Turkish Journal of Veterinary & Animal Sciences

Volume 30 | Number 1 Article 13

1-1-2006

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EKİN SUCU

İSMAİL FİLYA

Turk J Vet Anim Sci
30 (2006) 83-88

Research Article

Effects of Homofermentative Lactic Acid Bacterial Inoculants on the Fermentation and Aerobic Stability Characteristics of Low Dry Matter Corn Silages

Ekin SUCU*, İsmail FİLYA**

Department of Animal Science, Faculty of Agriculture, Uludağ University, 16059 Bursa - TURKEY

Received: 18.03.2005

Abstract: This study was carried out to determine the effects of homofermentative lactic acid bacterial inoculants on the fermentation and aerobic stability characteristics of low dry matter corn silages.

Corn was harvested at the milk stage. Inoculant-1188 (Pioneer®, USA; Inoculant A) and Maize-All (Alltech, UK; Inoculant B) were used as homofermentative lactic acid bacterial inoculants. Inoculants were applied to silages 1.5 x 10^6 colony forming units/g levels. Silages with no additive served as controls. After treatment, the chopped corn was ensiled in 1.5-l special anaerobic jars, equipped with a lid that enables gas release only. Three jars from each group were sampled for chemical and microbiological analysis on days 2, 4, 8, 15 and 50 after ensiling. At the end of the ensiling period, all silages were subjected to an aerobic stability test for 5 days.

Neither inoculant improved the fermentation characteristics of low dry matter corn silages. At the end of the ensiling period, inoculants increased the lactobacilli and decreased yeast and mold numbers of silages. However, Inoculant A led to higher CO_2 production and impaired the aerobic stability of silages. Inoculant B did not affect the aerobic stability of silages.

Key Words: Aerobic stability, corn, fermentation, lactic acid bacterial inoculant, silage

Homofermantatif Laktik Asit Bakteri İnokulantlarının Düşük Kuru Maddeli Mısır Silajlarının Fermantasyon ve Aerobik Stabilite Özellikleri Üzerine Etkileri

Özet: Bu çalışma homofermantatif laktik asit bakteri inokulantlarının düşük kuru maddeli mısır silajlarının fermantasyon ve aerobik stabilite özellikleri üzerine olan etkilerini belirlemek amacı ile düzenlenmiştir.

Araştırmada, mısır süt olum döneminde hasat edilmiştir. Homofermantatif laktik asit bakteri inokulantları olarak İnokulant-1188 (Pioneer®, USA; İnokulant A) ve Maize-All (Alltech, UK; İnokulant B) kullanılmıştır. İnokulantlar silajlara 1,5 x 10^6 koloni form unite/g düzeyinde katılmışlardır. Katkı maddesi içermeyen silajlar kontrol olarak değerlendirilmiştir. Uygulamadan sonra parçalanmış mısır, yalnızca gaz çıkışına olanak tanıyan 1,5 litrelik özel anaerobik kavanozlara silolanmıştır. Silolamadan sonraki 2, 4, 8, 15 ve 50. günlerde her gruptan üçer kavanoz açılarak kimyasal ve mikrobiyolojik analizler yapılmıştır. Silolama döneminin sonunda açılan tüm silajlara 5 gün süre ile aerobik stabilite testi uygulanmıştır.

Her iki inokulant da düşük kuru maddeli mısır silajlarının fermantasyon özelliklerini geliştirmemiştir. Silolama dönemi sonunda inokulantlar silajlarının lactobacilli sayılarını artırırlarken, maya ve küf sayılarını düşürmüşlerdir. Bununla birlikte, İnokulant A yüksek bir CO_2 üretimine yol açarak silajların aerobik stabilitesini düşürmüştür. İnokulant B, silajların aerobik stabilitesini etkilememiştir.

Anahtar Sözcükler: Aerobik stabilite, mısır, fermantasyon, laktik asit bakteri inokulantı, silaj

Introduction

Silage is the product formed when a crop, forage or agricultural by-product of sufficiently high moisture content liable to spoilage by aerobic microorganisms is stored anaerobically. Normally during ensiling the fodder undergoes an acid fermentation in which bacteria produce lactic, acetic and butyric acids from water-soluble carbohydrates (WSCs) present in the raw material. The net result is a reduction in pH, which prevents the growth of spoilage microorganisms, the majority of which are intolerant of acid conditions (1). In order to reduce the dependence of the ensiling process on epiphytic lactic acid

* This study is a part of the author's MSc thesis.

