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Effects of Inoculant Preparation Time and Doses on Fermentation and Aerobic Stability Characteristics of the Second Crop Maize Silages*

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This study was carried out to determine the effects of inoculant preparation time and doses on fermentation and aerobic stability characteristics of the second crop maize ensiled under laboratory conditions. Maize was harvested at the dough maturity stage. MICROBIOS (Cuprem®, USA) was used as lactic acid bacteria+enzyme mixture silage inoculant. The inoculant was applied at 1.0×10^5 cfu/g (RD1), a recommended dose, and 2.0×10^5 cfu/g (DD1) double the recommended dose. The chopped forages were ensiled in 1.0-l anaerobic jars (Weck, Wher-Oftlingen, Germany) equipped with a lid that enables only gas release. Three jars per treatment were sampled on days 4, 7, 14, 21 and 55. The jars were stored at 20-22°C under laboratory conditions. After 55 days of ensiling, the silages were subjected to an aerobic stability test for 5 days.

The study showed that doubling the rate of inoculant application was not effective than the recommended rate at enhancing the silage quality or aerobic stability. Moreover, preparation time did not improve the fermentation and aerobic stability of the second crop maize silage.

Keywords: Maize silage, dose, inoculant preparation time, silage fermentation

İnokulant Hazırlama Süresi ve Dozunun İkinci Ürün Mısır Silajlarının Fermantasyon ve Aerobik Stabilite Özellikleri Üzerine Etkileri

İnokulant hazırlama süresi ve dozunun hamur olum döneminde hasat edilen mısır silajının fermantasyon gelişimi üzerine etkilerinin laboratuvar koşullarında saptanması amacı ile düzenlenen çalışmada, laktik asit+enzim silaj inokulantı olarak MICROBIOS (Cuprem®, USA) kullanılmıştır. İnokulant silajlara 1.0×10^5 cfu/g ve 2.0×10^5 cfu/g düzeyinde katılmıştır. Uygulamalardan sonra muameleler yalnızca gaz çıkışına olanak tanıyan, 1,0 litrelik özel kavanozlara silolanmıştır. Kavanozlar laboratuvar koşullarında 20-22°C'de depolanmışlardır. Silolamadan sonraki 4., 7., 14., 21. ve 55. günlerde her gruptan 3'er kavanoz açılmıştır. Silolama döneminin sonunda açılan tüm silajlara 5 gün süre ile aerobik stabilite testi (20-22 °C) uygulanmıştır.

Sonuç olarak, ikinci ürün mısır silajlarında dozun artırılması ve bekletme süresinin silaj fermantasyonu ve aerobik stabilite üzerinde olumlu bir etkisi gözlenmemiştir.

Anahtar kelimeler: Mısır silajı, doz, inokulant hazırlama zamanı, silaj fermantasyonu

*Bu çalışma yüksek lisans tezinden üretilmiştir.

Introduction

Ensiling a common preservation method for moist forage crops, is based on conversion of water soluble carbohydrates (WSC) by lactic acid bacteria (LAB) into organic acids. As a result, pH decreases and the forage is preserved (McDonald et al., 1991).

Whole crop maize (*Zea mays*) is the most popular cereal crop conserved as silage in many parts of the world, and is regarded as an ideal crop for silage making because of its high yield, low buffering capacity and high WSC content (McDonald, 1981).

Trakya Region is the most important wheat and sunflower production area in Turkey with 600 mm yearly average rainfall. However, the rainfall in this region is often insufficient for maize. With better irrigation conditions, maize, especially second crop maize, become a good potential for farmers in Trakya Region. In Trakya Region, the second crop season includes a period of approximately 120 days between the beginnings of July and November after wheat harvesting. In recent years, due to the improvement in livestock production, silage maize on irrigated fields as a second crop in late summer has become popular (Bayhan, et al., 2006).

Lactic acid bacteria (LAB) are added to forages at the time of ensiling with the goal of improving the fermentation process. These inoculants are applied in a dry form or they are commonly mixed in water and held in applicator tanks. Unused liquids should be discarded after a period of 24 to 48 h because bacterial numbers begin to decline (Kung, 1998). Moreover, success in using these inoculants is highly dependent on the specific inoculant used and adding sufficient numbers of viable bacteria. The most commonly recommended inoculation rate for homofermentative LAB based inoculant results in final concentration of 100.000 (or 10^5) colony forming units (cfu) of this organism per gram of wet forage. There is limited evidence support the suggestion of some doubling or tripling this amount (e.g. 200.000-300.000 cfu) is more beneficial (Huisden et al., 2009).

