Kafkas Univ Vet Fak Derg
16 (5): 751-756, 2010
DOI:10.9775/kvfd.2009.1628

RESEARCH ARTICLE

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

Mehmet Levent OZDUVEN * Zeynep KURSUN ONAL * Fisun KOC *

* Namik Kemal University, Agricultural Faculty, Department of Animal Science, TR-59030 Tekirdağ - TÜRKİYE

Makale Kodu (Article Code): KVFD-2009-1628

Summary

This study was carried out to determine the effects of lactic acid bacteria inoculant, enzymes and lactic acid bacteria inoculant-enzymes mixture on the fermentation, cell wall content, aerobic stability and *in vitro* dry and organic matter digestibility characteristics of triticale (x*Triticosecale* Wittmack) silages. Triticale was harvested at the milk stage of maturity. Pioneer -1188 (Iowa, USA), enzyme (Global Nutritech, TR) and Sil-All (Alltech, UK) were used as lactic acid bacteria, enzyme and lactic acid bacteria+enzyme mixture inoculants. Inoculants were applied to silages at 6.00 $\log_{10}$ cfu/g levels. After treatment, the chopped triticale was ensiled in 1.0 liter special anaerobic jars, equipped with a lid enabling gas release only. Three jars from each group were sampled for chemical and microbiological analysis on days 2, 5, 8 and 45 after ensiling. At the end of the ensiling period (45^{th} day) all silages were subjected to an aerobic stability test for 5 days. In addition, *in vitro* dry and organic matter digestibility of these silages were determined. Both inoculants and enzymes increased characteristics of fermentation but impaired aerobic stability of triticale silages. Enzymes and lactic acid bacteria+enzymes mixture inoculant decreased neutral detergent fibre content and increased *in vitro* dry and organic matter digestibility of silages.

Keywords: *Triticale, Lactic acid bacterial inoculants, Enzyme, Fermentation, Aerobic stability*

Bakteriyal İnokulantlar ve/veya Enzimlerin Tritikale Silajlarında Fermantasyon, Aerobik Stabilite ve *in vitro* Kuru ve Organik Madde Sindirilebilirliği Üzerine Etkileri

Özet

Bu çalışma silaj katkı maddesi olarak kullanılan laktik asit bakteri inokulantı, enzim ve laktik asit bakteri+enzim karışımı inokulantların, tritikale (x*Triticosecale* Wittmack) silajlarının fermantasyon, aerobik stabilite ve *in vitro* kuru ve organik madde sindirilebilirlik özellikleri üzerindeki etkilerinin saptanması amacı ile düzenlenmiştir. Laktik asit bakteri inokulantı olarak Pioneer-1188 (Iowa, USA), enzim (Global Nutritech, TR) ve laktik asit bakteri+enzim karışımı inokulant olarak Sil-All (Allteck, UK) kullanılmıştır. İnokulantlar silajlara 6.00 $\log_{10}$ cfu/g düzeyinde katılmışlardır. Uygulamadan sonra parçalanmış tritikale, yalnızca gaz çıkışına olanak tanıyan 1.0 litrelik özel anaerobik kavanozlara silolanmıştır. Silolamadan sonraki 2, 5, 8 ve 45. günlerde her gruptan üçer kavanoz açılarak kimyasal ve mikrobiyolojik analizler yapılmıştır. Silolama döneminin sonunda (45. gün) açılan tüm silajlara 5 gün süre ile aerobik stabilite testi uygulanmıştır. Ayrıca bu silajların, *in vitro* kuru ve organik madde sindirilebilirlikleri saptanmıştır. Sonuç olarak her iki inokulant ve enzim, tritikale silajlarının fermantasyon özelliklerini artırmıştır. Enzim ve laktik asit bakteri+enzim karışımı inokulantları silajların nötral deterjanlarda çözünmeyen lif içeriklerini azaltmış ve *in vitro* kuru ve organik madde sindirilebilirliklerini artırmıştır.

