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Silage Quality, Fermentation Dynamics and Chemical Composition of Alfalfa Silage Prepared with Salt and Lactic Acid Bacteria Inoculants

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ABSTRACT

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The present study was carried out to investigate the silage quality, fermentation profile and chemical composition of alfalfa silage prepared with salt (NaCl) and lactic acid bacteria (LAB) as inoculants. After harvesting, fresh alfalfa samples were chopped into small pieces and the silage additives were manually applied to fresh alfalfa in a plastic basin. Four groups were established of salt and LAB inoculant i.e., CON (no supplementation); LAB (LAB supplementation), SALT (salt supplementation) and SALT-L (LAB inoculant and salt supplementation). Four silos from each group were opened for the analysis of silage quality, fermentation quality and chemical composition on 7, 14, 30 and 60th d of ensiling. The physical quality of silages revealed that good and excellent quality silages were obtained regardless of the duration of ensiling. All additives remarkably increased ($P<0.01$) the Flieg point during ensiling. After 7 d of ensiling, the pH value was linearly decreased in all silages throughout fermentation ($P<0.01$). There were no significant differences ($P>0.05$) among groups in terms of NH_3-N/TN content on all treatment days. All inoculants tended to increase acetate and lactate levels ($P<0.01$) and decrease propionate and butyrate levels ($P<0.01$) compared to CON- silage irrespective of the days of ensiling. The highest LAB numbers were observed in LAB-treated silages than CON-silage. Silage prepared with salt had greater ($P<0.01$) CO_2 production whereas those prepared with LAB exhibited lower CO_2 production ($P<0.05$) compared to CON-silage. There were significant differences ($P<0.01$) among groups in DM, CP, ash CF, NDF, ADF and hemicellulose at d 60 of ensiling. This study showed that LAB inoculant was more efficient than S and SALT-L inoculant to improve fermentation quality.

Keywords: Aerobic stability, Alfalfa, Fermentation, Lactic acid bacteria, Salt, Silage

INTRODUCTION

Modern dairy and beef farmers use many forages to feed their herd, however, silages and alfalfa hay are the most popular among these forages. According to the

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Turkish Statistical Institute data, an average of 23.152 million tons of corn silage and 17.561 million tons of alfalfa hay were produced in 2018 in Turkey (TUIK, 2018). Recently, the wilting of alfalfa for the preparation of hay has become more difficult because of climatic changes and irregular rainfall in Turkey that lie in the Mediterranean climatic region of the subtropical zone having a sensitive climate (Unal *et al.,* 2012). Consequently, the use of alfalfa silage is gradually increasing due to drying problems inflicting the loss of nutritive value. Therefore, forage preservation holds central importance to maintain the sustainability and economics of animal husbandry.

Ensiling or preservation is a natural fermentation process of forage conservation to improve the feed palatability and extend the storage time (Ni *et al.,* 2017). During this process, water-soluble carbohydrates (WSC) are converted into lactic acid (LA) via epiphytic lactic acid bacteria (LAB) resulting in a decline of pH (Liu *et al.,* 2016; Yan *et al.,* 2019). Alfalfa *(Medicago sativa)* is a perennial legume and is a good source of nutrients for animals (Silva *et al.,* 2016) including protein (Kim *et al.,* 2017). Greater buffer capacity, high protein content, and relatively low concentrations of WSC make it difficult to preserve. In addition, the moisture content of alfalfa is an important factor that should be reduced before ensiling to prevent secondary clostridial fermentation. However, several factors affect the silage fermentation from harvesting to ensiling. Therefore, many silage additives have been used for years to enhance silage preservation such as fermentation stimulants (microbial inoculants), fermentation inhibitors (organic salts), aerobic deterioration inhibitors (propionic acid), nutrients (urea, mineral), and absorbents (wheat, grain, sugar beet pulp) (Muck *et al.,* 2018). LAB are commonly used as silage additives (Silva *et al.,* 2016) and is reported to decrease ammonia nitrogen/total nitrogen (NH_3-N/TN) and pH level (Liu *et al.,* 2016) in addition to increasing lactic acid concentration and number of LAB in the silage. Obligate homofermentative LAB (facultative heterofermentative LAB) convert hexose mainly into lactic acid via the Embden-Meyerhof-Parnas pathway (Muck *et al.,* 2018) whereas obligate heterofermentative LAB can ferment pentose besides hexose to produce acetic acid, ethanol, and CO_2 as well as lactic acid (Muck *et al.,* 2018). The success of LAB as an additive depends on the type and characteristics of the crop, climatic conditions, environmental temperature, epiphytic population, ensiling method, and the qualities of LAB inoculant (Silva *et al.,* 2016). It has been reported that LAB inoculants provide an opportunity to swiftly decrease pH (Kim *et al.,* 2017) and NH_3-N/TN (Liu *et al.,* 2016) improving quality point and Flieg point (Dong *et al.,* 2017), improving number of LAB (Yan *et al.,* 2019), and aerobic stability of silage (Arriola *et al.,* 2015). Salt is commonly used to inhibit the growth of butyric acid bacteria (BAB) and increase fermentation. It is reported that the water activity of silage might be reduced by the addition of NaCl (Cai *et al.,* 1997).