The first objective of this study was to evaluate a recommended and double the recommended dose of homofermentative LAB required to improve the fermentation and aerobic stability of second crop maize silage ensiled in laboratory silos. Secondly, homofermentative (LAB) has been used to improve the fermentation of different silages, but to our knowledge, preparation time and doses of this strain have not been studied for second crop maize silages.

Materials and methods

Experimental design

Whole crop of maize (*Zea mays* L.) was harvested at the 2/3 milk line stage and 32% DM and radially chopped with a 1-row forage harvester to about 2.0 cm length. The chopped forages were ensiled in 1.0-liter anaerobic jars (Weck, Wier-Oflingen, Germany) equipped with a lid that enabled gas release only. Each jar was filled with about 550 g (wet weight) of chopped forage without a headspace. All of the jars were filled (18 per treatment), and they were stored at a temperature 20-22 °C to follow fermentation dynamics. Fresh and ensiled maize were sampled (on 4th, 7th, 14th, 21st and 55th days after ensiling, three jars for each time) for chemical and microbiological analyses. At the end of ensiling period (55th day) the silages were subjected to an aerobic stability test lasting 5 days in the bottle system developed by Ashbell et al. (1991). In this system, the numbers of yeast and moulds, change

in pH, and the amount of CO₂ produced during test, were used as indicators of aerobic deterioration. Visual appraisal of the samples exposed to air was performed by a panel of 3 according to the extent of mould cover, texture and their odour. The panel evaluation was converted into numeric scale from 1 to 5, with 1 being good quality silage with no apparent moulding and 5 being completely moulded samples (Filya et al., 2000).

A commercial inoculant MICROBIOS W/S (Cuprem®, USA) containing *Lactobacillus plantarum*, *Lactobacillus brevis*, *Propionibacterium shermanii*, *Enterococcus faecium*, *Bacillus subtilis*, *Pediococcus acidilactici* and *alpha-Amylase (A.oryzae)*, *cellulase* and *hemicellulase (A. niger)* was used in this study.

The following treatments were applied to fresh forages :

1. Control: no additive (C)
2. Preparation of 1.0×10^5 recommended dose inoculant just before (5 to 10 minutes) the application (RD1)
3. Preparation of 2.0×10^5 double dose inoculant just before (5 to 10 minutes) the application (DD1)
4. Preparation of 1.0×10^5 recommended dose inoculant 24 hour before the application (RD2)
5. Preparation of 2.0×10^5 double dose inoculant 24 hour before the application (DD2)

The application rate of LAB of the products was determined in accordance with manufacturer instructions. The inoculants were diluted with distilled water so that they were all applied at same rate (20 ml solution kg⁻¹ forage). The control treatment received 20 ml water kg⁻¹ forage. The amount of chopped maize for a given silo was weighed, sprayed with appropriate inoculant solution using a plant sprayer (one sprayer for each treatment), mixed by hand, and then placed into the silo by hand with periodic tamping. Equipment coming in contact with inoculated maize washed and wiped with ethanol between treatments to prevent cross-contamination. Silos were weighed before and after filling to determine the actual amount ensiled.

Analytical procedures

Dry matter (DM) was determined by oven drying for 48 h at 60°C. The pH in fresh material and

silage samples was measured according to the British Standard method (Anonymous, 1986). The ammonia nitrogen (NH₃-N) content of silages was determined—in according to Anonymous (1986). The water soluble carbohydrates (WSC) content of silages was determined by spectrophotometer (Shimadzu UV-1201, Kyoto, Japan) after reaction with antron reagent (Thomas, 1977). Crude protein (CP) and crude fiber (CF) contents were determined by a Kjeldahl method (AOAC, 1990). Lactic acid (LA) and acetic acid (AA) contents were determined by the spectrophotometric method (Koc and Coskuntuna, 2003). Fermentation losses during storage were estimated by weight loss, calculated separately for each jar by the difference in the weight at the beginning and end of the ensiling period.

