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RESEARCH ARTICLE

THE EFFECTS OF BACTERIAL INOCULANTS AND/OR ENZYMES ON THE FERMENTATION CHARACTERISTICS AND AEROBIC STABILITY OF ALFALFA ENSILED AT DIFFERENT STAGES OF MATURITY

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ABSTRACT

This study was implemented to determine the effects of lactic acid bacteria (LAB) inoculants and/or enzymes on the fermentation, aerobic stability and *in vitro* organic matter digestibility characteristics of alfalfa ensiled at different stages of maturity. Alfalfa was harvested at three levels including early, middle and full flowering stages. In this research, inoculant-1188 (Pioneer®, USA) and enzyme (Global Nutritech, TR) were used as lactic acid bacteria, enzyme and as lactic acid bacteria+enzyme mixture inoculants. Proceeding the treatments of these inoculants to silages at 6.00 $\log_{10}$ cfu/g levels, the chopped alfalfa was ensiled in 1.0 liter special anaerobic jars, equipped with a lid enabling gas release only. The jars were stored at 25±2°C under laboratory conditions. Microbiological and chemical analyses were done through four jars sampled from each group on the 45th day of ensiling process. At the end of the ensiling period, *in vitro* organic matter digestibility test was done and all silages were subjected to an aerobic stability test for 5 days. The results showed that LAB and/or enzymes increased the characteristics of fermentation but decreased cell wall content. There was no effect determined on aerobic stability and *in vitro* organic matter digestibility of alfalfa silages.

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INTRODUCTION

Leguminous is one of the most widely used feed sources in ruminant feeding as well as in other kinds of animal feeding. The most significant forage crop in this group is known as alfalfa (*Medicago sativa* L.). The alfalfa is both used as dry or silo forage, the latter of which is particularly composed of final form of alfalfa not available to dry in regions with high rainfall (Çiftçi *et al.,* 2005). It is hard to make good quality silage from the alfalfa due to its low dry matter (DM) levels, the difficulty of wilting, the limited concentration of water soluble carbohydrates (WSC) and its high buffering capacity (McAllister *et al*., 1998, Ozturk *et al*., 2006). Wilting of alfalfa before ensiling reduces plant respiration rate and enzyme activities, which cause a decrease in silo proteolysis leading to the preservation of plant proteins (Muck, 1987) and an improved nutritive value of the preserved forage for ruminants (Marsh, 1979). During wilting however, the risk of rainfall may lead to the substantial loss of leaves as well as soluble (and fermentable) organic matter and may decrease the nutritive value of the resulting silage (Collins, 1983).

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This nutrient loss in fermentation is usually overcome with the addition of silage additives (Tyrolová and Výborná, 2008) during ensiling process. Thus, the application of lactic acid bacteria (LAB) brings about adequate lactic acid production, reduces pH in silage and enhances safe feeding of the livestock. The fermentable substrates are conditional on this positive effect. In that sense, enzymes (E) are the most effective substrates on crops with low sugar content, such as alfalfa with low DM contents (Muck and Kung 1997). While increasing the rate of ruminal degradability of DM and neutral detergent fibre (NDF), enzymes also improve the availability of WSC to use as a substrate for LAB (McDonald *et al*., 1991; Weinberg *et al*., 1995). The study by Nadeau *et al*. (2000a,b) on the effects of inoculants and enzymes confirmed the increase in the quality of orchard grass and alfalfa silage via this technique. Within that scope, this study was implemented to test and analyse the effects of LAB, E and LAB+E mixture applications on the fermentation, aerobic stability and nutritive values of alfalfa silages harvested at various stages of maturity.

MATERIALS AND METHODS

For the present study, alfalfa forages were harvested by hand at early, mid and full flowering stages and chopped by a laboratory-type chopper at about 1.5 cm and were ensiled in 1.0-l special anaerobic jars (Weck, Wher-Oftlingen, Germany), which were equipped with a lid that enables gas release only.