However, the available literature that evaluates the use of salt and LAB inoculant together in order to improve the silage fermentation is scarce. Therefore, the present study was conducted to investigate the silage quality and fermentation

dynamics of alfalfa silages prepared with salt LAB inoculant. This study aimed to examine the effects of salt and LAB supplementation as inoculants on silage quality, fermentation dynamics and chemical composition of alfalfa silage.

MATERIALS AND METHODS

The study used alfalfa that was harvested by sickle 65 d after sowing at about 10% blooming phase for making silage. The DM, CP, CF, ash, NDF and ADF contents of the fresh alfalfa were 25.72, 22.14, 25.15, 12.85, 43.35 and 30.85 per cent, respectively.

Study design and silage preparation

The experiment was conducted in a completely randomized design using four experimental groups as CON (control), with no inoculants; SALT, with common salt as an inoculant; LAB, with LAB as an inoculant, and SALT-L, with both salt and LAB as inoculants. The LAB inoculant (Pioneer 11A44. Pioneer Hi-Bred International, Inc., Des Moines, IA, US) and commercial salt (Billur® Brand, Beþiktaþ, Istanbul) were applied to chopped fresh alfalfa at the rate of 1.0×10^6 cfu/g and at 3 g/kg fresh weight, respectively. *Lactobacillus buchneri* strain LN4637 is a heterofermentative bacteria, which produces lactic acid and acetic acid during fermentation. The inclusion levels of salt and LAB inoculant were based on a proper review of the existing literature.

Following harvesting, the fresh alfalfa samples were chopped into small pieces (~1.5-2 cm) by pruning shears for ensiling and the silage additive was manually applied to fresh alfalfa in the plastic basin. Approximately 800g of chopped alfalfa (fresh weight) was compressed by hand into a 1-L jar (100 mm diameter × 170 mm height). A total of 80 jars (20 jars per experimental group) were prepared and stored at ambient temperature (16 ± 2°C). Four silos from each treatment were randomly opened for the analysis of physical quality, fermentation dynamics (Flieg point, pH value, NH_3-N/TN content, VFA and lactate levels, microbial population) and chemical composition of silage on 7, 14, 30 and 60 d of ensiling.

Physical quality analysis

Physical quality analysis was assessed by using DLG scoring system (DLG, 1997). Each alfalfa silage samples were carefully opened and scored by 3 experts in terms of smell score (0-14 scale), structure score (0-4 scale) and colour score (0-2 scale) of the silage. According to score, silage was divided into the quality classes namely, excellent (16-20 points); good (10-15); mid (5-9) and too bad (0-4).

Fermentation profile analysis

Flieg point (Dong *et al.,* 2017) was determined using the DM content and pH values of the silages with the following equation:

$$\text{Flieg's point} = 220 + (2 \times \text{DM} - 15) - (40 \times \text{pH})$$

For the assessment of pH value and NH_3-N/TN content, a 25g fresh silage sample was blended with 100 ml distilled water in a mixer for 4-5 min and filtered through a cheesecloth. The pH value was measured with a glass electrode pH meter (ECPlaza, Guro-gu, Seoul, Korea). Approximately 10 ml of filtrate was distilled (Vapodest 10 Rapid Kjeldahl Distillation Unit; Gerhardt, Konigswinter, Germany) and titrated to determine NH_3-N/TN content using Kjeldahl methods (Broderick and Kang, 1980). Volatile fatty acids (acetate, propionate, butyrate) concentration (Suzuki and Lund, 1980) of the silage were measured using gas chromatography (Agilent Technologies, Inc., Berlin, Germany; column: HP-FFAP 30 m × 0.53 mm × 0.50 μm). The lactate concentration of silage was analyzed using the HPLC (Agilent Technologies, Inc., Berlin, Germany; column: interstil ODS-4. Sepax Technologies, Inc., Santa Clara, CA, USA; UV-VIS detector) as described by (Ni *et al.,* 2017). About 25g silage sample was blended in 225 ml of sterile peptone water for assaying enumeration of lactic acid bacteria, yeast and mould according to Assis *et al.* (2014). After opening the jars, the silage samples were exposed to oxygen for seven days to determine the aerobic stability of alfalfa silages (Ashbell *et al.,* 1991). For this analysis, two bottles were used, and two 1-cm diameter holes were bored, one on the top and one on the bottom of the upper part to enable air circulation. Silage was loosely packed in this part (250-300 g on a wet basis). The lower part of the unit, which was made from another bottle, was filled with 100 ml of 20% KOH. The upper and lower parts fit together and formed the system (Fig. 1).

