according to the method of Markham (1942). The samples for *in vitro* gas production were ground though a 1 mm screen. Chemical composition of alfalfa before ensiling are shown in Table 1.

The aerobic stability was measured using the method introduced by Adesogan et al. (2004). Based on this method, 200 g of fresh silage were poured into containers, a thermometer was placed in the center of each silage mass, and two thermometers were placed in two different points of the room (ambient temperature at 12° C). Silage and ambient temperature were recorded manually every two hours until heating occurred. When the silage mass temperature reached 2° C above the ambient temperature, silage was considered corrupted. The data were analyzed based on a completely randomized design. For the measurement of lactic acid, Borshchevskaya et al. (2016) method was applied using iron chloride solution.

***In vitro* gas production trial:**

Ruminal fluid was collected approximately 2 h after morning feeding from two cannulated sheep consuming 400 g alfalfa hay, 300 g barley, and 300 g soybean meal. Ruminal fluid was immediately squeezed through four layers of cheesecloth and transported to the laboratory in a sealed thermos. The ruminal fluid was then purged with deoxygenated CO_2 before use as the inoculum. Gas production parameters was measured by the method suggested by Fedorak and Hrudy (1983). Approximately 300 mg of dried and ground (2 mm) samples of the treatments were weighed and placed into serum bottles. Buffered rumen fluid with McDougal (1948) buffer (20 mL) was pipetted into each serum bottle. The gas production was recorded after 2, 4, 6, 8, 12, 16, 24, 36, and 48 h of incubation. Total gas volume was corrected for the blank incubation and reported as gas volume (mL g^{-1} of DM). The gas production profiles were measured in triplicate using the equation of $Y=A(1-e^{-ct})$, in which Y is the volume of gas production (mL g^{-1} DM) at time t, A is gas production from soluble and insoluble fraction, c is the gas production rate, and t is the incubation time (h). The metabolizable energy (ME) contents of gas production (GP) and organic matter digestibility (OMD) were calculated using equations of Menke et al. (1979) as:

$ME\ (MJ/kg\ DM) = 2.20 + 0.136 \times GP + 0.057 \times CP + 0.0029 \times CP^2$

$OMD\ (g/100g\ DM) = 14.88 + 0.889 \times GP + 0.45 \times CP + 0.0651 \times XA$

$NE_L\ (MJ/kg\ DM) = 0.54 + 0.096 \times GP + 0.0038 \times CP + 0.000173 \times CF^2$

where, XA is ash (g/100 g DM) and GP is the net gas production (mL) at 24 h, CP is crude protein, CF is crude fat. The prediction of short chain fatty acid production was calculated using below equation as:

$SCFA\ (mmol) = -0.00425 + 0.0222 \times GP$

where, GP: gas production at 24 h (ml/0.2 DM).

Statistical analysis: Data obtained from gas production studies were subjected to analysis of variance as a completely randomized design by the GLM procedure of SAS (2002) and treatment means were compared by the Duncan multiple range test ($P<0.05$).

Table1- Chemical composition (mean ± SD) of wilted alfalfa before ensiling (%DM)

Item	Chemical composition [1]						
	DM	pH	CP	ASH	WSC	NDF	ADF
Alfalfa	22.2±0.975	6.14±0.011	19.6±0.427	11.6±0.028	3.74±0.087	24.8±1.058	17±1.40

[1] DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; WCS: water soluble carbohydrate.

Results and discussion

The effects of experimental treatments on chemical properties of alfalfa silage are presented in Table 2. Addition of lemon seed essential oil with two levels of 60 and 120 mg/kg alfalfa silage decreased the silage pH compared with the control ($P<0.05$). The decrease in the pH of silage samples containing the essential oils probably is due to an increase in the amount of lactic acid produced and also total VFA in the silage. Lactic acid is the most important organic acid produced by fermentation in the silage and is responsible for increasing the acidity of the silage (Cheng et al. 2001). Some *in vitro* experiments have reported the effect of essential oils on the growth and development of acetic acid bacteria (Basirati et al. 2015). The effect of ensiling on forage carbohydrate concentration is complex, and the concentration of NDF is altered by hydrolysis, in addition to other effective factors such as plant respiration, effluent, and fermentation (Jokela and Russelle 2003).