** E-mail: ifilya@uludag.edu.tr

bacteria (LAB) and on chemical additives, inoculants containing selected strains of LAB have been developed. The function of these inoculants is to ensure a rapid and efficient fermentation of WSCs into lactic acid by homolactic fermentation. This results in an intensive build-up of lactic acid, rapid decrease in pH and improved silage preservation with minimal fermentation losses (2). Most available inoculants consist of selected strains of homofermentative LAB, such as *Lactobacillus plantarum*, Pediococcus and Enterococcus species (3). Many studies have shown the advantages of such LAB inoculants (4-6). However, some studies under laboratory conditions (2,7,8) indicated that the addition of homofermentative LAB inoculants impaired the aerobic stability of silages of mature cereal crops (wheat, sorghum, and corn). This was suggested by a rise in pH, visible mold growth and intensive production of CO_2 during aerobic exposure. Similar problems caused by the use of homofermentative LAB inoculants have also been observed in other studies (9-11). Earlier observations had resulted in the opposite, namely that homofermentative LAB inoculants improved the aerobic stability of silages (12,13).

The purpose of this study was to focus on the effects of homofermentative LAB inoculants on the fermentation and aerobic stability characteristics of low dry matter (DM) corn silages.

Materials and Methods

Materials and silage preparation

Corn (*Zea mays* L.) was harvested at the milk stage of maturity (23.7 ± 0.65% DM). Whole plants were chopped about 2.0 cm and ensiled in 1.5-l special anaerobic jars (Le Parfait, France), equipped with a lid that enables gas release only. Three jars from each group were sampled for chemical and microbiological analysis on days 2, 4, 8, 15 and 50 after ensiling. 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. (14). 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 following treatments were used in the experiment:

Control (no additive).

Inoculum A (IA): Inoculant-1188 (Pioneer®, USA) containing *Lactobacillus plantarum* and *Enterococcus faecium*. Final application rate of 1.5 x 10^6 colony forming units (CFU)/g of fresh corn.

Inoculum B (IB): Maize-All (Alltech, UK) containing *Lactobacillus plantarum*, *Pediococcus acidilactici* and amylase. Final application rate of 1.5 x 10^6 CFU/g of fresh corn.

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 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 were applied to the forages in a uniform manner with constant mixing.

Analytical procedures

Chemical analyses were performed in triplicate. The DM content of the fresh materials was determined by drying at 60 °C for 48 h in a fan-assisted oven (15). Wet samples stored at –20 °C were extracted for 3 min in a stomacher blender in the water (1:9) for WSCs analyses. WSCs were determined by the phenol sulfuric acid method (16). Lactic, acetic and butyric acids were determined by Lepper's method (15). Ammonia-N was determined by the Kjeldahl method without a digestion step but with the addition of base (17). Fermentation losses were evaluated according to weight loss (8).

Microbiological analysis was performed on pooled samples of the 3 replicate silos per treatment per time point except for replicate samples that differed considerably in appearance. Microbiological evaluation included enumeration of lactobacilli on pour-plate Rogosa agar (Oxoid CM627, Oxoid, Basingstoke, UK), and yeast and molds on spread-plate malt extract agar (Difco, Detroit, MI, USA) acidified with lactic acid to pH 4.0. Plates were incubated for 3 days at 30 °C. Since microbiological analysis was performed on a single sample per time point, no statistical analysis was possible. All microbiological data were transformed to $\log_{10}$. Visual appraisal was determined as described by Filya et al. (18).

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 Statistical Analysis System (19).

Results

The chemical composition of the fresh and ensiled low DM corn is given in Table 1. All silages were well preserved. In the experiment, neither LAB inoculant improved the fermentation parameters of corn silages. The pH of all silages decreased faster and to a greater extent. During fermentation, no significant difference was shown between the pH values of control and LAB inoculated silages ($P > 0.05$). In the experiment, the WSCs in all silages decreased with the decrease in pH. Both LAB inoculated corn silages had significantly lower WSCs compared with the control silage ($P < 0.05$). Inoculant treatments did not affect the concentration of ammonia-N of the silages. After 2 days of ensiling, the silages inoculated with IB had significantly lower lactic acid and higher acetic acid levels than the control and IA treated silages ($P < 0.05$). The same trend was shown at 4, 8, 15 and 50 days of ensiling. During fermentation, no butyric acid was present in the silages. After 8 days of ensiling, both LAB inoculants significantly increased the weight losses of silages ($P < 0.05$). At the end of the ensiling period, neither LAB inoculant affected the weight losses of silages.

The microbiological composition of the corn silages is given in Table 2. Lactobacilli numbers of corn silages increased during the fermentation. In the present study, both LAB inoculants increased lactobacilli and decreased yeast and mold numbers of low DM corn silages compared with the control silage.

Table 3 gives the results of the aerobic exposure test of low DM corn silages. Silage deterioration indicators are pH change, CO_2 production and an increase in yeast and mold numbers. The silages inoculated with IA had significantly higher pH and CO_2 production than the control and IB treated silages ($P < 0.05$).

Table 1. Chemical analyses of the corn silages (DM %)*.