Anahtar sözcükler: *Tritikale, Laktik asit bakteri inokulantları, Enzim, Fermantasyon, Aerobik stabilite*

INTRODUCTION

Maize silage is a major component of diets fed to dairy cows because of the high energy yield per unit area, relatively high palatability, the ease of mechanization and storage, and the uniformly high feeding value [1,2]. However, on drought prone sandy soils, and in years with insufficient rainfall the yield of maize is very low [7

İletişim (Correspondence)
☎ +90 282 2931442/185
✉ lozduven@nku.edu.tr

to 8 tons dry matter (DM)/ha]. In situations where water is a limiting factor for growing maize, triticale may be an alternative fodder crop. When triticale is harvested as whole crop triticale silage the DM yield ranges between 9 and 11 tons/ha. Therefore, under water limiting conditions it may be attractive to replace forage maize by whole crop triticale silage [3].

Silage additives had been used to improve the silage quality [4]. Inoculations and enzymes are the most popular silage additives. Bacterial inoculants are added to silage in order to stimulate lactic acid fermentation, accelerating the decrease in pH, and thus improving silage preservation [5,6]. Enzymes were originally added to silage to improve fermentation characteristics and animal performance. When fermentable water soluble carbohydrates (WSCs) are limiting, enzyme additives should improve fermentation characteristics by releasing WSCs for use by the lactic acid bacteria during the fermentation process, resulting in silage with a lower final pH and higher lactic acid content [7]. The mixtures of inoculations and enzymes had also been employed to improve silage fermentation quality [8].

Some researchers had also reported the effects of additives on the fermentation and nutritive value of triticale silage [9,10]. In contrast, there is limited study about the effects of inoculant, enzymes and inoculant-enzymes mixture on fermentation and nutritive value of triticale silage. The series of experiments was undertaken to examine the effect of inoculant, enzymes and inoculant-enzymes mixture on the fermentation, aerobic stability and nutritive value of triticale silage.

MATERIAL and METHODS

Triticale (x*Triticosecale* Wittmack) at the milk stage (36.9% DM) was harvested by hand and cropped with laboratory type cropped to about 2.0 cm size and ensiled in 1.0 liter special anaerobic jars (Weck, Wher-Oftlingen, Germany), equipped with a lid that enables gas release only. Each jar filled with about 450 g (wet weight) of cropped forage, without a headspace. There were 48 jars per crop, and they were stored at ambient temperature (22-26°C). Fresh and ensiled material (on days 2, 5, 8 and 45 after ensiled, three jars per treatment for each time) were sampled for chemical and microbiological analysis. 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.[11]. In this system, the numbers of yeasts and molds, change in pH and amount of CO_2 produced during the test are used as aerobic deterioration indicators.

The chopped triticale was mixed and divided into equal portions for application of four treatments: (1) distilled water, denoted as treatment control; (2) inoculant, a mixture of lactic acid bacteria (LAB) consisting of *Lactobacillus plantarum* and *Enterococcus faecium* applied at a rate of 6.00 $\log_{10}$ cfu LAB/g of fresh forage (Pioneer 1188, USA), treatment LAB; (3) enzymes, a mixture of enzymes consisting of cellulase, amylase, hemicellulase and pentosanase enzymes applied at a rate of 0.01 mg/g of fresh forage (Enzyme, Global Nutritech 41600 Kandira, Kocaeli-Turkey), treatment Enzyme; (4) 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 $\log_{10}$ cfu LAB/g of fresh forage (Sil All, Altech, UK), treatment LAB+enzyme. The application rate determined by the manufacturers stated the level of LAB and enzyme in the products. On the day of the experiment, inoculants and enzymes were suspended in 20 ml of tap water and the whole suspension was sprayed over 10 kg (wet weight) of the chopped forage spread over a 1 x 4 m area. All inoculants and enzymes were applied to the forages in a uniform manner with constant mixing.