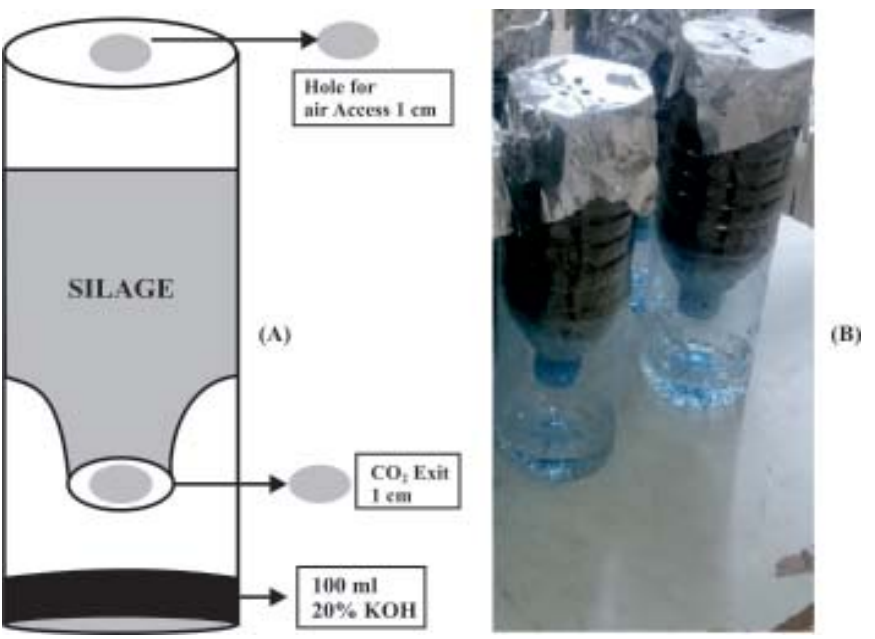

Fig. 1. (A) System for determination of aerobic stability [Adapted from Ashbell *et al.* (1991)], (B) an image from experiment.

Chemical composition analysis

The silage sample was dried in a forced ventilation oven at 60°C (Memmert GmbH Universal, Schwabach, Germany) for 48 h and milled at 1-mm sieve for the estimation of chemical composition. The DM (method 934.01), CP (method 984.13), ash (method 942.05) contents were estimated according to the methods outlined by AOAC (1990). The crude fibre content was determined by the method previously described by Crampton and Maynard (1938), whereas NDF and ADF were determined according to Goering and Van Soest (1970).

Statistical analyses

The data were analysed in a statistical software package SPSS (version 22.0. Armonk, NY, US). One-way ANOVA was applied using general linear model procedures of SPSS in order to assess the effect of salt and LAB inoculant on measured traits of silage quality, fermentation dynamics and chemical composition. Differences among the groups were calculated using the Tukey test (Dawson and Trapp, 2001) The confidence interval was set at 95% ($P<0.05$). Results were presented as Mean±SEM.

RESULTS

Silage quality

Physical quality of silages revealed that good and excellent quality silages were obtained regardless of the days (Table 1) which had a slightly acidic and aromatic scent, green-yellow colour and anatomical structures of the plant (stem and leaf) did not break down during the ensiling process. The smell score of silage (13.93; 13.86; 14.00) was not affected ($P>0.05$) by inoculant treatment at d 7, 30 and 60. At d 14 of ensiling, all inoculants significantly increased smell score ($P<0.01$). Colour score of silage was not affected ($P>0.05$) by inoculant treatment at d 7, 14 and 30, except at d 60 of ensiling. Structure score remained unaffected ($P>0.05$) at d 14, 30, and 60 of ensiling. At d of 60 ensiling, SALT-L silage had the lowest smell score ($P>0.05$), colour score ($P<0.05$) and structure score ($P>0.05$) resulted in a decline in total score (14.26; good) of silage ($P>0.01$) as compared with the other silages while that of increased in LAB-silage.

Fermentation dynamics

Regardless of the days, all additives remarkably increased ($P<0.01$) the Flieg point due to the increases in DM and decreases in pH. The highest Flieg point was recorded ($P<0.01$) in silage prepared with SALT-L inoculation, which was 81.97 at 60 d of ensiling (Table 2). There was a significantly different effect ($P<0.01$) between treatments for silage pH. After 7 d of ensiling, the pH value was linearly decreased in all silages throughout fermentation. Silage pH value was similar between LAB (4.59) and SALT-L (4.49), and both were lower ($P<0.01$) compared with control (5.91) at d 60 of ensiling (Table 2). There were no significant differences ($P>0.05$)

Table 1. Physical analysis of alfalfa silage prepared with salt and lactic acid bacteria inoculant