Each jar was filled with about 600 g (wet weight) of cropped forage, without a head space. The experiment was carried out at three different maturity stages (early, mid and full flowering) and in four treatments (untreated control, three inoculants) with four replicates (jars). In total, 48 jars were stored at ambient temperature (20±2°C). Fresh and ensiled materials (on the day 45th after ensiling, four jars per treatment for each maturity stage) were sampled for chemical and microbiological analyses. At the end of the ensiling period, the silages were subjected to an aerobic stability test for 5 days in a system developed by Ashbell *et al*. (1991). In this system, the number of yeasts and molds, the change in pH and the amount of CO_2 produced during the test were used as aerobic deterioration indicators. The treatment groups in the study were as follows: (1) Control (C), no additive, (2) Inoculation of lactic acid bacteria (LAB, 33 g t^{-1}, a mixture of *Lactobacillus plantarum* and *Enterococcus faecium* applied at the rate of 6.00 $\log_{10}$ CFU LAB g^{-1} to fresh material, Pioneer 1188, Iowa,USA), (3) Enzyme (E, 2 g t^{-1}, a mixture of cellulase (150000 CMCU kg^{-1}) and amylase (200000 SKB kg^{-1}), Silaid WSTM, Global Nutritech Co., USA) and (4) Inoculation of lactic acid bacteria plus enzymes mixture (LAB+E), the treatment LAB+E was applied in a manner that achieved the same concentrations of inoculants and enzymes as in treatments LAB and E. For the treatment, the LAB, E and LAB+E were dissolved in 20 ml of distilled water and applied to 10 kg of fresh alfalfa using a hand sprayer. Four mini-silos were prepared for each treatment and silages were ensiled for 45 days.

After the treatment, the pH values and ammonia nitrogen (NH_3-N) content of fresh and silage samples were determined regarding Anonymous (1986). The WSCs contents of silages were tested by the spectrophotometer (Shimadzu UV-1201, Kyoto, Japan) after the reaction with an antron reagent (Anonymous 1986). The lactic and acetic acid rates were determined via the spectrophotometric method (Koc and Coskuntuna 2003). Moreover, *lactobacilli*, yeast and mold numbers were obtained according to the method reported by Seale *et al*. (1986). Concerning that method, the microbiological examination included the enumeration of *lactobacilli* on pour plate Rogosa agar (Oxoid CM627 incubated at 30 °C for 3 days) whereas yeast and mold examinations were implemented on spread plate malt extract agar (acidified with LA to pH 4.0 and they were incubated at 30 °C for 3 days. The *lactobacilli,* yeast and mold counts of fresh and silages samples were converted into logarithmic coli form unit (CFU g^{-1}). In addition, the DM content of fresh and silage materials was determined by drying those at 60 °C for 48 h in a fan-assisted oven, proceeded by milling through a 1-mm screen and drying for another 4 h at 105 °C. The crude protein (CP) content however was analyzed via the Kjeldahl method by following the procedural steps of the Association of Official Analytical Chemists (1990). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were also performed according to Goering and van Soest (1970). Hemicellulose was calculated in accordance with the difference between NDF and ADF while cellulose being calculated regarding the difference between ADF and ADL. Finally, *in vitro* organic matter digestibility (OMD) of the silages was determined concerning the procedure explained by Aufrere and Michalet-Doreau (1988), through a three-stage technique which was initiated by the pre-treatment with pepsin in hydrochloric acid (0.2% pepsin in 0.1 N HCl), starch hydrolysis and the attack by cellulose (Onozuka R 10 from *trichoderma viride*, Merck). All the data obtained were analysed through the general linear model procedure of the SPSS (2007). The differences between the obtained and previously reported mean scores were determined using Duncan's multiple range tests with the 5% level of probability. The results of statistical analyses were presented in tables as mean values and in standard errors of those means.

The statistical model implemented was as follows:

$$Yijl = \mu + \tau i + \gamma j + \tau\gamma ij + eijl,$$

in which μ = overall mean; τi = effect of maturity i; γj = effect of treatment j; τγij =maturity×treatment interaction; and eijl = residual error.

RESULTS AND DISCUSSION

During the study, initially the chemical composition of the fresh alfalfa and alfalfa silages were analyzed. The chemical composition of the fresh alfalfa harvested at different stages of maturity is displayed in Table 1. Concerning the results, it is obvious that the content of DM increased from 20.13% to 25.04% at early and full flowering stages, respectively, whereas the NDF and ADF contents gradually increased during the experimental period of the crop growth. On the contrary, CP content was determined to decrease gradually during crop growth from 22.76% to 18.39% DM. The content of WSCs also decreased from 33.45 to 26.74 g kg^{-1} DM at early and full flowering, stages, respectively. The pH and buffer capacity values revealed a similar tendency as their levels increased from 6.05 to 7.17 and from 427 to 507 mE kg^{-1} DM at the same stages, respectively. Compared with the study of Knežević *et al*. (2011), which stated that the buffer capacity of alfalfa silomass at flowering stage ranged from 591 to 700 mE kg^{-1} DM., the chemical composition of the alfalfa used in this present study is consistent with the values reported by Nadeau *et al*. (2000a,b), Filya *et al*. (2001, 2007) and Kozelov *et al*. (2008).