pH values and ammonia nitrogen (NH_3-N) content of fresh and silage samples was determined, according to Anonymous [12]. The WSCs content of silages was determined by spectrophotometer (Shimadzu UV-1201, Kyoto, Japan) after reaction with an antron reagent [12]. Lactic and acetic acid were determined by the spectrophotometric method [13]. LAB, yeast and mold numbers were obtained according to the methods reported by Seale et al.[14]. The microbiological examination included enumeration of lactobacilli on pour plate Rogosa agar (Oxoid CM627 incubated at 30°C for 3 days), yeast and molds on spread plate malt extract agar (acidified with LA to pH 4.0 and incubated at 30°C for 3 days). The lactobacilli mold and yeast numbers of the silages were converted into logarithmic coli form unit (cfu/g). Fermentation losses were evaluated according to weight loss [15]. The DM content of the fresh and silage materials was determined by drying at 60°C for 72 h in a fan-assisted oven, followed by milling through a 1-mm screen and drying for another 3 h at 103°C. Ash content was obtained after 3 h at 550°C. Crude protein (CP) content were determined following the procedure of Association of Official Analytical Chemists [16]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) was performed according to Goering and Van Soest [17]. *In vitro* DM and organic matter (OM) digestibility of the silages was determined with the procedure reported by Aufrère and Michalet-Doreau [18], with a three-stage technique: Pre-treatment with pepsin in hydrochloric acid (0.2% pepsin in 0.1 N HCl), starch hydrolysis, attack by cellulase (Onozuka R 10 from *Trichoderma viride,* Merck).

The statistical analysis of the results included one-way analysis of variance and Duncan's multiple range tests, which were applied to the results using the Minitab statistical package program [19].

RESULTS

The chemical composition of the fresh and ensiled triticale is given in *Table 1*. The triticale used for ensiling was characterized by DM content of 36.9%, concentration of CP of 9.0% and concentration of WSCs of 63.7 g/kg DM. The composition of structural carbohydrate in the cell wall was 61.2% NDF and 38.3% ADF in DM. All silages were well preserved. The addition of LAB, enzyme and LAB + enzyme mixture at ensiling improved the fermentation parameters of triticale silages, with increasing lactic acid levels, and decreasing acetic acid, NH_3-N and pH values ($P<0.05$) compared to control silage. The WSCs in all silages decreased with the decrease in pH. The addition of LAB, enzyme and LAB + enzyme mixture at ensiling had significantly higher WSCs compared with the control silage ($P<0.05$). After 2 days of ensiling, control silages significantly increased the weight losses than the LAB, enzyme and LAB+enzyme mixture treated silages ($P<0.05$). The same trend was shown at 5, 8 and 45 days of ensiling.

The microbiological composition of the silages is given in *Table 2*. Lactobacilli numbers increased during the fermentation period. In the present study, the LAB and LAB + enzyme mixture treated silages increased lactobacilli and decreased mold numbers of triticale silages compared with the control silage ($P<0.05$). In contrast, lactobacilli and mold numbers of enzyme treated silages did not differ from control silage. The addition of LAB or enzyme had no influence on yeast numbers of the silages ($P>0.05$).

Table 2. *Results of the microbiological analysis of the triticale silages (log cfu/g DM)*

Tablo 2. *Tritikale silajlarının mikrobiyolojik analiz sonuçları (log cfu/g kuru madde)*

Days of Ensiling	Treatment	Lactobacilli	Yeast	Mold
0		3.8	4.0	2.5
2	Control	3.9±0.06 ^{b}	4.2±0.03	2.5±0.09 ^{a}
	LAB	4.0±0.03 ^{a}	4.3±0.06	0.7±0.67 ^{b}
	Enzyme	3.8±0.04 ^{b}	4.1±0.07	NF
	LAB+ Enzyme	4.1±0.02 ^{a}	4.2±0.03	NF
5	Control	4.0±0.03 ^{b}	4.6±0.03	NF
	LAB	4.4±0.04 ^{a}	4.5±0.04	NF
	Enzyme	4.2±0.05 ^{b}	4.3±0.10	0.7±0.67
	LAB+ Enzyme	4.4±0.05 ^{a}	4.5±0.03	NF
8	Control	4.3±0.04 ^{c}	4.9±0.04	0.8±0.77
	LAB	5.4±0.06 ^{a}	4.9±0.04	NF
	Enzyme	5.1±0.07 ^{b}	5.1±0.37	NF
	LAB+ Enzyme	5.4±0.04 ^{a}	4.8±0.06	NF
45	Control	4.6±0.01 ^{b}	5.2±0.08	3.2±0.13 ^{a}
	LAB	6.0±0.04 ^{a}	5.1±0.02	2.3±0.14 ^{b}
	Enzyme	5.7±0.21 ^{a}	5.2±0.04	2.8±0.19 ^{b}
	LAB+ Enzyme	6.1±0.06 ^{a}	5.1±0.06	2.2±0.14 ^{b}