The amount of volatile fatty acids in this experiment increased with the addition of essential oil, so that the highest amount of VFA was at the levels of 60 and 120, respectively, which was significantly different from control ($P<0.05$, Table 2). The amount of VFA is an indicator to determine the quality of silage based on the concentration of its fermentation products. This indicator actually indicates the positive effects of lactic acid and acetic acid on the negative effect of butyric acid in silage.

Determination or evaluation of DM content showed that the addition of essential oils to alfalfa silage had an effect on DM content, in which treatment inoculated with 120 lemon seed essential oil had higher DM compared with control. An increase in the DM content of silage containing lemon seed essential oil may be due to restrict of growth and development of a particular group of microorganisms in the silage and thus, the fewer loss of silage nutrients (Selwet 2009). There is an increasing tendency for crude protein as compared with control. The highest increase was related to level of 120 and then level of 60, which was significantly different from control ($P<0.05$).

An increase in crude protein content of the silage supplemented with lemon seed essential oil was in contrast with the results of Chaves et al. (2012), in which no change was observed in crude protein content of silage with orange essential oil. This difference is probably due to differences in the essential oil concentration and type of used silage (Hart et al. 2008).

Lemon seed essential oil at 60 and 120 mg concentrations had no significant effect on ammonia nitrogen compared with control treatment (Table 2). At the level of 60 mg/kg a numerical increase was observed in ammonia nitrogen of alfalfa silage. On the other hand, Foskolos et al. (2010) reported a decrease in the ammonia nitrogen concentration of grass silage containing high levels of thyme essential oil. Busquet et al. (2006) reported a reduction in the concentration of ammonia nitrogen under *in vitro* conditions with using oregano essential oil (3 g/l in the culture medium) or its main active ingredient (carvacol).In a study by Ando et al. (2003), the addition of 1 g of essential oils to dairy cows prevented the amino acid deamination. Newbold et al. (2004) reported similar results with the addition of 110 mg of plant essential oil mixture in sheep diet. Essential oils and monensin reduced rumen ammonia nitrogen concentrations with inhibitory effects on proteolysis and deamination (Nagaraja et al. 1997).

Table 2- Effect of lemon essential oil on chemical properties of alfalfa silage after 60 d of ensiling (%DM)

Treatments[1]	Chemical composition[2]										
	DM	NDF	ADF	WSC	tVFA	NH_3-N	CA	CP	LA	pH	EE
Control	24.44^{b}	49.07	22.67^{a}	4.08^{b}	12.63^{b}	84.93	11.40^{b}	11.62^{c}	69.38^{c}	4.66^{a}	4.26^{ab}
LSEO60	24.24^{b}	48.03	19.33^{b}	2.63^{c}	14.89^{a}	85.16	12.31^{a}	12.37^{b}	76.22^{b}	3.61^{b}	4.73^{a}
LSEO120	25.82^{a}	46.84	22.33^{a}	4.91^{a}	14.74^{a}	84.93	11.78^{a}	12.95^{a}	79.41^{a}	3.79^{b}	4.03^{b}
SEM	0.215	1.575	0.666	0.081	0.046	0.602	0.072	0.041	0.259	0.060	0.137
p-value	0.004	0.629	0.011	<.0001	<.0001	0.135	0.0003	<.0001	<.0001	<.0001	0.029

[1] Treatment are as follows: control: alfalfa silage without additives, LSEO60: alfalfa silage with 60 mg lemon seed essential oil/kg, LSEO120: alfalfa silage with 120 mg lemon seed essential oil/kg.

[2] Chemical composition: DM, dry matter; CP, crude protein; EE, ether extract; CA, crude ash; NDF, neutral detergent fiber; ADF, acid detergent fiber; NH3-N: ammonium nitrogen (% of total nitrogen), tVFA: total volatile fatty acid (mm), LA: lactic acid. WSC: water soluble carbohydrate.

Means within the same column with different superscripts differ significantly (P<0.05).