As shown in Table 2, the present study confirmed the significant changes in the concentrations of individual substances. It was significant that the pH values for full flowering stage decreased more than other silages ($P<0.01$), whereas the pH of silage with LAB and LAB+E was the lowest ($P<0.01$) regarding the analyses. The results of this study is in agreement with the results of Kozelov *et al*. (2008), who reported that the addition of LAB and LAB+E inoculants to alfalfa at ensiling caused a decrease in pH values compared with untreated and E treatment silages. In contrast to these findings, Schmidt *et al*. (2001) observed similar terminal pH in LAB and/or E treatment and in untreated non-wilted alfalfa silages. In the present study, the DM content was found out to increase from 19.61% to 24.41% at early and full flowering stages, respectively. The DM content of the LAB and/or E treated silages tended to be higher but those differences were not significant ($P>0.05$). The concentration of WSC was increased by the inclusion of LAB+E ($P<0.05$). On the other hand, the CP content of alfalfa silage was found to decrease significantly from 21.86% to 18.30% dry matter at early and full flowering stages, respectively. The CP content of the alfalfa silage with LAB and/or E treatment was higher than that of untreated silage but those differences were not remarked as significant ($P>0.05$).

Table 1. The chemical composition of the fresh alfalfa harvested at different stages of maturity

	Early flowering	Mid flowering	Full flowering
DM, %	20,13	22,67	25,04
ADF, % DM	28,20	31,99	34,59
NDF, % DM	39,03	44,59	48,51
WSC, g/kg KM	33,45	29,57	26,74
CP, % DM	22,76	21,03	18,39
pH	6,05	6,14	7,17
Bc, meq NaOH/kg	427,00	448,00	507,00
Lactobacilli, log_{10} cfu/g	3,26	3,74	3,40

DM: dry matter; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; WSC: water soluble carbohydrates; Bc: buffer capacity