LAB: *lactic acid bacteria;* ***NF:*** *not found*
$^{a\text{-}b\text{-}c:}$ *Within a column means followed by different letter differ significantly (P<0.05)*

Table 1. *Results of the chemical analyses of the triticale silages*

Tablo 1. *Tritikale silajlarının kimyasal analiz sonuçları*

Days of Ensiling	Treatment	pH	DM, %	WSCs, g/kg DM	NH_3-N, g/kg TN	LA, %	AA, %	Weight Loss, %	CP, %	NDF, %	ADF, %
0		5.8	36.9	63.7	22.9	0.9	0.3	-	9.0	61.2	38.3
2	Control	5.6±0.04 ^{a}	36.6±0.56	44.2±1.50	37.6±3.12	3.4±0.12 ^{b}	0.3±0.06	1.6±0.12 ^{a}	9.1±0.14	62.1±0.68	38.0±0.80
	LAB	4.2±0.06 ^{c}	36.4±0.53	47.0±1.25	30.5±3.48	5.0±0.24 ^{a}	NF	0.5±0.02 ^{b}	9.0±0.07	60.5±0.60	37.9±0.71
	Enzyme	5.3±0.04 ^{b}	36.4±0.35	47.9±0.72	31.4±1.96	3.9±0.17 ^{b}	0.4±0.05	0.5±0.03 ^{b}	9.0±0.12	60.9±0.24	37.8±0.53
	LAB + Enzyme	4.2±0.02 ^{c}	36.6±0.26	49.0±1.34	27.5±3.30	4.7±0.10 ^{a}	NF	0.4±0.07 ^{b}	9.0±0.10	61.0±0.27	37.3±0.29
5	Control	5.3±0.05 ^{a}	36.5±0.35	33.2±1.01 ^{b}	54.8±2.81 ^{a}	4.2±0.13 ^{b}	1.6±0.09 ^{a}	2.7±0.05 ^{a}	8.7±0.08	60.8±1.18	38.2±1.03
	LAB	4.1±0.02 ^{b}	36.4±0.16	39.1±1.08 ^{a}	25.4±1.42 ^{c}	6.3±0.25 ^{a}	0.7±0.08 ^{b}	0.9±0.10 ^{b}	9.0±0.06	60.8±1.10	38.3±0.80
	Enzyme	5.0±0.04 ^{a}	36.8±0.24	41.9±0.88 ^{a}	36.9±1.07 ^{b}	6.8±0.17 ^{a}	0.8±0.15 ^{b}	1.0±0.13 ^{b}	9.0±0.07	60.4±0.54	38.2±0.44
	LAB + Enzyme	4.0±0.03 ^{b}	36.5±0.55	43.0±1.12 ^{a}	23.9±2.02 ^{c}	5.8±0.29 ^{a}	1.0±0.13 ^{b}	1.0±0.11 ^{b}	9.0±0.12	59.0±1.06	37.4±0.45
8	Control	5.0±0.03 ^{a}	36.4±0.33	25.9±1.04 ^{b}	82.4±3.73 ^{a}	5.5±0.20 ^{c}	2.5±0.13 ^{a}	3.8±0.09 ^{a}	8.5±0.12	60.6±0.93	38.2±0.53
	LAB	4.1±0.04 ^{c}	36.9±0.42	33.7±1.22 ^{a}	27.6±3.26 ^{c}	7.5±0.34 ^{ab}	1.8±0.08 ^{b}	1.9±0.59 ^{b}	8.8±0.06	60.6±1.76	37.7±0.88
	Enzyme	4.5±0.04 ^{b}	37.0±0.29	35.4±1.03 ^{a}	45.0±1.63 ^{b}	6.6±0.12 ^{b}	1.9±0.08 ^{b}	2.1±0.16 ^{b}	8.9±0.10	59.4±0.38	37.3±1.00
	LAB + Enzyme	4.0±0.03 ^{c}	36.2±0.27	36.6±0.97 ^{a}	38.0±3.53 ^{bc}	8.0±0.23 ^{a}	1.8±0.08 ^{b}	2.1±0.25 ^{b}	9.0±0.11	57.9±1.04	36.1±1.19
45	Control	4.5±0.02 ^{a}	35.5±0.37	14.7±1.41 ^{b}	103.1±7.53 ^{a}	7.3±0.36 ^{b}	5.0±0.18 ^{a}	5.8±0.09 ^{a}	8.5±0.08	60.4±0.68 ^{a}	38.0±0.80
	LAB	3.8±0.04 ^{c}	35.4±0.21	22.9±0.78 ^{a}	57.0±4.11 ^{b}	10.2±0.23 ^{a}	1.9±0.18 ^{b}	3.6±0.59 ^{b}	8.6±0.06	60.8±0.60 ^{a}	37.9±0.71
	Enzyme	4.1±0.03 ^{b}	35.8±0.30	20.4±0.93 ^{a}	53.4±2.12 ^{b}	9.3±0.28 ^{a}	2.6±0.11 ^{b}	3.5±0.11 ^{b}	8.7±0.05	58.7±0.24 ^{ab}	37.1±0.53
	LAB + Enzyme	3.7±0.02 ^{c}	35.5±0.30	25.0±1.01 ^{a}	48.8±4. 20 ^{b}	10.5±0.30 ^{a}	2.4±0.19 ^{b}	3.2±0.25 ^{b}	8.6±0.05	56.8±0.27 ^{b}	36.2±0.29