Day	Groups†	Smell	Structure	Colour	Total	Quality
7th	CON	13.73±0.14	3.86^{a}±0.18	1.93±0.14	19.53±0.29	Excellent
	SALT	13.93±0.14	4.00^{a}±0.01	1.93±0.14	19.86±0.43	Excellent
	LAB	13.86±0.29	3.93^{a}±0.14	1.93±0.14	19.73±0.18	Excellent
	SALT-L	14.00±0.01	3.46^{b}±0.29	2.00±0.01	19.46±0.29	Excellent
	Significance‡	NS	*	NS	NS	
14th	CON	9.53^{b}±0.73	3.40±0.82	1.80±0.44	14.53^{b}±1.42	Good
	SALT	11.86^{a}±0.29	3.53±0.38	1.40±0.36	16.86^{ab}±0.14	Excellent
	LAB	12.26^{a}±0.72	3.60±0.54	1.80±0.44	17.86^{a}±1.26	Excellent
	SALT-L	11.93^{a}±1.09	3.06±0.72	1.40±0.43	16.33^{ab}±2.13	Excellent
	Significance‡	**	NS	NS	*	
30th	CON	12.40±0.27	3.86±0.184	1.88±0.18	18.13±0.38	Excellent
	SALT	12.40±0.27	4.00±0.01	2.00±0.01	18.40±0.40	Excellent
	LAB	12.00±0.33	3.93±0.14	2.00±0.01	17.93±0.27	Excellent
	SALT-L	12.26±0.27	3.86±0.18	2.00±0.01	18.13±0.44	Excellent
	Significance‡	NS	NS	NS	NS	
60th	CON	12.00^{ab}±0.74	3.86±0.89	2.00^{a}±0.01	17.60^{a}±1.16	Excellent
	SALT	11.53^{ab}±1.80	3.73±0.36	2.00^{a}±0.01	16.73^{ab}±2.61	Excellent
	LAB	12.93^{b}±0.76	3.93±0.36	2.00^{a}±0.001	18.33^{a}±0.80	Excellent
	SALT-L	10.00^{a}±1.33	3.46±0.55	1.46^{b}±0.29	14.26^{b}±2.04	Good
	Significance‡	NS	NS	**	**	

†CON: no additive; SALT: silage treated with salt inoculant (3 g/kg of fresh weight); LAB: silage treated with lactic acid bacteria inoculant (1.0×10^{6} cfu/g); SALT-L: silage treated with salt (3 g/kg of fresh weight) and lactic acid bacteria inoculant (1.0×10^{6} cfu/g)

^{ab}Means within a column with different letters are significantly different.

‡Significance: *$P<0.05$; **$P<0.01$; NS, not significant

among groups in terms of NH_3-N/TN content on all treatment days. LAB inoculant had the lowest ($P>0.05$) NH_3-N/TN content during ensiling (Table 2). Acetate and lactate levels in silage increased ($P<0.01$) due to all inoculants irrespective of the days of ensiling (Table 2). The addition of inoculant increased ($P<0.01$) the propionate levels in silage at d 7 and 14 that decreased rapidly on d 30 and 60 ($P<0.01$). The butyrate levels were lower in silages treated with all inoculant than the control silage. During the ensiling period, except at d 7 of ensiling, LAB silage had the highest ($P<0.05$) lactate levels among all silages. The highest LAB numbers were observed in LAB-silages, while the lowest LAB numbers in control silage. The numbers of mould and yeast were below the detection after 7 d of ensiling among groups (Fig. 2).

Table 2. Fermentation dynamics of alfalfa silage prepared with salt and lactic acid bacteria inoculant

Day	Groups†	Flieg Point	pH value	NH_3-N/TN (g/kg)	Acetate (g/kg)	Propionate (g/kg)	Butyrate (g/kg)	Lactate (g/kg)
7th	CON	5.39^{d}±0.72	6.07^{a}±0.05	109.98±7.80	6.35^{d}±0.03	0.18^{b}±0.03	4.23^{a}±0.04	4.46^{d}±0.07
	SALT	26.90^{c}±0.86	5.61^{b}±0.23	89.96±12.88	7.93^{c}±0.10	0.19^{b}±0.01	0.96^{b}±0.06	11.33^{b}±0.06
	LAB	59.41^{b}±1.28	4.79^{c}±0.06	78.64±6.43	11.58^{b}±0.06	0.11^{b}±0.02	0.70^{c}±0.05	10.25^{c}±0.05
	SALT-L	60.45^{a}±1.51	4.78^{c}±0.09	93.90±8.68	13.68^{a}±0.81	0.53^{a}±0.02	0.89^{bc}±0.04	14.13^{a}±0.05
	Significance‡	**	**	NS	**	**	**	**
14th	CON	16.94^{c}±1.72	6.01^{a}±0.7	94.98±6.83	6.39^{d}±0.05	0.03^{b}±0.01	3.62^{a}±0.07	24.07^{d}±0.11
	SALT	49.76^{b}±3.81	5.22^{b}±0.23	75.91±11.09	12.29^{c}±0.35	0.22^{c}±0.02	0.92^{b}±0.06	27.59^{c}±0.09
	LAB	67.67^{a}±0.99	4.78^{c}±0.15	66.00±5.52	14.19^{b}±0.18	0.20^{c}±0.03	0.44^{c}±0.04	71.53^{a}±0.13
	SALT-L	69.74^{a}±2.57	4.74^{c}±4.74	83.15±7.50	19.63^{a}±0.14	0.39±0.04	0.55^{c}±0.05	47.64^{b}±0.08
	Significance‡	**	**	NS	**	**	**	**
30th	CON	22.34^{c}±1.40	5.94^{a}±0.07	50.38±3.31	9.96^{d}±0.26	2.22^{a}±0.03	2.82^{a}±0.08	43.98^{d}±0.09
	SALT	48.35^{b}±3.06	5.28^{b}±0.16	47.85±4.55	12.86^{c}±0.29	0.04^{c}±0.01	0.44^{b}±0.04	70.58^{c}±0.12
	LAB	75.44^{a}±1.97	4.66^{c}±0.12	38.64±3.13	18.57^{b}±0.17	0.01^{c}±0.01	0.22^{c}±0.03	93.72^{a}±2.02
	SALT-L	75.01^{a}±1.56	4.62^{c}±0.08	40.35±0.78	20.11^{a}±0.04	0.33^{b}±0.03	0.11^{c}±0.01	81.83^{b}±0.06
	Significance‡	**	**	NS	**	**	**	**
60th	CON	24.37^{d}±1.83	5.91^{a}±,010	36.93±1.92	15.76c±0.21	2.502^{a}±0.05	1.22^{a}±0.05	63.87^{d}±0.08
	SALT	51.61^{c}±1.95	5.25^{b}±0.11	34.58±4.13	21.76^{b}±0.15	0.01^{b}±0.01	0.35^{b}±0.04	85.79^{c}±0.11
	LAB	80.34^{b}±1.44	4.59^{c}±0.58	30.70±1.85	24.75^{a}±0.08	0.007^{b}±0.001	0.12^{c}±0.02	103.28^{a}±0.12
	SALT-L	81.97^{a}±0.40	4.49^{c}±0.08	31.06±1.61	24.29^{a}±0.08	0.10^{ab}±0.03	0.11^{c}±0.01	91.83^{b}±0.08
	Significance‡	**	**	NS	**	**	**	**