Table 2. Results of the chemical composition of the alfalfa silages

Factor		pH	DM % FM	WSCs % DM	NH_3-N g/kg TN	LA g/kg DM	AA g/kg DM	CP % DM	NDF % DM	ADF % DM	ADL % DM	HCel % DM	Cellulose % DM
Maturity (M)													
Early flowering		4.30^{a}	19.61^{c}	15.77^{b}	67.58	47.89^{a}	17.84^{a}	21.86^{a}	36.89^{c}	27.06^{c}	4.17^{b}	9.84^{b}	22.88^{c}
Mid flowering		4.33^{a}	22.14^{b}	15.43^{b}	71.17	42.02^{b}	19.33^{a}	20.34^{b}	43.50^{b}	31.77^{b}	6.78^{a}	11.73^{a}	24.99^{b}
Full flowering		4.15^{b}	24.41^{a}	19.11^{a}	62.37	37.51^{c}	14.88^{b}	18.30^{c}	45.28^{a}	33.94^{a}	6.97^{a}	11.34^{a}	26.97^{a}
SEM		0.02	0.20	0.40	2.10	0.51	0.48	0.19	0.23	0.28	0.12	0.32	0.30
Treatment (T)													
Control		4.54^{a}	21.86	15.80^{b}	85.91^{a}	35.46^{b}	22.29^{a}	19.93	43.21^{a}	32.15^{a}	6.08	11.06	26.07^{a}
LAB		4.11^{c}	22.13	15.62^{b}	58.14^{c}	46.81^{a}	14.27^{c}	20.15	41.48^{b}	30.83^{b}	6.16	10.64	24.67^{b}
E		4.32^{b}	22.02	17.44^{ab}	66.06^{b}	42.78^{a}	17.45^{b}	20.11	41.72^{b}	30.64^{b}	5.80	11.08	24.84^{b}
LAB+E		4.08^{c}	22.20	18.21^{a}	58.04^{c}	44.84^{a}	15.40^{bc}	20.48	41.16^{b}	30.06^{b}	5.87	11.10	21.19^{b}
SEM		0.06	0.23	0.47	2.47	0.59	0.56	0.22	0.27	0.33	0.13	0.37	0.35
M x T													
Early flowering	Control	4.55^{b}	19.62^{c}	16.56^{de}	82.93^{ab}	39.83^{cd}	24.38^{a}	22.02^{a}	38.45^{d}	28.50^{e}	4.16^{c}	9.95^{cd}	24.34^{cd}
	LAB	4.13^{de}	19.61^{c}	13.98^{fg}	65.68^{de}	52.47^{a}	13.40^{d}	21.54^{a}	36.52^{e}	27.03^{ef}	4.29^{c}	9.49^{d}	22.74^{de}
	E	4.35^{c}	19.40^{c}	14.70^{efg}	65.33^{de}	49.79^{a}	18.38^{c}	22.04^{a}	36.71^{e}	26.81^{ef}	4.13^{c}	9.90^{cd}	22.68^{de}
	LAB+E	4.18^{c}	19.79^{c}	17.83^{bcd}	56.40^{def}	49.48^{ab}	15.19^{d}	21.84^{a}	35.90^{e}	25.89^{f}	4.13^{c}	10.00^{cd}	21.77^{e}
Mid flowering	Control	4.66^{a}	21.13^{b}	13.77^{g}	94.24^{a}	33.06^{f}	20.52^{bc}	20.08^{b}	42.91^{c}	32.50^{cd}	7.38^{a}	10.41^{bd}	25.12^{bc}
	LAB	4.15^{de}	22.30^{b}	13.09^{g}	57.93^{def}	46.70^{b}	19.13^{bc}	20.26^{b}	43.60^{bc}	31.17^{d}	6.89^{ab}	12.43^{ab}	24.29^{cd}
	E	4.41^{c}	22.34^{b}	18.50^{abcd}	69.87^{cd}	41.82^{c}	19.49^{bc}	20.03^{b}	43.73^{bc}	31.80^{cd}	6.39^{b}	11.93^{ac}	25.42^{bc}
	LAB+E	4.13^{de}	22.20^{b}	16.38^{def}	62.63^{def}	46.51^{b}	18.18^{c}	21.00^{ab}	43.77^{bc}	31.60^{cd}	6.47^{b}	12.18^{b}	25.12^{bc}
Full flowering	Control	4.41^{c}	24.21^{a}	17.08^{cde}	80.56^{bc}	33.50^{f}	21.97^{ab}	17.70^{c}	48.28^{a}	35.46^{a}	6.70^{ab}	12.82^{a}	28.76^{a}
	LAB	4.06^{e}	24.50^{a}	19.80^{ab}	50.82^{f}	41.28^{cd}	10.26^{e}	18.66^{c}	44.31^{bc}	34.30^{ab}	7.31^{a}	10.02^{cd}	26.99^{b}
	E	4.21^{c}	24.31^{a}	19.13^{abc}	62.99^{def}	36.74^{e}	14.48^{d}	18.26^{c}	44.72^{b}	33.31^{bc}	6.88^{ab}	11.42^{ad}	26.43^{b}
	LAB+E	3.94^{f}	24.62^{a}	20.43^{a}	55.10^{ef}	38.52^{de}	12.83^{de}	18.60^{c}	43.81^{bc}	32.69^{bd}	7.00^{ab}	11.12^{ad}	25.69^{bc}
SEM		0.04	0.39	0.81	4.28	1.02	0.97	0.39	0.46	0.57	0.23	0.64	0.60
Maturity (M)		<0.01	<0.01	<0.01	0.23	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	0.23	<0.01
Treatment (T)		<0.01	0.98	0.05	<0.01	<0.01	<0.01	0.88	<0.01	0.98	0.05	<0.01	0.88
M x T		<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01

DM: dry matter; FM:fresh matter; WSC: water soluble carbohydrates; NH_3-N: ammonia-nitrogen; TN: Total nitrogen; LA: lactic acid, AA: acetic acid; CP: crude protein ; ADF: acid detergent fiber; NDF: neutral detergent fiber; ADL: acid detergent fiber; HCel: hemicellulose

Table 3. Results of the microbiological analyses of the alfalfa silages (log CFU g^{-1} DM)