LAB: *lactic acid bacteria;* ***DM:*** *dry matter;* ***WSCs:*** *water-soluble carbohydrates;* ***NH₃-N:*** *ammonia-nitrogen;* ***TN:*** *total nitrogen;* ***LA:*** *lactic acid;* ***AA:*** *acetic acid;* ***CP:*** *crude protein;* ***NDF:*** *Neutral detergent fiber;* ***ADF:*** *Acid detergent fiber;* ***NF:*** *not found*
$^{a\text{-}b\text{-}c:}$ *Within a column means followed by different letter differ significantly (P<0.05)*

Table 3 gives the results of the aerobic exposure test. pH change, CO_2 production and an increase in yeast and mold numbers are indicators of silage deterioration. In the present study, the LAB or LAB + enzyme mixture treated silages increased significantly CO_2 production and yeast numbers in the triticale silages compared to the control and enzyme silage (P<0.05).

Table 3. *Results of the aerobic stability test (5 days) of the triticale silages*

Tablo 3. *Tritikale silajlarının aerobik dayanıklılık test (5 gün) sonuçları*

Treatment	pH	CO_2, g/kg DM	Yeast log cfu/g DM	Mold log cfu/g DM
Control	5.6±0.14	40.8±1.01 ^{c}	5.7±0.63 ^{b}	4.9±0.19
LAB	5.7±0.14	58.6±2.10 ^{a}	7.5±0.19 ^{a}	5.3±0.48
Enzyme	5.7±0.11	48.8±2.93 ^{bc}	6.9±0.53 ^{ab}	5.0±0.15
LAB + Enzyme	5.8±0.11	55.2±0.83 ^{ab}	7.1±0.17 ^{a}	5.2±0.15

LAB: *lactic acid bacteria*
$^{a\text{-}b\text{-}c:}$ *Within a column means followed by different letter differ significantly (P<0.05)*

Values for *in vitro* DM and OM digestibility are given in *Table 4*. The addition of enzyme (enzyme and LAB + enzyme mixture) at ensiling had significantly higher *in vitro* DM and OM digestibility compared with the control silage (P<0.05). Inoculation with the LAB did not affect *in vitro* DM and OM digestibility (P>0.05).