†CON: no additive; SALT: silage treated with salt inoculant (3 g/kg of fresh weight); LAB: silage treated with lactic acid bacteria inoculant (1.0×10^{6} cfu/g); SALT-L: silage treated with salt (3 g/kg of fresh weight) and lactic acid bacteria inoculant (1.0×10^{6} cfu/g)

[abcd]Means within a column with different letters are significantly different.

‡Significance: *P<0.05; **P<0.01; NS, not significant

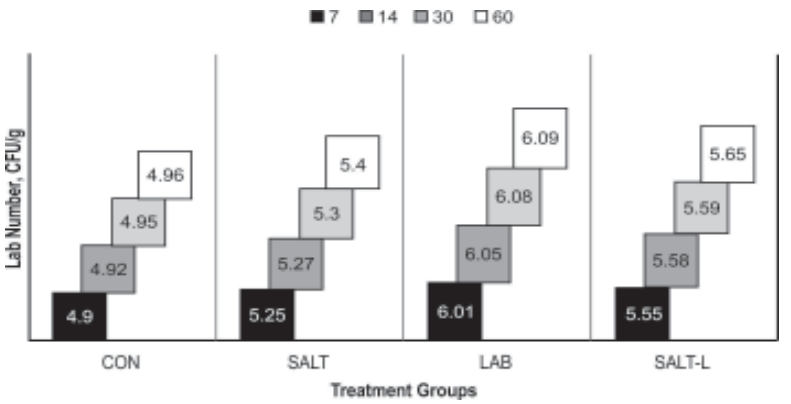

Fig. 2. Lactic acid bacteria numbers (1.0×10^6 cfu/g FW) of silage prepared with salt and lactic acid bacteria inoculant

CON: no additive; SALT: silage treated with salt inoculant (3 g/kg of fresh weight); LAB: silage treated with lactic acid bacteria inoculant (1.0×10^6 cfu/g); SALT-L: silage treated with salt (3 g/kg of fresh weight) and lactic acid bacteria inoculant (1.0×10^6 cfu/g).

After exposure to air, pH value, CO_2 production and DM loss in silage treated with LAB were significantly lower than those of untreated silage. Silage prepared with salt had greater CO_2 production whereas those prepared with LAB exhibited lower CO_2 production ($P<0.01$). All inoculants lowered the silage pH value. DM loss decreased ($P<0.05$) in silage prepared with LAB or SALT-L (Table 3).

Chemical composition

At d 7 of ensiling, the DM content was highest ($P<0.01$) in silages prepared with LAB inoculant. Silage prepared with salt showed lower CP content ($P<0.05$). Ash content increased due to salt or SALT-L inoculant and decreased with LAB inoculant ($P<0.01$). The CF content decreased ($P<0.05$) in silage prepared with all

Table 3. Aerobic stability of alfalfa silage prepared with salt and lactic acid bacteria inoculant (Mean±SEM)

Groups†	pH	CO_2 production (g/kg DM)	DM Loss (%)
CON	$5.99^{a}\pm0.11$	$9.17^{b}\pm0.26$	$7.08^{a}\pm0.89$
SALT	$5.29^{b}\pm0.95$	$10.53^{a}\pm0.58$	$6.48^{a}\pm0.97$
LAB	$4.80^{c}\pm0.77$	$8.82^{b}\pm0.78$	$3.04^{c}\pm0.28$
SALT-L	$4.89^{c}\pm0.08$	$9.03^{b}\pm0.82$	$4.93^{b}\pm1.35$
Significance‡	*	**	*

†CON: no additive; SALT: silage treated with salt inoculant (3 g/kg of fresh weight); LAB: silage treated with lactic acid bacteria inoculant (1.0×10^6 cfu/g); SALT-L: silage treated with salt (3 g/kg of fresh weight) and lactic acid bacteria inoculant (1.0×10^6 cfu/g)

[abcd]Means within a column with different letters are significantly different.