Factor		Lactobacilli	Yeast	Mold
Maturity (M)				
Early flowering		5.54^{b}	2.62^{b}	2.51
Mid flowering		5.48^{b}	2.60^{b}	2.47
Full flowering		6.23^{a}	3.33^{a}	2.42
SEM		0.09	0.11	0.10
Treatment (T)				
Control		5.29^{b}	2.81	2.49^{ab}
LAB		6.33^{a}	2.9	2.34^{b}
E		5.24^{b}	3.03	2.72^{a}
LAB+E		6.15^{a}	2.66	2.31^{b}
SEM		0.11	0.12	0.11
M x T				
Early flowering	Control	5.47^{ab}	2.5	2.4
	LAB	6.06^{a}	2.79	2.42
	E	5.06^{b}	2.79	2.86
	LAB+E	5.59^{ab}	2.42	2.37
Mid flowering	Control	4.53^{c}	2.6	2.60^{a}
	LAB	6.14^{a}	2.57	2.22^{b}
	E	5.27^{b}	2.81	2.67^{a}
	LAB+E	5.99^{a}	2.43	2.39^{ab}
Full flowering	Control	5.86^{b}	3.35	2.48
	LAB	6.79^{a}	3.36	2.38
	E	5.40^{b}	3.48	2.65
	LAB+E	6.86^{a}	3.12	2.16
SEM		0.46	0.57	0.23
Maturity (M)		<0.01	<0.01	<0.01
Treatment (T)		<0.01	0.98	0.05
M x T		<0.01	0.01	<0.01

Table 4. Results of the aerobic stability test (5 days) of the alfalfa silages

Factor		pH	CO_2, g kg^{-1} DM	Yeast, log CFU g^{-1} DM	Molds, log CFU g^{-1} DM
Maturity (M)					
Early flowering		5.19[b]	26.88[ab]	5.82	5.73
Mid flowering		5.61[a]	25.71[b]	5.92	5.79
Full flowering		5.73[a]	27.58[a]	5.94	5.63
SEM		0.23	0.28	0.12	0.32
Treatment (T)					
Control		5.53	28.29[a]	6.00	5.80
LAB		5.39	25.75[b]	5.81	5.71
E		5.54	26.26[b]	5.92	5.73
LAB+E		5.58	26.59[b]	5.86	5.63
SEM		0.27	0.33	0.13	0.37
M x T					
Early flowering	Control	5.30	29.57	5.92	5.92
	LAB	5.12	24.28	5.82	5.68
	E	5.16	27.23	5.68	5.73
	LAB+E	5.17	26.63	5.88	5.60
Mid flowering	Control	5.65	27.22	6.00	6.01
	LAB	5.45	23.22	5.67	5.71
	E	5.71	28.33	6.10	5.64
	LAB+E	5.65	24.54	5.92	5.81
Full flowering	Control	5.64	26.29	6.07	5.46
	LAB	5.61	22.00	5.94	5.74
	E	5.76	23.17	5.97	5.82
	LAB+E	5.91	21.35	5.79	5.48
SEM		0.04	1.89	0.06	0.11
Maturity (M)		<0.01	0.01	0.34	0.38
Treatment (T)		0.12	0.01	0.29	0.68
M x T		0.47	0.03	0.26	0.35

Table 5. *In vitro* OM digestibility of the ensiled alfalfa after 45 days of ensiling (%)

Factor		OMS
Maturity (M)		
Early flowering		62.26[a]
Mid flowering		61.52[a]
Full flowering		58.37[b]
SEM		0.31
Treatment (T)		
Control		60.02[b]
LAB		60.50[ab]
E		60.94[ab]
LAB+E		61.39[a]
SEM		0.36
M x T		
Early flowering	Control	62.08
	LAB	61.97
	E	62.58
	LAB+E	62.40
Mid flowering	Control	60.74
	LAB	61.23
	E	61.50
	LAB+E	62.60
Full flowering	Control	57.23
	LAB	58.32
	E	58.73
	LAB+E	59.18
SEM		0.63
Maturity (M)		<0.01
Treatment (T)		0.63
M x T		0.841

The previous literature emphasizes that the NH_3-N in silages indicates the level of protein degradation as extensive proteolysis has an adverse effect on the ruminants' nitrogen utilization (McDonald *et al*., 1991). The NH_3-N was also recorded as lower in silages treated with LAB, E and LAB+E compared to that in untreated silage. It was remarkable that the levels of NH_3-N formation in all treated silages in the present study were determined to be under the threshold level of 80 g/kg of total nitrogen per good quality silages (McDonald *et al*., 1991).