Microbiological evaluation included enumeration of lactobacilli on pour-plate Rogosa agar (Oxoid CM627, Oxoid, Basingstoke, UK). Yeast and moulds were determined by pour plating in malt extract agar (Oxoid CM59) that had been acidified, after autoclaving, by the addition of 85% lactic acid at a concentration of 0.5% vol/vol. Plates were incubated aerobically at 32°C for 48 to 72 h (Seale et. al., 1990).

Statistical analysis of the silage chemical analysis results included a one-way analysis of variance and Duncan's multiple range test performed with

the Statistical Analysis System (2005) Software (SAS, Cary, NC).

Results

The fresh second crop of maize contained 325.1, 93.0 and 75.90 g kg⁻¹ DM, CP and WSC respectively, and the pH was 5.31. The log numbers of cfu g⁻¹ FM of LAB and yeasts in the fresh material were 5.20 and 6.55, respectively.

The chemical and microbiological composition of the fresh and ensiled maize silages were given in Table 1. In the experiment, neither inoculant dose nor inoculant preparation time improved the fermentation parameters of second crop maize silages. The pH values of all silages were lower than that of fresh maize. During fermentation, no significant difference were shown between the pH values of control and treatment silages (P>0.05). In the experiment, the WSCs in all silages decreased with the decrease in pH. Treatment silages had significantly higher WSCs compared with control silage (P<0.001, Fig. 1).

Inoculant treatments did not affect the concentration of NH₃-N of the silages. (P>0.05). After 4 days of ensiling the silages inoculated with RD1 had significantly higher lactic acid than those of the control and DD1, RD2, DD2 treated silages (P<0.001, Fig. 2).

