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RESEARCH ARTICLE

The Effects of Lactic Acid Bacteria and Enzyme Mixture Inoculants on Fermentation and Nutrient Digestibility of Sunflower Silage

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Summary

This study was carried out to determine the effects of lactic acid bacteria and lactic acid bacteria+enzyme mixture inoculants as silage additives, on the fermentation and nutrient digestibility in lambs fed sunflower silage. Pioneer 1174 (Iowa, USA), and Sil-All (Alltech, UK) were used as lactic acid bacteria and lactic acid bacteria+enzyme mixture inoculants, respectively. Inoculants were applied at 6.00 log10 cfu/g silage levels. Sunflower was harvested at the dough stage and ensiled in 120 litre capacity plastic containers. Three plastic container from each group were sampled for chemical and microbiological analyses on day 60 after ensiling. In addition, in vivo nutrition digestibility of silages were determined. Both inoculants increased fermentation qualities of sunflower. Lactic acid bacteria+enzyme decreased (P<0.002) neutral detergent fiber content and increased in vivo organic matter and acid detergent fiber digestibility of silages (P<0.05).

Keywords: *Sunflower silages, Lactic acid bacterial inoculants, Lactic acid bacteria + enzyme inoculants, Fermentation, Digestibility*

Laktik Asit Bakterisi ve Enzim Karışımı İnokulantlarının Ayçiçeği Silajlarının Fermantasyonu ve Besin Maddelerinin Sindirilebilirliği Üzerine Etkileri

Özet

Bu çalışma silaj katkı maddesi olarak kullanılan laktik asit bakterisi ve laktik asit bakterisi +enzim karışımı inokulantların, ayçiçeği silajlarının fermantasyon ve toklularda besin maddelerinin sindirilebilirlik özellikleri üzerindeki etkilerinin saptanması amacı ile düzenlenmiştir. Laktik asit bakterisi inokulantı olarak Pioneer 1174 (Iowa, USA) ve laktik asit bakterisi +enzim karışımı inokulantı olarak ise Sil-All (Allteck, UK) kullanılmıştır. İnokulantlar silajlara 6.00 log10 cfu/g düzeyinde katılmışlardır. Ayçiçeği, hamur olum döneminde hasat edilmiş ve 120 litrelik plastik bidonlarda silolanmıştır. Silolamadan sonraki 60. günde her gruptan 3' er plastik bidon açılarak silajlarda kimyasal ve mikrobiyolojik analizler yapılmıştır. Ayrıca bu silajların, besin maddelerinin sindirilebilirlikleri saptanmıştır. Sonuç olarak her iki inokulant da, ayçiçeği silajlarının fermantasyon özelliklerini artırmıştır. Laktik asit bakterisi +enzim karışımı inokulantı nötral deterjanlarda çözünmeyen lif içeriklerini azaltmış (P<0.002), in vivo organik madde ve asit deterjanlarda çözünmeyen lif sindirilebilirliklerini artırmıştır (P<0.05).

Anahtar sözcükler: *Ayçiçeği hasılı silajı, Laktik asit bakteri inokulantı, Laktik asit bakteri + Enzim inokulantı, Fermantasyon, Sindirilebilirlik*

INTRODUCTION

Ensiling is a preservation technology for moist whole-plant forage crops which is based on lactic acid fermentation under anaerobic conditions, whereby lactic acid bacteria (LAB) convert water-soluble carbohydrates (WSC) into organic acids, mainly lactic acid. As a result, pH decrease and thus forage is preserved for a long time [1]. The application of silage additives has become the conventional implement to control the ensiling process. Although the main objective in using silage additives is to ensure the fermentation process to produce well preserved silages, attention is also paid to methods of reducing ensiling losses and improving aerobic stability of silages during the feed-out period [2]. In order to improve the ensiling process various chemical and biological

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additives have been developed. Biological additives are advantageous because they are safe and easy to use, are non-corrosive to machinery, do not pollute the environment, and are natural products [3]. Bacterial inoculants generally increase lactic acid and reduce pH, acetic acid, butyric acid and ammonia-nitrogen levels in silage [4,5]. Inoculation of forage crops with homofermentative LAB can improve silage fermentation if sufficient fermentable substrate (WSC) is available. Enzyme (E) mixture can partially degrade plant carbohydrates (cellulose, hemicellulose, pectin and starch) to release sugars for bacteria fermentation and should, therefore, act additively with inoculants LAB [6]. When LAB is combined with cell wall degrading enzymes a stronger effect should be expected by releasing fermentable sugars to produce more lactic acid in proportion to other products [7-10].