Table 4. *In vitro DM and OM digestibility of the ensiled triticale after 45 days of ensiling, (%)*

Tablo 4. *Silolamanın 45. gününde tritikale silajlarının in vitro kuru ve organik madde sindirilebilirliği, (%)*

Treatment	DM Digestibility	OM Digestibility
Control	57.6±0.34 ^{b}	60.10±2.11 ^{b}
LAB	58.3±0.54 ^{ab}	61.47±0.99 ^{ab}
Enzyme	60.9±0.20 ^{a}	63.91±0.57 ^{a}
LAB+enzyme	60.7±0.49 ^{a}	64.30±0.84 ^{a}

DM: *Dry matter;* ***OM:*** *Organic matter*
$^{a\text{-}b:}$ *Within a column means followed by different letter differ significantly (P<0.05)*

DISCUSSION

Biological additives such as bacterial inoculants have been added to silage in order to stimulate lactic acid fermentation, accelerating the decrease in pH and thus improving silage preservation [20]. The same trend was shown in this experiment. Both LAB inoculants (LAB and LAB+enzyme) ensured rapid and vigorous fermentation that resulted in faster accumulation of lactic acid, lower pH values at an earlier stage of ensiling, and improved forage preservation. These findings are in agreement with those reported by Sucu and Filya [6], Williams et al.[21], Zahiroddini et al.[22]. Acetic acid and NH_3-N concentration in silage are also important criterions evaluating silage fermentation quality. High concentration of acetic acid (>3-4% of DM) probably leads to poor energy and DM recovery [23]. In the present study, the concentrations of acetic acid and weight losses of triticale silages were significantly increased in control silage compared with other groups. Silage NH_3-N concentration, which reveals the extent of proteolysis in silage, was significantly lower in silage treated with LAB, enzyme and LAB + enzyme compared with control. The low NH_3-N concentration may attribute to the pH sharp decline which made aerobic microorganism and plant enzymes inhibit rapidly, resulting in reduction in protein degradation during fermantation process [24]. Cell wall degrading enzymes, such as cellulases and hemicellulases, applied to herbage before ensiling decreased the cell wall content of ensiled crops [25,26]. Including cell wall degrading enzymes in silage additives has been used to increase WSCs available to LAB and as a method to degrade cell wall and subsequently improve the digestibility of OM and fiber [24,27-29]. In some studies, enzyme and LAB+enzyme mixture inoculants decreased cell wall contents of silages [30-35]. In contrast to these researcher's findings, some reports show that inoculants did not decrease significantly cell wall contents of silages [22,36,37]. At the end of the ensiling period, treatment with enzyme and LAB + enzyme mixture significantly decreased NDF concentration triticale silages compared with the control and LAB silages in present study. The addition of LAB or enzyme had no influence on ADF concentration. WSCs content of triticale silages significantly increased with treatment LAB or enzyme compared to control silage. However, LAB, enzyme and LAB + E mixture inoculants improved microbiological composition of triticale silages compared with control silage. At the end of the ensiling period all the treatment (LAB, enzyme and LAB + enzyme) increased lactobacilli numbers and decreased mold numbers of triticale silages compared with the control silage. The addition of LAB or enzyme had no influence on yeast numbers. The lack of effects in the present study is in agreement with previous findings [6,28,33].

Aerobic deterioration of silage is a complex process which depends on many factors. Usually it is initiated by aerobic yeasts that can use either residual WSCs or lactic acid for their metabolism. Aerobic deterioration usually results in production of CO_2 and consequent DM losses [38]. Both LAB inoculants (LAB and LAB+enzyme mixture) had high contents of both residual WSCs and lactic acid and therefore, tended to spoil more upon aerobic exposure, as indicated by more intensive CO_2 production. These findings are agreement with those

reported by Sucu and Filya [6], Filya [33], Meeske et al.[39].

There are various reports indicating that LAB or enzyme did not effect ruminal DM and OM degrabilities or digestibility of silages [5,32,40]; however in some studies, LAB or enzymes treated silage improved, degradability or digestibility [28,31]. In the present study, the *in vitro* DM and OM digestibility were higher in silages treated with enzymes (enzyme and LAB + enzyme mixture). In contrast, the addition of LAB had no effect on *in vitro* DM and OM digestibility. The lack of effects was in agreement with other studies in which the addition of LAB did not show significant effects on *in vitro* DM or OM digestibility of silage [5,32,40].

In conclusion, the result of this study show that both LAB inoculants and enzyme increased characteristics of fermentation, but impaired aerobic stability of triticale silages. Enzyme and LAB+enzyme mixture inoculants decreased NDF content and increased *in vitro* DM and OM digestibility of triticale silages.