The lactic and acetic acid content in alfalfa silage at full flowering stage was lower ($P<0.01$) than those observed at the other stages. The concentration of lactic and acetic acid of silages ranged from 33.06 to 52.47 and 10.26 to 24.38 g/kg DM, respectively. Regarding those data, it was noted in the study that the acetic acid concentration was lower ($P<0.05$), whereas the lactic acid concentration was higher ($P<0.05$) in silages treated with LAB when compared with the control group. The lactic and acetic acid concentrations were significantly higher at early and mid flowering stages than

those observed at full flowering stage (P<0.05). Those findings comply with the data indicated by Kozelov *et al*. (2008), who reported an increase in lactic acid concentration in LAB and LAB+E treated low-dry matter alfalfa silage when compared with the untreated silage. The acetic acid content was significant as it was also lowered in LAB+E and LAB treated silages compared with E-treated or untreated silages (P<0.01). These data were also confirmatory to the results of Sucu and Aydogan Ciftci (2016), who mentioned the decrease in acetic acid content due to LAB+E treatment at triticale silage. The NDF content however was found to increase from 36.89% to 45.28% at the stages of early and full flowering, respectively. The ADF, ADL, hemicellulose and cellulose contents were observed to have similar tendencies to increase as their levels were higher at the same stages of crop development. It was significant that LAB and/or enzyme treatments lowered the NDF, ADF and cellulose (P<0.05). The least amounts of NDF, ADF and cellulose were observed for silages treated with LAB+enzymes at early flowering stage (P<0.05). In some studies, E or LAB+E mixture inoculants were indicated to decrease cell wall contents of silages (Nadeau *et al*., 2000a,b, Filya *et al*., 2001, Ozduven 2010, Paya *et al*., 2015). In contrast to those findings, some other authors mentioned the lowering effect of inoculants on cell wall contents of silages as in significant (Meeske *et al*., 1993; Basmacioglu *et al*., 2003).

As a third step in analyses, the microbiological compositions of the silages were determined, which was proceeded by aerobic stability test and finalized by *in vitro* OMD tests. The microbiological composition of the silages is given in Table 3. Regarding the table above, after 45 days of ensiling, *lactobacilli* counts were noted to increase (P<0.05) by both LAB inoculants (LAB and LAB+E treatment) though yeast counts were not affected by the treatments (P>0.05). Both LAB inoculants (LAB and LAB+E) decreased mold counts of alfalfa silages compared to the E-treatment silages. However, there were higher amounts of *lactobacilli* and yeast in silages at early flowering stage than those observed at other stages. Those findings agree with the data revealed by Spoelstra (1991), Filya (2003), Sucu and Filya (2006), Hassanat *et al*. (2007) and Ozduven *et al*. (2009). Table 4 displays the results of the aerobic exposure test for the alfalfa silages. The literature emphasizes that the pH change, CO_2 production and an increase in yeast and mold figures are the indicators of silage deterioration. As seen in Table 4, the present research displays no change in pH value, yeast and mold numbers in the alfalfa silages (P>0.05), yet CO_2 production was lower (P<0.05) in silages with LAB and/or E treatment when compared with the control groups. It was remarkable that CO_2 production was seen in all stages of silage including the control groups. The yeast population observed particularly at this stage had a negative effect on the aerobic stability of silages, resulting in CO_2 production. This finding is in agreement with the research by Seale (1986), which mentions the primary reason for CO_2 production to be the yeast numbers. Furthermore, there was a slight increase detected in pH values of alfalfa during that 5-day period when silage deterioration occurred. The values for *in vitro* OMD are specified in Table 5. Table 5 indicates that *in vitro* OMD was significantly higher (P<0.05) in silages treated with LAB+E when compared with the control groups. However, there were higher amounts of *in vitro* OMS in silages at the early and mid flowering stages than the amounts at full flowering stage. There were no interactions (P>0.05) observed between the maturity and the treatments.

In contrast to some researches expressing no effect of LAB on ruminal OM degradability or on the digestibility of silages (Nadeu *et al*., 2000a, Hristov and McAllister, 2002); some other authors underlines the positive effect of LAB treated silage which improved degradability or digestibility (Weinberg *et al*., 1995, Nadeu *et al*., 2000b). In the present research, there was no effect observed on *in vitro* OMD of silages due to the addition of LAB and/or E. This finding confirms the concluded idea in literature stating that LAB or E treatment did not cause significant effects on *in vitro* OMD of silages (Weinberg *et al*., 1995, Nadeu *et al*., 2000b).

Conclusion

The results of this study confirmed that LAB and/or E inoculants increased the characteristics of fermentation and aerobic stability, while decreasing NDF, ADF and cellulose contents of alfalfa silages. The study revealed that there was no treatment effect on any variables measured for *in vitro* OMD.

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REFERENCES

Anonymus, 1986. The Analysis of Agricultural Materials: A Manual of the Analytical Methods Used by the Agricultural Development and Advisory Service. 3rd Edn., H.M. Stationery Office, London, ISBN: 9780112427629, pp: 427-428.