Borchers (1965) showed that the addition of thymol to rumen fluid resulted in the accumulation of amino acids and a decrease in ammonia nitrogen concentrations. He suggested that the thymol prevents the degradation of amino acids by rumen bacteria. Cardozo et al. (2004) in an experiment using a continuous culture system investigated the effect of some natural plant extracts on protein breakdown and ruminal fermentation *in vitro*. The researchers reported that garlic oil reduces the concentration of nitrogen-ammonia and increases the amount of amino-acid and peptide proteins (Cardozo et al. 2004). This result indicates the inhibitory effect of garlic oil on deamination (Cardozo et al. 2004). Also, in another experiment by Ferme et al. (2004), garlic oil caused a change in microbial population in continuous culture medium so that the amount of protella species was decreased in relation to the whole microbial population, in which these species are mainly responsible for protein breakdown and deamination of amino acids. Reduction in the population of protella bacteria that are responsible for protein degradation and amino acid deamination is one of the mechanisms of action of garlic oil on protein metabolism (Forme et al. 2004). However, the results of this experiment was not in line with the results of Chaves et al. (2012), who reported that the addition of oregano, cinnamon, and orange essential oils at values of 37.5, 75 and 120 mg/kg DM of barley silage had no significant effect on ammonia nitrogen in barley silage. However, oregano essential oil in the amount of 75 mg/kg DM of barley silage reduced the amount of ammonia nitrogen. The concentration of the essential oil used in the experiment is an important factor. The results of studies by Busquet et al. (2006) showed that when essential oils used in high concentrations (300 to 3000 mg/L), essential oils were more effective on ruminal fermentation than those used at low concentrations (0.22 to 2.2 mg/l of culture medium).

The effect of adding lemon seed essential oil on gas production is presented in Table 3. Treatment with 120 mg lemon seed essential oil from 2 h to the end of incubation had the highest volume of gas production compared with the other treatments (P<0.05). Treatment with 60 mg from 2 h to the end of incubation showed less gas production than control. At the end of incubation, the highest volume of produced gas was in the treatment with 120 mg lemon seed essential oil (147.14 ml/g DM) and the lowest amount was in the treatment of lemon seed essential oil with 60 mg (124.97 ml/g DM). These results indicate that the addition of 60 mg essential oil had a significant effect on the rate of gas production by its inhibitory effects. The results of this experiment were in agreement with the findings of Hodjatpanah et al. (2016) and Chaves et al. (2012).

Ghorbani and Vakili (2014) showed that peppermint and fennel essential oils at 150 mg level reduced the amount of produced gas from the fermentable fraction of corn silage

compared with the control group. The effects of using thyme essential oils in alfalfa silage of ruminants by in vitro method showed that thyme essential oil reduced the amount of gas production compared with non-supplemented group (Amini Pour et al. 2017). Using cinnamon essential oil reduced the amount of gas produced in 24 h of incubation (Fraser et al. 2007). In another study, it was shown that the use of garlic essential oil for 17 h of incubation reduced the amount of gas production and decreased *in vitro* gas production by increasing the amount of essential oil (Busquet et al. 2005).

The effect of different levels of lemon seed essential oil on the estimation of gas production parameters is shown in Table 4. The gas production potential (b) was significantly different between the experimental treatments. The highest average gas production was obtained from the fermentable fraction in the 120 mg treatment and the lowest amount in the 60 mg treatment compared with the control (Table 3). The treatment LSEO120 increased gas production from the early hours of incubation compared with the control group and continued until the end. The organic matter digestibility (OM) digestibility, metabolizable energy, ammonia nitrogen, VFA, short-chain fatty acids, and digestible organic matter in dry matter are shown in Table 4. Addition of essential oils had a significant effect on ammonia nitrogen and total volatile fatty acids (P<0.0001). The highest OM digestibility and metabolizable energy was observed in LSEO120 and control, but the lowest amounts in LSEO60 treatment as compared with control. In a study of Patra and Yu (2006), fennel and clove extracts (*extracted using ethanol or methanol*) reduced the amount of produced gas in the *in vitro* environment, while garlic extracts (extracted using water) had an increasing effect on gas production (Patra and Yu 2006). Ginger extract also had no effect on gas production (Patra and Yu 2006). It has been reported that there is a strong correlation between the amounts of estimated metabolizable energy, the amount of *in vitro* gas production in 24 h incubation time, and the chemical composition of the feeds (Menke and Steingass 1988).