REFERENCES

1. Oshita T, Takayama H, Otsuka H, Igarashi H, Nonaka K, Kume S: Effects of maturing stage of corn hybrids on silage yield, feeding value for dairy cows and milk production in a cold region of Japan. *Asian-Aust J Anim Sci,* 20, 511-516, 2007.

2. Vatandoost M, Danesh Mesgaran M, Valizadeh R, Nasiri Moghaddam H: Effect of whole crop silages (triticale or barley) versus corn silage on performance of Holstein lactating dairy cows. *J Anim Vet Advances,* 6, 344-348, 2007.

3. van Duinkerken G, Zom RLG, Bleumer EJB: The effects of replacing maize silage by triticale whole crop silage in a roughage mixture with grass silage on feed intake and milk production by dairy cows. *Proceedings of the British Society of Animal Science Annual Meeting.* p. 78. Scarborough, 1999.

4. Cai YM, Benno Y, Ogawa M, Ohmomo S, Kumai S, Nakase T: Influence of *Lactobacillus spp.* from an inoculant and of Weissella and *Leuconostoc spp.* from forage crops on silage fermentation. *Appl Environ Microbiol,* 64, 2982-2987, 1998.

5. Filya İ: Silaj kalitesinin arttırılmasında yeni gelişmeler. *International Animal Nutrition Congress'2000,* s. 243-250. Isparta. 2000.

6. Sucu E, Filya I: 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, 2006.

7. Sheperd AC, Kung L: An enzyme additive for corn silage: Effects on silage composition and animal performance. *J Dairy Sci,* 79, 1760-1766, 1996.

8. Chen J, Stokes MR, Wallace CR: Effects of enzyme-inoculant systems on preservation and nutritive value of hay crop and corn silages. *J Dairy Sci,* 77, 501-512, 1994.

9. Guedes CM, Rodrigues MAM, Gomes MJ, Silva SR, Ferreira LM, Mascarenhas-Ferreira A: Urea treatment of whole-crop triticale at four growth stages: Effects on chemical composition and on *in vitro* digestibility of cell wall. *J Sci Food Agr,* 86, 964-970, 2006.

10. Nadeau E: Effects of plant species, stage of maturity and additive on the feeding value of whole-crop cereal silage. *J Sci Food Agric,* 87, 789-801, 2007.

11. Ashbell G, Weinberg ZG, Azrieli A, Hen Y, Horev B: A simple system to study the aerobic deterioration of silages. *Can Agric Eng,* 33, 391-393, 1991.

12. Anonymus: The Analysis of Agricultural Material. Reference Book. pp. 427-428, London. 1986.

13. Koç F, Coşkuntuna L: Silo yemlerinde organik asit belirlemede iki farklı metodun karşılaştırılması. *J Anim Prod,* 44, 37-47, 2003.

14. Seale DR, Pahlow G, Spoelstra SF, Lindgren S, Dellaglio F, Lowe JF: Methods for the microbiological analysis of silage. *Proceeding of The Eurobac Conference,* 147, Uppsala, 1990.

15. Filya I: The effect of *Lactobacillus buchneri* and *Lactobacillus plantarum* on the fermentation, aerobic stability, and ruminal degradability of low dry matter corn and sorghum silages. *J Dairy Sci,* 86, 3575-3581, 2003.

16. AOAC: Official Methods of Analysis. 15th ed., Association of Official Analytical Chemists, Arlington, Virginia, USA, 1990.

17. Goering HK, Van Soest PJ: Forage Fiber Analyses. Agricultural Handbook, No 379, Washington. 1983.

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

19. MINITAB: Minitab Incoporation. Minitab for Windows, Release 13 for Windows. User's Guide 2-Data Analysis and Quality Tools, Minitab Inc, USA, 2000.

20. Sucu E, Filya I: Effects of homofermentative lactic acid bacterial inoculants on the fermentation and aerobic stability characteristics of low dry matter corn silages. *Turk J Vet Anim Sci,* 30, 83-88, 2006.

21. Williams CC, Froetschel MA, Ely LO, Amos HE: Effects of inoculation and wilting on the preservation and utilization of wheat forage. *J Dairy Sci,* 78, 1755-1765, 1995.