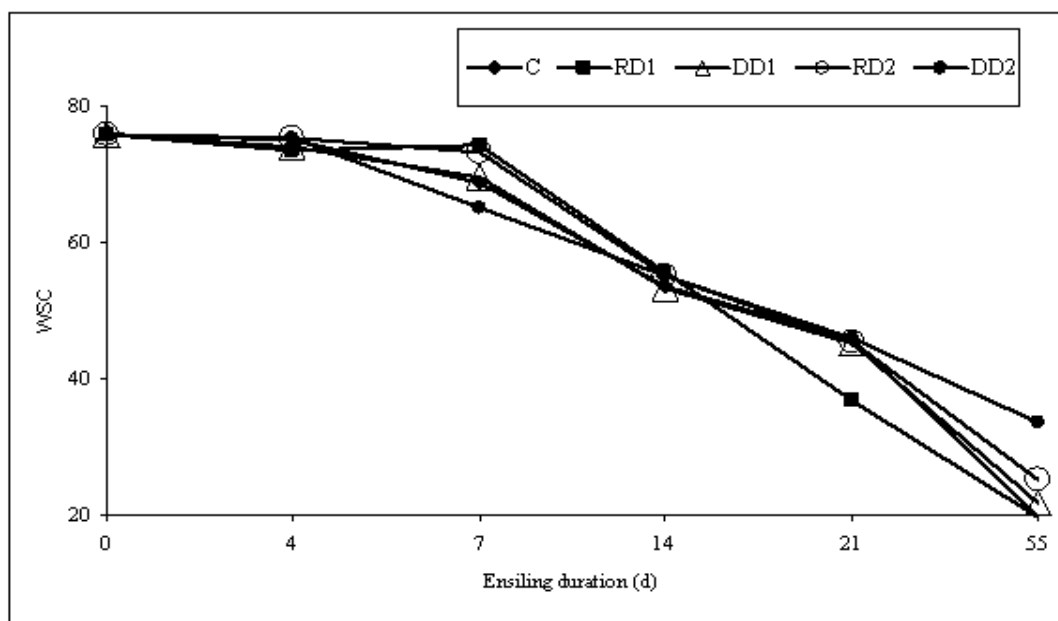


Figure 1. Water-soluble carbohydrates content in second crop maize silages

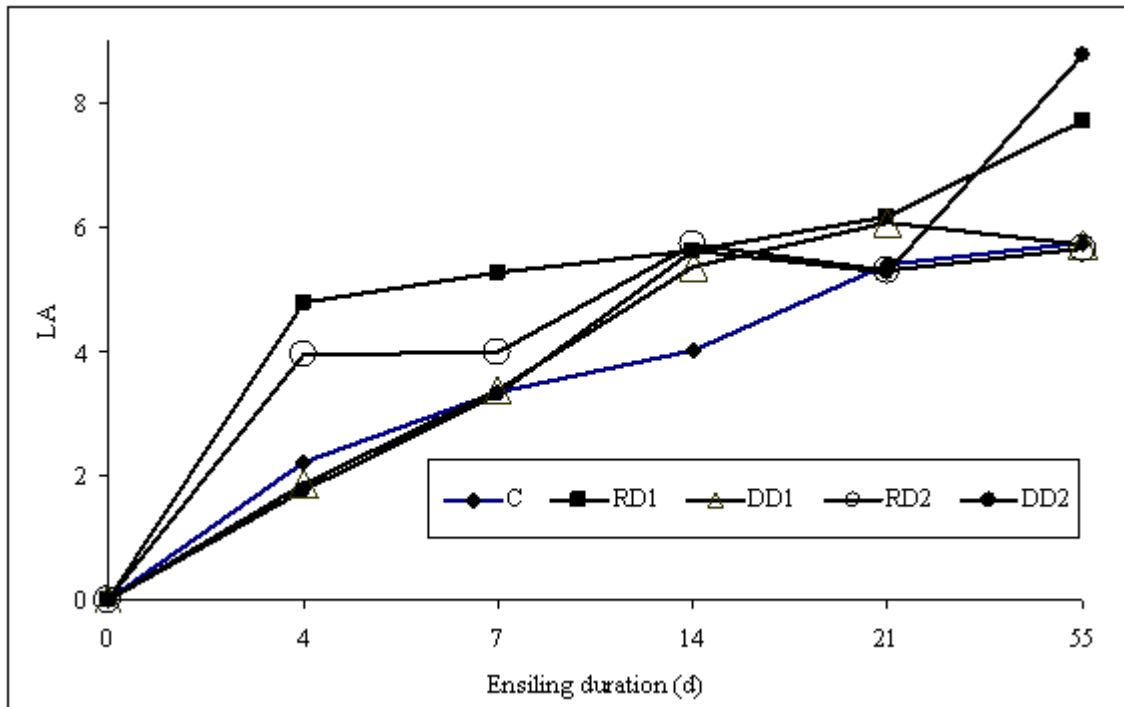


Figure 2. Lactic acid concentration (%) change in second crop maize silages

The same trend was shown at 14th, 21st and 55th days of ensiling. At the end of the ensiling period, inoculant dose affected the weight losses of silages ($P<0.005$). Inoculant preparation time effects were only observed for WSC and AA contents ($P<0.001$). The silages RD2 and DD2 had higher WSC and AA contents than the control, RD1 and RD2 treated silages. Significant doses x inoculant preparation time interactions for NH₃-N

($P<0.009$), WSC ($P<0.014$) and LA ($P<0.001$) were obtained in present study.

The microbial composition of the maize silages was given in Table 2. LAB numbers of second crop maize silages increased during the fermentation. In the present study, both recommended dose and double the recommended dose increased LAB and decreased yeast numbers of second crop maize silages compared with the control silage.

Table 1. Results of the chemical and microbiological analysis (log₁₀ cfu/g) of the second crop maize silages

Treatment	pH	DM, % in FM	CP, g/kg DM	NH ₃ -N, g/kg TN	WSC, g/kg DM	LA,% DM	AA,% DM	LAB	Yeast	Mould	Weight loss %, DM
FM	5.31	32.51	9.30		75.9	-	-	5.20	6.55	NF	-
Control	4.09	31.06	9.53	28.29	19.70e	5.73e	2.50a	5.67e	2.17	NF	1.71a
RD1	4.11	31.00	9.22	29.99	19.80d	7.73b	1.80e	6.48b	NF	NF	1.00d
DD1	4.11	31.26	9.46	26.74	21.80c	6.87c	1.84c	5.99c	NF	NF	1.26c
RD2	4.08	30.92	9.38	25.43	25.31b	6.65d	1.87d	5.83d	NF	NF	1.00d
DD2	4.11	27.66	9.53	30.45	33.73a	8.78a	1.90b	6.88a	NF	NF	1.34b
SEM	0.017	0.714	0.127	1.456	1.390	0.176	0.021	-	-	-	0.131
		Source of variation					<i>P level</i>				
Dose	NS	NS	NS	NS	0.003	0.001	0.046				0.005
Preparation time	NS	NS	NS	NS	0.001	NS	0.000				NS
Interaction	NS	NS	NS	NS	0.014	0.0000	NS				NS

Note: Values with different letters in the same column are statistically different (P<0.05). DM: dry matter; FM: fresh matter; NH₃-N: ammonia nitrogen; WSC: water-soluble carbohydrate.