Sunflowers have been grown successfully as silage crop in many parts of the world. Sunflower, in comparison to corn, provides high dry matter yield and has better cold tolerant and more drought resistant. High fiber content of sunflower silage cause decreases in digestibility of nutritient matters [11].

The aim of this study was to determine the effects of LAB and LAB+E mixture silage inoculants on sunflower silage fermentation characteristic, cell wall contents and digestibility of nutrients in lambs.

MATERIAL and METHODS

Sunflower was harvested at the dough stage, to a dry matter (DM) content of approximately 238 g/kg. The chopped sunflower was mixed and divided into equal portions for application of three treatments: (1) distilled water, denoted as treatment control; (2) inoculant, a mixture of LAB consisting of *Lactobacillus plantarum* and *Enterococcus faecium* applied at a rate of 6.00 log10 cfu LAB/g of fresh forage (Pioneer 1174, USA), treatment LAB; (3) inoculant+enzymes, a mixture of LAB consisting of *Pediococcus acidilactici, Lactobacillus plantarum, Streptococcus faecium* and cellulase, amylase, hemicellulase and pentosanase enzymes applied at a rate of 6.00 log10 cfu LAB/g of fresh forage (Sil All, Altech, UK), treatment LAB+E. The application rate determined by the manufacturers stated the level of LAB in the products. On the day of the experiment, inoculants were suspended in 600 ml of tap water and the whole suspension was sprayed over 360 kg (wet weight) of chopped forage spread over a 5x6 m area. All inoculants were applied to the forages in a uniform manner with constant mixing. The control silage was treated with an equivalent amount of water. After sufficient mixing, silage materials were ensiled in nine plastic containers (120 liter, 3 replicates). After 60 days, the silo was opened and silage samples were taken for chemical and microbiological analysis.

To determine the digestibility of the sunflower silage, three Turkgeldi lambs with average 42.4±1.9 kg body weight were used in the study. The animals were offered sunflower silage and ad libitum intake throughout all study. The animals housed in individual cages were fed on a daily base at 08:00 in the morning and at 17:00 in the evening as two meals with water ad libitum. The experimental design was a 3x3 latin square design in which 10 days of dietary adaptation was followed by 7 days of faeces collection in each period. Lambs were equipped with the bags for the faeces collection. For digestibility trial, each animal's faeces was weighed daily and a 10% aliquot retained, composited and frozen. Composited samples were subsequently dried in a forced air oven at 60°C at 48 h. The fresh faeces were then completely mixed and a sample taken for chemical analysis.

pH in fresh material and silage samples was measured according to the British standard method [12]. Buffering capacity (Bc) in fresh material was estimated as described by Playne and McDonald [13]. The ammonia nitrogen (NH_3-N) content of silages was determined, according to Anonymus [12]. The WSC content of silages was determined by spectro-photometer (Shimadzu UV-1201, Kyoto, Japan) after reaction with an antron reagent [12]. Lactic and acetic acid were determined by the spectro-photometric method [14]. LAB, yeast and mould counts were obtained according to the methods reported by Seale et al.[15]. The microbiological examination included enumeration of LAB on pour plate Rogosa agar (Oxoid CM627 incubated at 30°C for 3 days), yeast and moulds on spread plate malt extract agar (acidified with LA to pH 4.0 and incubated at 30°C for 3 days). The LAB, mould and yeast counts of the silages were converted into logaritmic coli-form unit (cfu/g). DM content of the fresh material, silage and faces samples was determined by oven drying for 72 h at 60°C, followed by milling through

a 1-mm screen and drying for another 3 h at 103°C. Then, contents of the DM, organic matter (OM), crude protein (CP), ether extract (EE) and ash were determined following the procedure of Association of Official Analytical Chemists [16]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to the method of Goering and Van Soest [17]. Moreover, values of the DM, OM, CP, EE, NDF and ADF of the faeces samples were analyzed by the same method [16,17].