22. Zahiroddini H, Baah J, Absalom W, McAllister TA: Effect of an inoculant and hydrolytic enzymes on fermentation and nutritive value of whole crop barley silage. *Anim Feed Sci Technol,* 117, 317-330, 2004.

23. Kung LM, Shaver R: Interpretation and use of silage fermentation analysis reports. *Focus Forage,* 3, 1-5, 2001.

24. Xing L, Chen J, Han LJ: The effect of an inoculant and enzymes on fermentation and nutritive value of sorghum straw silages. *Bioresour Technol,* 100, 488-491, 2009.

25. Jaakkola S: The effect of cell wall degrading enzymes on the preservation of grass and on the silage intake and digestibility in sheep. *J Agric Sci Finland,* 62, 51-62, 1990.

26. Selmer-Olsen I, Henderson AR, Robertson S, McGinn R: Cell wall degrading enzymes for silage. 1. The fermentation of enzyme-treated ryegrass in laboratory silos. *Grass Forage Sci,* 48, 45-54, 1993.

27. McDonald P, Henderson AR, Heron SJE: The Biochemistry of Silage. Chalcombe Publications, 13 Highwoods drive, Marlow Bottom, Marlow, Bucks, UK, 1991.

28. Weinberg ZG, Ashbell G, Yaira H, Azrieli A: The effect of cellulase and hemicellulase plus pectinase on the aerobic stability and fiber analysis of peas and wheat silages. *Anim Feed Sci Technol,* 55, 287-293, 1995.

29. Ozduven ML, Koc F, Polat C, Coskuntuna L: The effects of lactic acid bacteria and enzyme mixture inoculants on fermentation and nutrient digestibility of sunflower silage. *Kafkas Univ Vet Fak Derg,* 15(2): 195-199, 2009.

30. Sheperd AC, Maslanka M, Quinn D, Kung L: Additives containing bacteria and enzymes for alfalfa silage. *J Dairy Sci,* 78, 565-572, 1995.

31. Nadeau EMG, Buxton DR, Russell JR, Allison MJ, Young JW: Enzyme, bacterial inoculant, and formic acid effects on silage composition of orchardgrass and alfalfa. *J Dairy Sci,* 83, 1487-1502, 2000.

32. Nadeau EMG, Russell JR, Buxton DR: Intake, digestibility, and composition of orchardgrass and alfalfa silages treated with cellulase, inoculant, and formic acid fed to lambs. *J Animal Sci,* 78, 2980-2989, 2000.

33. Filya I: Laktik asit bakteri ve laktik asit bakteri + enzim karışımı silaj inokulantlarının mısır silajı üzerine etkileri. *Turk J Vet Animal Sci,* 26, 679-687, 2002.

34. Colombatto D, Mould FL, Bhat MK, Morgavi DP, Beauchemin KA, Owen E: Influence of fibrolytic enzymes on the hydrolysis and fermentation of pure cellulose and xylan by mixed ruminal microorganisms *in vitro*. *J Anim Sci,* 81, 1040-1050, 2003.

35. Polat C, Koç F, Özdüven ML: Mısır silajlarnda laktik asit bakterileri ve laktik asit bakteri+enzim karışımı inokulantların fermantasyon ve toklularda ham besin maddelerinin sindirilme dereceleri üzerine etkileri. *J Tekirdag Agricultural Faculty,* 2, 13-22, 2005.

36. Kung L, Tung RS, Maciorowski KG, Buffum K, Knutsen K, Aimutis WR: Effects of plant cell-wall-degrading enzymes and lactic acid bacteria on silage fermentation and composition. *J Dairy Sci,* 74, 4284-4296, 1991.

37. Meeske R, Basson HM, Cruywagen CW: The effects of a lactic acid bacteria inoculant with enzymes on the fermentation dynamics, intake and digestibility of Digitaria eriantha silage. *Anim Feed Sci Technol,* 81, 237-248, 1999.

38. Weinberg ZG, Szakacs G, Ashbell G, Hen Y: The effect of temperature on the ensiling process of corn and wheat. *J Appl Microbiol,* 90, 561-566, 2001.

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

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