AOAC, 1990. Official Methods of Analysis. 15th Edn., Association of Official Analytical Chemists, Washington, DC., USA., Pages: 684.

Ashbell, G., Weinberg, Z.G., Azrieli, A., Hen, Y. and Horev, B., 1991. A simple system to study the aerobic deterioration of silages. *Can. Agric. Eng.*, 34: 171-175.

Aufrere, J. and Michalet-Doreau B., 1988. Comparison of methods for predicting digestibility of feeds. *Anim. Feed Sci. Technol.*, 20: 203-218.

Basmacioglu, H., Ergul, M. and Karaayvaz, B. K., 2003. Effect of Bacteria and Enzyme Mixture Inoculant on Quality and Feeding Value of Maize Silage. *J. Applied Anim. Research*, 24:1, 49-58.

Collins, M., 1983.Wetting and maturity effects on the quality of legume hay. *Agron J.,* 75:523–527.

Çiftçi, M., Çerçi, I. H., Dalkılıç, B., Güler, T., Ertaş, O. N., 2005. Elmanın karbonhidrat kaynağı olarak yonca silajına katılma olanağının araştırılması. *YYÜ Vet. Fak. Derg.*, 16 (2):93-98.

Filya, I., 2003. The effect of *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria, on the fermentation, aerobic stability and ruminal degradability of wheat, sorghum and maize silages. *J.Appl. Microbiol.*, 95:1080-1086.

Filya, I., Ashbell, G., Weinberg, Z. G., Hen, Y. 2001. Hücre duvarını parçalayıcı enzimlerin yonca silajlarının fermantasyon özellikleri, hücre duvarı kapsamı ve aerobik stabiliteleri üzerine etkileri. *Tarim Bilimleri Dergisi*, 7 (3), 81-87.

Filya, I., Muck, R. E. and Contreras-Govea, F. E., 2007. Inoculant effects on alfalfa silage: fermentation products and nutritive value. *J. Dairy Sci.*, 90:5108–5114.

Goering, H.K. and van Soest, P. J., 1970. Forage Fiber Analyses (Apparatus, Reagents, Procedures and Some Applications). *Agricultural Handbook*, No. 379, U.S. Government Printing Office, Washington, DC.

Hassanat, F., Mustafa, A.F. and Seguin, P., 2007. Effects of inoculation on ensiling characteristics, chemical composition and aerobic stability of regular and brown midrib millet silages. *Anim. Feed Sci. Tech.*, 139: 125-140.

Hristov, A.N. and McAllister, T.A., 2002. Effect of inoculants on whole-crop barley silage fermentation and dry matter disappearance in situ . *J. Anim. Sci.,* 80: 510-516.

Knežević, J., Milenković, M., Milošević, B., Beković, D., Aksić, M., Jašović, B., 2011. Suitability of alfalfa and orchard grass for ensilage. *Biotechnology in Animal Husbandry*, 27(3):1315-1320.

Koc, F. and Coskuntuna, L., 2003. The comparison of the two different methods on the determination of organic acids in silage fodders. *J. Anim. Prod.,* 44: 37-47.

Kozelov, L. K., Iliev, F., Hristov, A. N., Zaman, S. and McAllister, T. A., 2008. Effect of fibrolytic enzymes and an inoculant on *in vitro* degradability and gas production of low-dry matter alfalfa silage. *J Sci Food Agric,* 88:2568–2575.

Marsh, R., 1979.The effects of wilting on fermentation in the silo and on the nutritive value of silage. *Grass Forage Sci* 34:1–10 (1979).

McAllister, T. A., Fenjuk, R., Mir, Z., Selinger L. B. and Cheng, K. J., 1998. Inoculants for alfalfa silage: Effects of aeorobic stability, digestibility and the growth performance of feedlot steers. *Livestock Prod. Sci.*, 53:171-181.

McDonald, P., Henderson A.R. and Heron, S.J.E., 1991. The Biochemistry of Silage. *2nd Edn., Chalcombe Publications*, Marlow, Bucks, UK., ISBN-13: 9780948617225, Pages: 340.

Meeske, R., Ashbell, G., Weinberg, Z.G. and Kipnis, T., 1993. Ensiling forage sorghum at two stages of maturity with the addition of lactic acid bacterial inoculants. *Anim. Feed Sci. Technol.*, 43:165-175.

Muck, R. E., and Kung, Jr. L., 1997. Effects of silage additives on ensiling. Proc. form the Silage: *Field to Feedbunk North American Conference. NRAES -99,* Pp 187-199.