‡Significance: *$P<0.05$; **$P<0.01$; NS, not significant

inoculants. The NDF content increased in silage prepared with salt and decreased in that prepared with LAB inoculant ($P<0.05$). The ADF content was lower ($P<0.01$) in silages prepared with all inoculants. Hemicellulose content was greater ($P<0.01$) in silages prepared with salt or LAB inoculant (Table 4). At d 14 of ensiling the DM content of silages was unaffected ($P<0.05$) regardless of the treatments. Silages prepared with salt had lower CP whereas those prepared with LAB inoculant showed greater CP ($P<0.01$). Ash was greater ($P<0.01$) in silage prepared with salt or SALT-L. The CF content lowered ($P<0.01$) in silages prepared with SALT-L inoculant. The NDF increased in silage prepared with salt and decreased in those prepared with LAB inoculant ($P<0.01$). All inoculant significantly reduced the ADF content and increased ($P<0.01$) the hemicellulose content of alfalfa silage (Table 4). At d 30 of ensiling, silage prepared with salt or SALT-L showed lower DM content whereas LAB silage had higher DM ($P<0.01$). Ash increased in silage prepared with salt and lowered in LAB or SALT-L silage ($P<0.05$). Silage prepared with salt exhibited lower CP content whereas LAB silage had higher CP ($P<0.05$). The CF content was lowered ($P<0.01$) in silages prepared with all inoculants. The NDF remained unaffected in silages prepared with salt or LAB inoculant. All inoculant resulted in lower ADF in silages ($P<0.01$). Hemicellulose increased in silage prepared with salt ($P< 0.05$) whereas LAB inoculant had no effect ($P<0.05$) on hemicellulose content of silage (Table 4). At d 60 of ensiling, DM content was higher in silages prepared with all inoculants ($P<0.01$). Ash was greater ($P<0.01$) in silage prepared with salt. The CP content was highest in silages prepared with LAB inoculant ($P<0.05$). CF lowered in silages prepared with all inoculant ($P < 0.01$). NDF lowered in silages prepared with salt or LAB inoculant ($P<0.01$). ADF content decreased and hemicellulose content increased ($P<0.01$) in silages prepared with all inoculant (Table 4).

DISCUSSION

The evaluation of smell, colour and structure of silage is the best and simple method for determining the physical quality of silage (Zhao *et al.,* 2019). In the present study, all inoculants increased the smell, colour, and structure score compared with the control group all ensiling days, except at d 60 of ensiling. The total score in SALT-L silage was lower than that of CON silage. The reason for this is unclear, however, it might be that the physical analyses vary from person to person in determining sensory analysis. Turan and Önenç (2018) reported that no significant differences were observed in alfalfa silage treated with cumin essential oil for colour, smell, and structure score. Fermentation quality depends on certain factors such as nutritive value and type of silage (Yan *et al.,* 2019), inoculant type, environmental temperature, and LAB characteristics (Wang *et al.,* 2017). The current study indicated that silage prepared with LAB improved the total score. These findings are in line with those of Yan *et al*. (2019) who expressed that LAB inoculation is necessary to provide good fermentation that produces high-quality silage.

Table 4. Chemical composition of alfalfa silage (DM% basis) prepared with salt and lactic acid bacteria inoculant (Mean±SEM)