In general, sour lemon seed essential oil had the greater ability in GP, ME, SCFA, DOMD and OM digestibility at 120 mg level than control group. It seems that lemon essential oil has been able to protect nutrients well during the silage processing. Busquet et al. (2005) reported that the amount of gas produced can be affected by the total values and the pattern of VFA. The rate of gas production can indicate the rate of digestion in the rumen followed by the passage rate and dry matter intake (DMI).

Table 3- The effect of different levels of lemon seed essential oil on gas production (ml/g DM)

Trt[1]	Incubation times (h)											
	2	4	6	8	12	16	24	36	48	72	96	120
Control	17.61^{b}	32.39^{b}	40.06^{b}	51.72^{b}	65.17^{b}	82.27^{b}	103.98^{b}	111.98^{b}	123.10^{b}	130.90^{b}	134.42^{b}	136.52^{b}
LSEO60	13.59^{c}	27.03^{b}	37.50^{b}	50.49^{b}	60.74^{b}	73.12^{c}	89.15^{c}	102.21^{c}	112.15^{c}	118.94^{c}	122.86^{c}	124.97^{c}
LSEO120	22.64^{a}	40.49^{a}	50.54^{a}	65.01^{a}	78.85^{a}	94.95^{a}	113.39^{a}	125.32^{a}	136.06^{a}	142.44^{a}	146.24^{a}	147.01^{a}
SEM	1.064	1.995	1.656	1.891	2.125	2.043	2.478	2.642	3.227	3.097	3.061	3.165

[1]Trt-control: Alfalfa silage without additives, LSEO60: alfalfa silage with 60 mg lemon seed essential oil/kg, LSEO120: alfalfa silage with 120 mg lemon seed essential oil/kg.

Means within the same column with different superscripts differ significantly (P<0.05).

Table 4- The effect of different levels of lemon seed essential oil on estimated parameters from gas production

Treatments[1]	Items[2]									
	pH	NE_L	SCFA	ME	OMD	DOMD	tVFA	NH_3-N	b	C
Control	6.60	1.27^{a}	0.152^{a}	3.22^{a}	27.23^{ab}	24.01	8.62^{a}	47.18^{c}	133.36^{b}	0.059^{b}
LSEO60	6.50	1.11^{b}	0.114^{b}	3.00^{b}	26.05^{b}	23.01	3.26^{b}	51.10^{b}	121.56^{c}	0.058^{b}
LSEO120	6.60	1.23^{ab}	0.143^{ab}	3.18^{ab}	27.45^{a}	24.17	7.20^{a}	56.80^{a}	143.44^{a}	0.069^{a}
SEM	0.041	0.042	0.009	0.060	0.396	0.366	0.426	0.400	3.134	0.00161
p-value	0.215	0.045	0.045	0.046	0.060	0.089	<.0001	<.0001	0.0013	0.0006

[1]Treatment-control: Alfalfa silage without additives, LSEO60: alfalfa silage with 60 mg lemon seed essential oil/kg, LSEO120: alfalfa silage with 120 mg lemon seed essential oil/kg.

[2] ME: metabolizable energy (MJ/Kg DM); SCFA: short chain fatty acid (mmol/0.2 g DM); DOMD: digestible organic matter in dry matter (%); NE_L: net energy lactation (MJ/Kg DM); tVFA: total volatile fatty acids (mmol/l); NH_3-N: ammonium nitrogen (mg/l); OMD: organic matter digestibility (%); b: Potential gas production (mL/g DM); c: Rate constant of gas production during incubation (mL/h)

Means within the same column with different superscripts differ significantly (P<0.05).