Muck, R.E., 1987. Dry matter level effect on alfalfa silage quality: I. Nitrogen transformations. *Trans ASAE,* 30:7–14.

Muck, R.E., Filya, I., Contreras-Govea, F.E:, 2007. Inoculant effects on alfalfa silage: In vitro gas and volatile fatty acid production. *J. Dairy Sci.*, 90, 5115-5125.

Nadeau, E.M.G., Buxton, D.R., Russell, J.R., Allison, M.J. and Young, J.W., 2000a. Enzyme, bacterial inoculant, and formic acid effects on silage composition of orchardgrass and alfalfa. *J. Dairy Sci.,* 83:1487-1502.

Nadeau, E.M.G., Russell J.R. and Buxton, D.R., 2000b. Intake, digestibility, and composition of orchardgrass and alfalfa silages treated with cellulase, inoculant, and formic acid fed to lambs. *J Anim. Sci*, 78: 2980-2989.

Nkosi, B. D., Vadlani, P. V., Brijwani, K., Nanjunda, A. and Meeske, R., 2012. Effects of bacterial inoculants and an enzyme on the fermentation quality and aerobic stability of ensiled whole-crop sweet sorghum *South African J. Anim.Sci.,* 2012, 42 (3):232-240.

Ozduven, M. L., Koc, F., Polat C. and Coskuntuna, L., 2009. The effects of lactic acid bacteria and enzyme mixture inoculants on fermentation and nutrient digestibility of sunflower silage. Kafkas Univ. Vet. Fak. Derg, 15: 195-199.

Ozduven, M. L., Kursun Onal, Z., Koc, F., 2010. The effects of bacterial inoculants and/or enzymes on the fermentation, aerobic stability and *in vitro* dry and organic matter digestibility characteristics of triticale silages. *The Journal of the Faculty of the Veterinary Medicine University of Kafkas*, 16 (5): 751-756.

Ozturk, D., Kizilsimsek, M., Kamalak, A., Canbolat, O.,Ozkan, C.O., 2006. Effects of ensiling alfalfa with whole-crop maize on the chemical composition and nutritive value of silage mixtures. Asian-Australasian Journal of Animal Sciences, 19(4): 526-532.

Paya, H., Taghizadeh, A., Lashkari, S., 2015. Effects of *Lactobacillus plantarum* and hydrolytic enzymes on fermentation and ruminal degradability of orange pulp silage. *J. BioSci. Biotechnol.*, 4(3): 349-357.

Schmidt, J., Szakacs, G., Cenkvari, E., Sipocz, J., Urbanszki K., Tengerdy, R.P., 2001. Enzyme assisted ensiling of alfalfa with enzymes by solid substrate fermentation. *Bioresource Technology*, 76:207-212.

Seale, D., 1986. Bacterial inoculants as silage additives. *J. Appl. Bacteriol.,* 61(Suppl): 9-26.

Spoelstra, S.F., 1991. Chemical and biological additives in forage conservation. In: Pahlow, G., Honig, H., Eds. Forage Conservation Towards 2000. Inst. Grassl. Forage Res., Braunschweigh, Germany, 48-70.

SPSS, 2007, SPSS 15 for Windows. SPSS Inc.

Sucu, E. and Filya, I., 2006. The effects of bacterial inoculants on the fermentation, aerobic stability and rumen degradability characteristics of wheat silages. *Turk J. Vet. Anim. Sci.*, 30, 187-193.

Sucu, E., Aydoğan Çifci, E., 2016. Effects of lines and inoculants on nutritive value and production costs of triticale silages. *R. Bras. Zootec*., 45(7):355-364.

Tengerdy, R. P., Weinberg, Z. G., Szakacs, G., Wu, M., Linden, J. C., Henk, L. L. and Johnson, D. E., 1991. Ensiling alfalfa with additives of lactic acid bacteria and enzymes. *J. Sci. Food Agric.*, 55:215-228.

Tyrolová, Y., Výborná, A., 2008. Effect of the stage of maturity on the leaf percentage of lucerne and the effect of additives on silage characteristics *Czech J. Anim. Sci.,* 53, 2008 (8): 330–335.

Weinberg, Z.G., Ashbell, G., Hen, Y. and Azrieli, A. 1995. The effect of cellulase and hemicellulase plus pectinase on the aerobic stability and fibre analysis of peas and wheat silages. *Anim. Feed Sci. Technol*., 55: 287-293.

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