The statistical analysis was performed by using a one-way analysis of variance and 3x3 Latin-Square. Moreover, Duncan's test was used for multiple range tests [18]. Fermentation characteristic and nutritient contents of the sunflower silage were investigated with one factor analysis variance. In vivo nutritient digestibility of the sunflower silage was studied 3x3 Latin-Square experimental designs. At the same time, the statistical analysis performed with the Minitap statistical package programme [19].

RESULTS

The chemical and microbiological composition of the fresh and ensiled sunflower silage is given in *Table 1*.

Both inoculants significantly increased the lactic acid levels (P<0.005) and decreased pH (P<0.005), acetic acid (P<0.018) and NH_3-N (P<0.01) levels. Silages treated with LAB and LAB+E mixture inoculants had a higher (P<0.01) lactic acid/acetic acid ration than that of control silages. No butyric acid was present in any of the silages. WSC levels was higher (P<0.05) in silages treated with LAB+E mixture inoculants than in control silages. The counts of LAB and yeast were higher (P<0.01) on the both inoculants treated silage compared to control silage. OM, CP and EE contents of silages were generally similar (P>0.05). NDF contents were lower (P<0.002) in silages treated with LAB+E mixture inoculants than control and LAB silages

Table 1. *Chemical and microbiological analysis of the sunflower silages*
Tablo 1. *Ayçiçeği silajlarına ait kimyasal ve mikrobiyolojik analiz sonuçları*

Item	At time of ensiling	Control	LAB	LAB+E	SEM	P
pH	5.74	4.22a	3.99b	3.96b	0.06	0.005
Bc, mEq NaOH/kg DM	146.50	-	-	-	-	-
DM, % in FM	24.17	23.92	23.41	23.64	0.52	0.525
NH_3-N, g/kg TN	-	81.34a	68.47b	65.46b	4.40	0.010
WSC, g/kg DM	52.35	19.68b	21.29ab	25.16a	1.95	0.034
Lactic acid, % DM	-	5.96b	7.56a	7.94a	0.48	0.005
Acetic acid, % DM	-	1.57a	1.47ab	1.42b	0.04	0.019
Lactic/acetic acid ratio	-	3.79b	5.12a	5.59a	0.39	0.003
LAB, log_{10} cfu/g DM	3.06	3.90b	6.70a	6.32a	0.38	<0.001
Yeast, log_{10} cfu/g DM	2.59	3.28b	3.89a	3.79a	0.16	0.007
Moulds, log_{10} cfu/g DM	3.15	2.98a	1.72b	1.76b	0.26	0.002
CA, % DM	11.46	11.87	11.12	10.91	0.61	0.207
OM, % DM	88.54	88.13	88.88	89.09	0.61	0.207
CP, % DM	9.82	9.91	9.53	9.56	0.31	0.329
EE, % DM	9.77	9.72	10.12	9.91	0.62	0.758
NDF, % DM	46.30	44.97a	43.62a	40.25b	0.96	0.002
ADF, % DM	35.32	36.53	36.54	34.57	1.90	0.403

LAB: *Lactic acid bacteria;* ***LAB+E:*** *Lactic acid bacteria+enzyme;* ***Bc:*** *Buffering Capacity;* ***DM:*** *Dry Matter;* ***NH3-N:*** *Ammonia Nitrogen;* ***FM:*** *Fresh material;* ***NH_3-N****: Ammonia nitrogen;* ***WSC:*** *Water Soluble Carbohydrate;* ***cfu:*** *Colony forming unit;* ***CA:*** *Crude ash;* ***OM:*** *Organic Matter;* ***CP:*** *Crude Protein; EE:Ether extract;* ***NDF:*** *Neutral detergent fiber;* ***ADF:*** *Acid detergent fiber*
a,b: *Within a column means followed by a different letter differ significantly, P<0.05*

Table 2. *In vivo digestibility of nutritients of the sunflower silages, %*
Tablo 2. *Ayçiçeği silajlarının in vivo besin maddeleri sindirilebilirlikleri %*

Digestibility of nutrients (%)	Control	LAB 15	LAB+E	SEM	P
DM	53.32	53.59	55.49	0.92	0.053
OM	54.23b	55.79ab	57.20a	0.80	0.012
CP	55.54	57.71	60.22	1.89	0.061
EE	83.98	79.95	82.52	1.57	0.051
NDF	46.75	47.02	48.08	1.60	0.590
ADF	35.39b	34.90b	38.46a	1.11	0.015

LAB: *Lactic acid bacteria;* ***LAB+E:*** *Lactic acid bacteria+enzyme;* ***DM:*** *Dry Matter;* ***OM:*** *Organic Matter;* ***CP:*** *Crude Protein;* ***EE:*** *Ether extract;* ***NDF:*** *Neutral detergent fiber;* ***ADF:*** *Acid detergent fiber*
a,b: *Within a column means followed by a different letter differ significantly, P<0.05*

(P<0.05). However, the amount of OM, CP, EE and ADF (%) contents of silages were similar between groups (P>0.05).