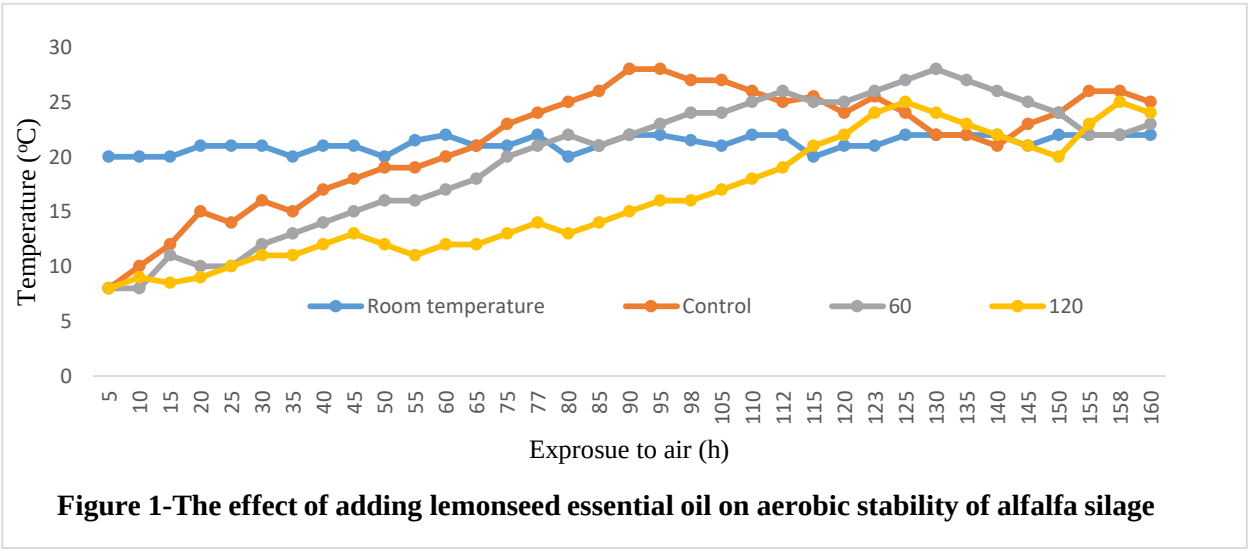

Figure 1-The effect of adding lemonseed essential oil on aerobic stability of alfalfa silage

Lemon seed essential oil effects on aerobic stability of alfalfa silages are presented in Figure 1. Addition of lemon seed essential oil to alfalfa silage increased aerobic stability compared with control and other treatments. Exposure to air in silos may result in silage corruption. The increase in temperature is the result of the metabolism of organic acids and nutrients left over by aerobic microorganisms. Changes in temperature can be an indicator of the development of aerobic corruption of silages. In a study of Chaves et al. (2012), the silages supplemented with 120 mg pineapple or thyme essential oil/kg DM remained stable to air for two weeks. Some secondary plant metabolites have been shown to inhibit the growth of some yeast species associated with aerobic degradation (Navarro-Villa et al. 2013). In a study, addition of three different herbal essential oils (cinnamon leaf, oregano, and sweet orange at 120 mg/kg DM) to barley silage increased aerobic stability compared with the control group (Chaves et al. 2012). In the experiment of Hodjatpanah et al. (2016), by adding different herbal essential oils (oregano, thyme, cumin, and cinnamon) to corn silage, they observed an increase in aerobic stability of silages. The increase in aerobic stability in silages containing plant essential oils was probably due to their inhibitory effects on the growth and activities of species from yeast that initiate spoilage in

the silage. Kung et al. (2000) reported that propionic acid, subic acid, benzonic acid, acetic acid, and ammonia are among the substances that increase the stability of silage against air.

Conclusion

The results of this study showed that addition of lemon seed essential oil to alfalfa silage increased aerobic stability of silage compared with control. Lemon seed essential oil had a significant effect on ammonia nitrogen and crude protein. Adding different levels of lemon seed essential oil was associated with reducing in pH as compared with control. Addition of sour lemon seed essential oil at high level increased the amount of gas production and fermentation parameters of gas production.

Conflict of Interest Declaration

The authors have not any conflict of interest.

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تاثیر اسانس هسته لیمو روی ترکیبات، خصوصیات شیمیایی و فراسنجه‌های تولید گاز سیلاژ یونجه