Day	Groups	DM (65°C)	CP	Ash	CF	NDF	ADF	Hemicellulose
7th	CON	21.67^{b}±0.21	22.60^{a}±0.67	11.69^{b}±0.27	19.77^{a}±0.22	40.05^{b}±0.20	25.95^{a}±0.10	14.09^{d}±0.11
	SALT	23.15^{a}±0.26	21.75^{b}±0.21	12.88^{a}±,015	18.59^{c}±0.33	41.26^{c}±0.21	22.89^{b}±0.09	18.42^{a}±0.12
	LAB	23.17^{a}±0.12	22.26^{a}±0.34	11.58^{b}±,17	19.07^{b}±,0.21	38.38^{a}±0.14	21.95^{d}±0.08	16.43^{c}±0.13
	SALT-L	23.44^{a}±0.17	22.33^{ab}±0.34	13.07^{a}±0.03	17.64^{d}±0.22	40.16^{b}±0.09	22.65^{c}±0.16	18.12^{b}±0.82
	Significance‡	**	*	*	*	*	**	**
14th	CON	26.25±0.22	22.19^{a}±0.10	11.77^{b}±0.26	19.54±0.17	39.25^{b}±0.21	25.76^{a}±0.45	13.91^{d}±0.12
	SALT	26.87±0.37	21.60^{b}±0.20	12.47^{a}±0.34	18.53±0.32	41.12^{d}±0.06	22.84^{b}±0.09	18.23^{a}±0.10
	LAB	27.01±0.04	22.44^{b}±0.14	11.85^{b}±0.89	18.77±0.19	37.36^{a}±0.13	21.23^{d}±0.19	16.13^{c}±0.06
	SALT-L	27.33±0.34	22.21^{c}±0.26	12.38^{a}±0.16	17.61±0.25	40.18^{c}±0.09	22.06^{c}±0.11	17.51^{b}±0.12
	Significance‡	NS	**	**	NS	**	**	**
30th	CON	27.63^{b}±0.07	22.15^{a}±0.08	11.47^{c}±0.27	19.56^{a}±0.19	38.52±0.22	24.75^{a}±0.44	13.77^{c}±0.15
	SALT	27.39^{b}±0.16	21.59^{b}±0.19	12.43^{a}±0.29	19.11^{b}±0.07	38.52±0.12	20.74^{c}±0.12	17.78^{a}±0.21
	LAB	28.50^{a}±0.20	22.86^{a}±0.11	11.19^{c}±0.06	17.63^{c}±0.20	35.55±0.16	20.93^{c}±0.14	14.62^{c}±0.09
	SALT-L	27.44^{a}±0.19	22.32^{a}±0.32	12.04^{b}±0.08	17.49^{c}±0.28	38.38±0.19	21.83^{b}±0.21	16.55^{b}±0.08
	Significance‡	**	*	*	**	NS	**	*
60th	CON	27.88^{b}±,024	21.27^{c}±0.18	11.22^{b}±0.19	19.55^{a}±0.21	38.24^{c}±0.28	24.61^{a}±0.40	13.63^{d}±0.25
	SALT	28.42^{b}±0.10	22.68^{bc}±0.30	12.01^{a}±0.05	18.18^{b}±0.16	36.05^{b}±0.36	20.19^{c}±0.25	15.86^{b}±0.05
	LAB	29.63^{a}±0.17	22.97^{a}±0.25	11.17^{b}±0.08	17.45^{c}±0.18	35.36^{a}±0.28	20.12^{c}±0.01	15.24^{c}±0.16
	SALT-L	28.44^{a}±0.21	22.45^{b}±0.25	11.99^{a}±0.10	17.38^{c}±0.35	38.89^{c}±0.16	21.15^{b}±0.06	16.74^{a}±0.22
	Significance‡	**	*	**	**	**	**	**

†CON: no additive; SALT: silage treated with salt inoculant (3 g/kg of fresh weight); LAB: silage treated with lactic acid bacteria inoculant (1.0×10^{6} cfu/g); SALT-L: silage treated with salt (3 g/kg of fresh weight) and lactic acid bacteria inoculant (1.0×10^{6} cfu/g)

[abcd]Means within a column with different letters are significantly different.

‡Significance: *P<0.05; **P<0.01; NS, not significant

The present findings showed that the Flieg point was lower in the control group than that of other groups. An increase of 26.52% in Flieg point found in treatment groups compared with the control group is in agreement with Dong *et al.* (2017). Flieg point was higher in LAB-silages than SALT-silages throughout the study. It is considered that the addition of LAB inoculant results in good fermentation of silage and causes a decline in pH and increase the DM compared with the salt inoculant. Therefore, this situation could be interpreted in the sense that low pH and high DM have a significant positive correlation with the Flieg point.

Acidity (pH) is an important factor to consider a good silage fermentation. In the present study, pH value ($P<0.01$) was lower in silage prepared with SALT-L compared to other treatments. The low pH in silage is desirable, which could inhibit the spoilage by microorganisms resulting in lower DM loss (Silva *et al.,* 2016).

Liu *et al.* (2016) reported a positive relationship between pH and NH_3-N/TN in silage. The protein utilization could be enhanced because of proteolysis that was significantly limited by the addition of LAB in silage that caused a quick drop in pH (Silva *et al.,* 2016). It is significant that a lower pH value ($P<0.01$) was obtained in silage prepared with LAB inoculant, which had a low NH_3-N/TN ($P<0.05$). The decline of protein degradation was attributed to the low pH of silage (Kim *et al.,* 2017). Similarly, previous studies have shown positive effects of LAB inoculant to alfalfa silage (Liu *et al.,* 2016), fresh rice straw silage (Kim *et al.,* 2017), and soybean silage (Ni *et al.,* 2017) in terms of pH value and NH_3-N/TN content of silage which were decreased. The current study revealed that inoculation of salt and LAB decreased the NH_3-N/TN being 34.58 and 30.70 g/kg, respectively, at d 60 of ensiling. Muck *et al.* (2018) suggested that NH_3-N/TN content fewer than 50 g/kg in ensiled silage is an optimal value. However, high NH_3-N/TN in silages indicates that that fraction of protein was gradually degraded to NH_3. This would be considered as a sign of insufficient fermentation mainly due to high pH and NH_3. which could be classified as poor quality silage (Borreani *et al.,* 2018). Silages prepared with SALT had lower pH value ($P<0.01$) and NH_3-N/TN ($P<0.05$) than that of control silage. Cai *et al.* (1997) reported that inoculation with NaCl to alfalfa silage significantly decreased the pH value and NH_3-N/TN, probably because of the good fermentation of the silage.