Days of ensiling	Treatment	pH	WSCs	NH_3-N	Lactic acid	Acetic acid	Butyric acid	Weight loss
0	Fresh Corn	6.9±0.07	6.8±0.26	0.8±0.15	0.8±0.05	0	0	0
	Silage							
2	Control	4.9 ± 0.08	6.2 ± 0.21^{a}	0.9 ± 0.09	1.0 ± 0.06^{a}	0.2 ± 0.04^{b}	0	0.4 ± 0
	IA	4.0 ± 0.03	5.7 ± 0.10^{b}	0.9 ± 0.09	1.0 ± 0.06^{a}	0.2 ± 0.01^{b}	0	0.4 ± 0.03
	IB	5.0 ± 0.02	5.6 ± 0.21^{b}	0.9 ± 0.10	0.7 ± 0.03^{b}	0.3 ± 0.01^{a}	0	0.3 ± 0
4	Control	4.2 ± 0.01	5.0 ± 0.15^{a}	1.2 ± 0.16	1.7 ± 0.03^{a}	0.4 ± 0.01^{b}	0	0.7 ± 0.03
	IA	4.0 ± 0.01	4.0 ± 0.15^{b}	1.2 ± 0.09	1.6 ± 0.03^{a}	0.4 ± 0.01^{b}	0	0.7 ± 0.06
	IB	4.2 ± 0.03	4.2 ± 0.15^{b}	1.2 ± 0.15	1.3 ± 0.06^{b}	0.5 ± 0.01^{a}	0	0.8 ± 0.03
8	Control	4.0 ± 0.02	4.1 ± 0.21^{a}	1.3 ± 0.12	1.9 ± 0.21^{a}	0.6 ± 0.06^{b}	0	1.1 ± 0.06^{c}
	IA	3.9 ± 0.01	2.8 ± 0.06^{b}	1.3 ± 0.12	2.0 ± 0.03^{a}	0.5 ± 0^{b}	0	2.1 ± 0.03^{b}
	IB	4.0 ± 0.04	2.6 ± 0.06^{b}	1.4 ± 0.18	1.6 ± 0.03^{b}	0.7 ± 0.02^{a}	0	3.1 ± 0.23^{a}
15	Control	3.9 ± 0.03	2.7 ± 0.15^{a}	1.5 ± 0.20	2.2 ± 0.06^{a}	0.8 ± 0.03^{b}	0	2.2 ± 0.03^{b}
	IA	3.8 ± 0.01	1.7 ± 0.10^{b}	1.5 ± 0.26	2.3 ± 0.03^{a}	0.7 ± 0.01^{b}	0	2.3 ± 0.34^{b}
	IB	3.9 ± 0.06	1.4 ± 0.12^{b}	1.6 ± 0.20	1.8 ± 0.06^{b}	0.9 ± 0.02^{a}	0	3.6 ± 0.04^{a}
50	Control	3.8 ± 0.03	1.8 ± 0.06^{a}	1.7 ± 0.18	5.0 ± 0.06^{a}	0.9 ± 0.01^{b}	0	2.7 ± 0.06
	IA	3.8 ± 0.02	0.5 ± 0.12^{b}	1.6 ± 0.18	5.2 ± 0.09^{a}	0.8 ± 0^{b}	0	2.7 ± 0.33
	IB	3.8 ± 0.04	0.6 ± 0.07^{b}	1.6 ± 0.15	4.1 ± 0.10^{b}	1.3 ± 0.02^{a}	0	2.7 ± 0

* Except pH and weight loss all the chemical analyses results are given in DM.

DM, dry matter; WSCs, water-soluble carbohydrates; NH_3-N, ammonia-nitrogen; IA, inoculum A; IB, inoculum B.

$^{a-b-c}$Within a column means followed by different letter differ significantly ($P < 0.05$).

Table 2. Microbiological analysis of the corn silages (log CFU/g DM).

Days of ensiling	Treatment	Lactobacilli	Yeast	Mold
	Fresh			
0	Corn	3.6	4.1	3.4
	Silage			
2	Control	3.8	4.2	0
	IA	4.3	3.8	0
	IB	4.5	4.1	0
4	Control	4.1	4.6	0
	IA	6.8	4.3	0
	IB	6.5	4.2	0
8	Control	5.3	4.5	1.8
	IA	7.9	4.0	1.1
	IB	7.7	4.4	1.4
15	Control	5.0	5.3	2.9
	IA	8.6	4.4	1.9
	IB	8.8	3.9	2.1
50	Control	5.8	5.3	2.9
	IA	9.3	5.0	2.6
	IB	9.5	5.1	2.5

Log CFU, logarithm colony forming unit; DM, dry matter; IA, inoculum A; IB, inoculum B. Microbiological analysis was performed on a single sample each time, no statistical analysis was possible.