Table 2. Results of the microbiological analysis of the second crop maize silages (log10 cfu/g FM)

Treatment	pH	CO ₂	Yeasts	Mould	Visual appraisal
Control	6.69	17.52c	3.07c	>2.0	2
RD1	6.31	15.39d	>2.0e	NF	1
DD1	6.48	23.28b	3.96b	NF	1
RD2	6.18	10.75e	2.60d	NF	1
DD2	6.92	22.72a	4.00a	>2.0	2
SEM	0.187	0.491	0.072	-	-
Source of variation			<i>P level</i>		
Dose	NS	0.000	0.001	-	-
Preparation time	NS	0.005	0.001	-	-
Interaction	NS	0.000	0.001	-	-

NF: Not found. CO₂ g/kg DM.

Note: Values with different letters in the same column are statistically different (P<0.05).

Table 2 gives the result of the aerobic exposure test of second crop maize silages. Silage deterioration indicators are pH change, CO₂ production and increase in yeast and mold numbers. The silages DD1 and DD2 had higher CO₂ production and yeasts numbers than the control, RD1, RD2 treated silages.

Discussion

Effect of doubling the rate of inoculant application

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 (Henderson, 1984). 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 (Sucu and Filya, 2006).

Compared to the recommended dose treatment, doubling the rate of inoculant application increased WSC and acetic acid concentrations and weight losses. However, the chemical composition, fermentation product concentrations of silages treated with recommended and double inoculant application rates were similar, indicating that doubling the application rates was unwarranted. Similar results were reported by some researchers (Ranjit and Kung, 2000; Ranjit et al. 2002; Neylon and Kung, 2003; Filya et al., 2006). Moreover, doubling the rate of inoculant application did not improve the aerobic stability of the second crop maize silage. DD1 and DD2 increased pH and CO₂ production of

maize silages compared RD1 and RD2 treated maize silages. Therefore double dose treatment silages were more susceptible to aerobic exposure than the RD1 and RD2 treated silages. This was evident from intensive CO₂ production and development of yeast in the DD1 and DD2 treated silages. Similar results were obtained in other studies (Weinberg, 1993; Filya et al., 2002, Filya, 2003, Huisden et.al., 2009).

The effect of inoculant preparation time

Inoculant preparation time did not affect silage pH and DM, CP, NH₃-N but RD2 and DD2 led to higher lactic acid ratios (Table 1) and higher residual WSC concentrations compared to RD1 and DD1 treatment silages. DD2 also tended to increase ammonia-N concentrations and increase DM losses. WSC concentration, lactic and acetic acid content in RD1 and DD1 increased in the treated silages. Moreover, preparation time improved the aerobic stability of the second crop maize silage. RD2 decreased pH and CO₂ production of maize silages compared to all of the treatment. Therefore RD2 treatment silages were less susceptible to aerobic exposure than the RD1, DD1, DD2 and control silages. There are not so many references to approve the effects of preparation time of silage inoculant on ensiling of whole crop maize. However, there are some references on application rate of inoculants in ensiling other forages (Filya et al., 2006; Adesogan, 2006). Mulrooney and Kung (2008), tested the viability of several commercial silage inoculants when they were exposed to water of different temperatures. The effects were variable depending on temperature and inoculant. Most inoculants were relatively stable when exposed to 30 and 35°C for 3 to 6 h. However, exposure to 40

and 45°C resulted in marked reductions in viable cells within 3 h for some inoculants.

Conclusions

The chemical composition—and fermentation product concentrations of silages treated with recommended and double inoculant application rates were similar, indicating that doubling the

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- application rates—was unwarranted. This study therefore refutes anecdotal suggestions that doubling inoculant application rates will improve their efficiency. Producers should be strongly discouraged from embracing this practice because it is expensive and ineffective. Moreover, preparation time didn't improve the fermentation and aerobic stability of the second crop maize silage.
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