In vivo digestibility of the sunflower silages are presented in *Table 2*.

The in vivo digestibility of OM and ADF were higher (P<0.05) in silages treated with LAB+E mixture inoculants than control and LAB silages (P<0.05). However, in vivo DM, CP, EE and NDF digestibility of silages were similar between groups (P>0.05).

DISCUSSION

The sunflower forage used for ensiling was characterized by DM content of 24.17% (in fresh matter), CP content of 9.82% (in DM) and WSC content of 52.35% (in DM). The composition of structural carbohydrate in the cell wall was 46.30% NDF (in DM) and 35.20% ADF (in DM). The chemical composition of the sunflower forage used in the present study is consistent with values reported by Demirel et al.[18]. pH and Bc value was 5.74 and 146.50 mEq NaOH/kg DM, respectively. The higher the Bc of forage, the longer it takes to ensile and more WSC are required. The Bc of fresh material was quite low. These findings are agreement with those reported by Özdüven and Öğün [20].

There are different reports about the effect of LAB and LAB+E mixture inoculants on silage fermentation [21-29]. It is generally reported that microbial inoculation to silage has a positive effect on the silage fermentation. When forages are inoculated with LAB and LAB+E before ensiling, resulting silage usually has a lower pH, and a higher concentration of lactic acid, but lower concentrations of acetic acid and NH_3-N than control silage [21-27]. The DM content of sunflower silages was not significantly different, but the DM content of control silage (23.92%) was higher than that of both inoculants silage. These findings are agreement with those reported by Kung et al.[28] and Kamarlory and Teimouri Yansari [29]. The result indicates that silage treated with LAB and LAB+E mixture inoculants had lower pH, NH_3-N and acetic acid contents than that of control silage and a higher lactic acid concentration and lactic acid/acetic acid ration than that of control silages. However, LAB and LAB+E mixture inoculants improved microbiological composition of sunflower silages compared with control silage. Both inoculants increased LAB and decreased mould number of sunflower silages compared with the control silage. The lower count of yeasts in the control silage, as compared to inoculated silage, is likely the result of the higher concentration of acetic acid in the control silage. These findings are agreement with those reported by Filya [25,30] and Baskavak et al.[31]. Including cell wall degrading enzymes in silage additives has been practise as a means of increasing the concentration of WSC available to LAB, and as a method to degrade cell wall and subsequently improve the digestibility of OM and fiber [2,32]. In some studies, LAB+E mixture inoculants decreased cell wall contents of silages [4,9,10,25,27]. In contrast to these researcher's findings, some reports show that inoculants did not decrease significantly cell wall contents of silages [7,26,33,34]. In the present study, compared to control and LAB silage, treatment with LAB+E significantly decreased NDF content, while WSC content significantly increased.

In vivo ADF digestibility of LAB+E silages was significantly higher than those of control and LAB silages. In vivo OM digestibility of LAB+E silages (57.20%) was different from control silages, but not from LAB silages. Increase in OM digestibility of LAB+E silages could arise from decrease in NDF content of silage materials [11]. DM, CP, EE and NDF digestibility were not affected by inoculation. However, DM, CP and NDF digestibility between LAB+E and control silages were numerically higher (P>0.05). Results with regard to digestibility and degradability have varied. Some reports show that LAB and LAB+E mixture inoculants did not improve DM and OM digestibility or degradability of silages [3,27,35,36], however in some other studies, these inoculants provided an improvement in digestibility or degradability [25,29,34].

In conclusion, the results of this study show that both inoculants increased lactic acid levels and decreased pH, acetic acid and NH3-N levels of sunflower silages. LAB+E mixture inoculants decreased NDF content and increased in vivo OM and ADF digestibility of sunflower silages in lambs.

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