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چکیده

زمینه مطالعاتی: یونجه یکی از مهمترین گیاهان علوفه‌ای است که در تغذیه دام به شکل علوفه خشک در ایران استفاده می‌شود. با این حال، مقدار قابل توجهی از مواد مغذی یونجه به دلیل تیمارهای مکانیکی در طی فرآیند خشک کردن و ذخیره‌سازی از بین می‌روند. تولید سیلوی یونجه در سال‌های اخیر مورد توجه بیشتر دامداران قرار گرفته است. یونجه یکی از علوفه‌هایی است که به دلیل داشتن سطح بالای پروتئین، ظرفیت بافری بالا و کربوهیدرات محلول در آب پایین سیلو کردن آن مشکل می‌باشد و افزودنی‌های سیلو در زمان سیلو کردن باید مورد استفاده قرار گیرد. از مواد افزودنی با خاصیت محافظتی (برپایه اسیدهای آلی) برای جلوگیری از رشد قارچ و مخمر در سیلاژ و افزایش پایداری هوازی استفاده شده است. اخیراً استفاده از گیاهان معطر و محصولات آنها به دلیل اثرات ضد قارچ، ضد باکتری و آنتی‌اکسیدان آنها افزایش یافته است. **هدف:** این آزمایش به منظور بررسی اثرات اسانس‌هسته‌لیموترش بر خصوصیات شیمیایی و پایداری-هوازی سیلاژ یونجه انجام شد. **روش کار:** یونجه در مرحله گلدهی برداشت شد و با یک چاپر به اندازه‌های ۳ تا ۵ سانتی متر خرد شد و در آزمایشگاه به مدت ۲۴ ساعت پژمرده شد. پس از دوره پژمردگی، به سه زیر نمونه تقسیم شد. تیمارهای آزمایشی شامل تیمار یونجه بدون افزودنی (شاهد)، یونجه بعلاوه ۶۰ میلی‌گرم در کیلوگرم اسانس لیموترش و یونجه بعلاوه ۱۲۰ میلی‌گرم در کیلوگرم اسانس هسته‌لیمو بودند که به مدت ۶۰ روز در دمای اتاق سیلو شدند. در پایان روز ۶۰، سیلوها باز شدند و بلافاصله pH و ماده خشک اندازه‌گیری و باقیمانده نمونه‌ها برای اندازه‌گیری خاکستر، ADF، NDF و CP در فریزر با دمای ۲۰- درجه سانتیگراد ذخیره شد. آزمایش تولید گاز با استفاده از روش آزمایشگاهی با ۵ تکرار در ۲، ۴، ۶، ۸، ۱۲، ۱۶، ۲۴، ۳۶، ۴۸، ۷۲، ۹۶ و ۱۲۰ ساعت اندازه گیری شد. داده‌های بدست‌آمده در قالب طرح‌آماری کاملاً تصادفی آنالیزگردید. **نتایج:** نتایج نشان داد که افزودن اسانس‌هسته لیمو در هر دو سطح ۶۰ و ۱۲۰میلی‌گرم بر کیلوگرم به سیلاژ یونجه میزان pH سیلو را به طور معنی‌داری ($P<0/05$) نسبت به شاهد کاهش یافت. افزودن اسانس در سطح ۱۲۰ میلی‌گرم غلظت کربوهیدرات محلول در آب را افزایش داد (۴/۹۱ ٪). میزان پروتئین خام در تیمار مکمل شده با اسانس هسته لیمو ئر سطح ۱۲۰ میلی‌گرم در مقایسه با تیمار کنترل افزایش یافت ($P<0/05$). اسانس‌هسته لیمو باعث افزایش پایداری‌هوازی سیلو نسبت به شاهد شد. افزودن اسانس در سطح ۶۰ میلی‌گرم گاز تولیدی را نسبت به تیمار شاهد کاهش داد. در کل، داده‌های بدست آمده نشان دهنده اثر مثبت اسانس هسته لیمو روی کیفیت سیلاژ یونجه و خصوصیات تخمیری است. **نتیجه‌گیری‌کلی:** نتایج این مطالعه نشان داد که افزودن اسانس هسته لیمو بر سیلاژ یونجه باعث افزایش پایداری هوازی سیلو نسبت به شاهد شد. اسانس هسته لیمو تاثیر معنی‌داری بر روی ازت امونیاکی و پروتئین خام داشت.

سطوح مختلف اسانس هسته لیمو میزان pH را تحت تاثیر قرار داد و به طور قابل‌توجهی کاهش یافت. افزودن اسانس هسته لیمو ترش در سطح بالا باعث افزایش میزان تولید گاز و فراسنجه‌های تخمیری تولید گاز گردید.

واژگان کلیدی: اسانس‌گیاهی، پایداری‌هوازی، سیلاژیونجه، هسته لیمو