Acetate and lactate levels were remarkably increased ($P<0.01$), while propionate and butyrate levels were decreased ($P<0.01$) by all inoculant treatments during the ensiling. Zhao *et al.* (2019), indicated that the addition of *L. plantarum* could improve the fermentation quality and increase the lactic acid concentration of rice straw silage. High-level acetate production might be attributed to the fact that the lactic acid was converted into acetic acid during the ensiling (Ni *et al.,* 2017). The homofermentative LAB ferment hexoses mainly to lactic acid while heterofermentative LAB produces acetic acid, CO_2. as well as lactic acid (Muck *et al.,* 2018). At 60 d of ensiling, lactate levels were remarkably increased by SALT

and LAB inoculant compared to untreated silage, which was 85.79 and 103.28 g/kg respectively. This was in accordance with the results reported by Cai *et al.* (1997), who revealed that the silages treated with NaCl stimulated the production of lactic acid; and therefore, BAB was inhibited during ensiling. Butyric acid was detected at quite a low level in all silages after 60 d of ensiling in the current study. Usually, no or low levels of butyric acid are desired in the silage as it negatively affects the silage quality by reducing the nutritional value of silages (Nkosi and Meeske, 2010). The current study showed that the LAB silage had the highest LAB number followed by SALT-L and SALT silage while CON silage having the lowest LAB number ($P<0.01$). Similarly, Yang *et al.* (2018) found *Lactobacillus* species increased by *L. plantarum*, due to its high activity in the low pH. A similar finding was also indicated by Ni *et al.* (2017), who ensiled alfalfa silage inoculated with *L. plantarum* and *Pecliococcus pentosaceus* tended to have greater LAB number than control silage.

Aerobic stability could be defined as the stability of silage against spoilage after it has been exposed to air. There was another description of aerobic stability according to the Liu *et al.* (2016) who stated that the silages producing $CO_2<10$ g/kg DM or showing a change of <0.5 units in pH over 5 days are deemed to be stable. The current results showed that the lowest pH ($P<0.05$), production of CO_2 ($P<0.01$) and DM loss ($P<0.05$) in LAB-silage was observed after 7 d of opening compared to SALT, which was compatible with results of Liu *et al.* (2018) who stated that lower VFA content and higher lactic acid content in silage might limit the aerobic deterioration after exposing to air. Arriola *et al.* (2015) reported that bermudagrass silage treated with LAB inoculant improved the fermentation strength resulting from the decline of silage pH that causes a low DM loss and high aerobic stability by inhibiting yeast fermentation. In the current study, the lower pH value and higher CO_2 production in SALT silage were observed than the control group. It might be attributed to the increased acetic acid concentration that suppressed the yeast fermentation during the ensiling, resulting in CO_2 production.

At 60 d of ensiling, the LAB silage had the highest CP content followed by the salt group, SALT-L silage while CON silage having the lowest CP content ($P<0.01$). This could be attributed to a reduction in NH_3-N/TN concentration of silages during the ensiling. Zhao *et al.* (2019) reported that higher CP content was observed in LAB-silage than in the control silage due to lower proteolysis in LAB-silages (Liu *et al.,* 2016) that caused the inhibition growth of Clostridia (Silva *et al.,* 2016). The significantly lower ash content was indicated in LAB-silage, but higher in salt and S-LAB silage due to mineral level of salt ($P<0.01$). Results obtained in this study are in agreement with those of Zhao *et al.* (2019) who found that the ash content in LAB silage was lower than that of the control group. Besides, the salt or LAB inoculant silage had lower NDF and ADF content, as well as CF content and higher hemicellulose content. It is possible that the lower pH, NH_3-N/TN/TN content, propionate and higher lactate observed in silages prepared with salt or LAB inoculant

resulted in an improved fermentation. Yan *et al*. (2019) stated that NDF and ADF content was lower in silage treated with LAB inoculant than that of untreated silage due to the fibrinolytic enzymes generally produced by the microorganism.

CONCLUSION

The addition of SALT or LAB improve the silage quality and affected fermentation pattern and chemical composition of alfalfa silage. The SALT-L or SALT inoculant had a similar fermentation to that of LAB inoculant overall ensiling days; however, obtained results from LAB were more successful to ensure desired fermentation, thereby providing the high-quality silage. According to these results, in the future, there will not be a requirement to apply LAB and salt together for a good fermentation, which will reduce expenses, result in more profitable silage production.

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