Table 3. Results of the aerobic stability test of the corn silages.

Treatment	pH	CO_2 (g/kg DM)	Yeast*	Mold*	Visual appraisal**
Control	3.8 ± 0.03[b]	7.5 ± 0.52[b]	9.8	5.4	0
IA	4.2 ± 0.02[a]	16.9 ± 0.99[a]	29.5	6.3	1
IB	3.9 ± 0.04[b]	8.2 ± 0.55[b]	11.7	6.1	0

DM, dry matter; IA, inoculum A; IB, inoculum B.

*Microbiological analysis was performed on a single sample each time, no statistical analysis was possible.

** Visual appraisal is expressed using a scale 1 to 5 where 1: good quality silage with no visible molding, 2: a few small mold spots, 3: scattered mold spots, 4: silage with partially covered molds, lumpy silage, 5: completely mold covered samples, unpleasant odor and silage particles sticking together.

[a- b]Within a column means followed by different letter differ significantly ($P < 0.05$).

Discussion

The success of a bacterial inoculant as a silage additive depends on many factors, such as the type and properties of the crops to be ensiled, climatic conditions, epiphytic microflora, ensiling technique and the properties of the inoculant (20). Until now homofermentative LAB inoculants have been added to silage in order to stimulate lactic acid fermentation, accelerating the decrease in pH and thus improving silage preservation. In this experiment, neither homofermentative LAB inoculant improved lactic acid production of low DM corn silages. During fermentation, IB decreased lactic acid and increased acetic acid production of silages. However, IA did not affect lactic and acetic acid levels of silages. However, lactic acid levels of silages were sufficient to assure high-quality silage. Bolsen et al. (21) concluded that whole crop corn was fermented rapidly and that bacterial inoculants had little effect on the rate and efficiency of silage fermentation. Observations reported by other researchers (22,23) were similar, and the present findings further confirm these earlier conclusions. Seale (4), in his review on bacterial inoculants for silages, reported that suitable fast acid producing strains in sufficient numbers might be as effective as silage additives if the DM and WSCs of the crop are high enough. In the present study, all silages had lower pH values at an earlier stage of ensiling. A lower pH in high moisture silage was expected because of higher concentrations of WSCs and more extensive fermentation (24). Filya (25) concluded that extensive fermentation in low DM corn silages made at the milk stage led to higher fermentation losses. The same trend was shown in this experiment. After 8 days of ensiling, both inoculants increased the weight losses of silages. This result shows high fermentation losses in silages, especially at the beginning of fermentation. Neither LAB inoculant affected concentrations of ammonia-N of corn silages compared with the control silage. McDonald et al. (24) reported that lower pH values inhibited protein degradation in silages. Therefore, concentrations of ammonia-N of all corn silages were low in the experiment.

At the end of the ensiling period, LAB inoculants improved the microbiological composition of low DM corn silages as expected. Both LAB inoculants increased lactobacilli and decreased yeast and mold numbers of corn silages compared with the control silage. These findings are agreement with those reported by Spoelstra (5), Filya (7,8,10,11), Saarisola et al. (6) and Filya et al. (26).

The results in the present study clearly indicated that both LAB inoculants showed different effects on the aerobic stability of low DM corn silages. IB did not affect the pH and CO_2 production of corn silages. However, IA increased significantly pH and CO_2 production of corn silages compared with the control and IB treated corn silages. Therefore, corn silages inoculated with IA were more susceptible to aerobic exposure than the control and IB treated silages. In this regard, there were differences between the effects of the 2 inoculants used. This was evident from intensive CO_2 production and development of yeasts in the silages inoculated with IA. A high level of yeasts impaired the aerobic stability of IA treated corn silages. Similar results were obtained in other studies (2,7-11). Filya et al. (18) hypothesized that homofermentative LAB inoculants produced mainly lactic acid, which could serve as a substrate for lactate-assimilating yeasts upon aerobic exposure. Thus, only small amounts of short-chain volatile fatty acids (VFAs) such as acetic, propionic and butyric acids are produced. These VFAs can inhibit yeasts and molds, making silages treated with homofermentative LAB inoculants deteriorate faster upon exposure to air. This difference between our results and those published by Ohyama et al. (12) and Pahlow (13) is probably due to the fact that these researchers infiltrated air into the silage during the ensiling period from the beginning. However, Filya et al. (18) reported that the presence of low concentrations of oxygen in silage results in a shift of homolactic fermentation to heterolactic metabolism, leading to production of VFAs, which possess antimycotic activity and inhibit the development of yeasts and molds.

In conclusion, the results of the present study showed that homofermentative LAB inoculants did not improve the fermentation parameters or aerobic stability of low DM corn silages. However, both LAB inoculants improved the microbiological compositions of low DM corn silages.

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