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The Effect of a Lactic Acid Bacteria Inoculant on Corn Silage Ensiled at the Different Stages of Vegetation

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Abstract: The study was carried out to investigate the effect of a lactic acid bacteria inoculant applied to fresh corn crop harvested at early milk, milk and dough stages on silage quality and some chemical composition of corn silage. Corn crop was harvested at early milk stage (19.3% dry matter content), milk stage (1/2 milk line in corn grain, 27.2% dry matter content) and dough stage (36.9% dry matter content). A total of six experimental groups including one control and one treatment group containing a Lactic Acid Bacteria inoculant (LAB) were used for each stage. The silage material was tightly filled in 192 glass jars. Eight jars of each group were opened on days 15, 30, 45 and 60th of ensiling and crude protein, metabolisable energy, the pH value, ammonia nitrogen, lactic acid, acetic acid, propionic acid and butyric acid concentrations were determined. Although, there was a difference ($p<0.05$) in pH value of corn silage ensiled at early milk stage between control and treatment groups on the 45 and 60th days of ensiling, the pH values of them were <4.0. On the 45 and 60th days of ensiling, there was no difference in ammonia nitrogen and organic acid levels of control and treatment groups of corn silage ensiled at early milk stage, milk stage and dough stage. In conclusion, a lactic acid bacteria inoculant had no beneficial effects compared to controls on corn silages ensiled at early milk stage, milk stage and dough stage.

Key words: Corn silage, bacterial inoculant, silage fermentation, organic acid, dough, milk

INTRODUCTION

Ensiling is a conservation method based on lactic acid fermentation of moist crops under anaerobic conditions. Lactic acid bacteria convert water-soluble carbohydrates into organic acids especially to lactic acid and the pH value decreases during this conservation method thus nutrient value of the ensiled material is preserved (Weinberg and Muck, 1996). Silage microflora has an important role for the success of ensiling process. Microorganism population in the ensiling is consisted of beneficial (homo-fermentative lactic acid bacteria) and harmful bacteria (such as clostridia, enterabacteria) and undesired yeasts and moulds. Microorganisms causing spoilage, decrease nutrient value of silage and also have harmful effects on animal health and performance (McDonald *et al*., 1991).

Microbial silage inoculants including lactic acid bacteria are generally used as silage additives to improve fermentation (Elferink *et al*., 2011). Muck and Kung (1997) indicated that 60% of the researches mentioned that silages with inoculant had lower pH, ammonia formation and higher lactic acid content than silages without inoculant. Filya *et al*. (2007) mentioned that most of the inoculants (but not all) used to improve silage fermentation decreased pH and increased lactic acid content compared to uninoculated silages.

The aim of the study was to investigate the effect of a lactic acid bacteria inoculant applied to fresh corn crop harvested at early milk, milk and dough stages on silage quality and some chemical composition of corn silage.

MATERIALS AND METHODS

Corn crop was harvested at the three different stages as early milk stage (19.30% dry matter content), milk stage (1/2 milk line in corn grain, 27.20% dry matter content) and dough stage (36.90% dry matter content). Corn crops used as silage material obtained from the field of a private company for ensilage in the study. A total of six

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experimental groups including one control and one treatment group containing Lactic Acid Bacteria inoculant (LAB) were used for each stage. The bacterial inoculant (Biomin® BioStabil Mays) containing homofermentative and heterofermentative lactic acid bacteria (*Enterococcus faecium*, *Lactobasillus brevis* and *Lactobasillus plantarum*) used in the study contained 2.5×10^{10} cfu g^{-1} of bacteria. The inoculant was added by dissolving 0.4 g of inoculant into 100 mL of water and spraying for each 100 kg of silage material in the treatment groups. The silage material was tightly filled in 1 L glass jars. A total of 192 glass jars were used in the study; 32 glass jars for the control and the treatment group of each stage. The glass jars closed with lid and sealed. Then, jars were turned upside down to enable gas, fluid output and silo water was drained for 2 or 3 days via the punctured jar lids and then holes on the lids were closed with plaster to avoid oxygen and placed for fermentation in room temperature.

Eight jars of each group were opened on the 15, 30, 45 and 60th days of ensiling. About 25 g of sample for each ensiling day was taken from upper, central and bottom of each silage jar and homogeneously mixed with 100 mL distilled water by blender for pH and organic acid analyses. Afterwards, the pH value of the filtrate obtained by filtering this mixture was measured with a digital pH meter (Jenco®). The remaining of filtrate was centrifuged for 15 min at 4000 rpm and the supernatant was stored in -20°C for organic acid analysis. Organic acid concentrations were analyzed by Shimadzu trademark full HPLC device at 210 nm wavelength (PDA detector) by using Bio-Rad Aminex HPX-87-H colon with 0.004 M H_2SO_4 mobile pHase at 0.06 mL min^{-1} flowing speed suggested by Bell *et al.* (1991). Ammonia nitrogen concentrations of the silage samples on the 15, 30, 45 and 60th days of the study were analyzed (Markham, 1942). The crude protein analysis of silage samples on the 30 and 60th days of the study were done according to AOAC (1984) and the metabolisable energy level of silage samples on the 30 and 60th days of the study was calculated according to TSE.

Statistical analysis: Factorial experiment pattern prepared according to randomized parcels experimental design was used in the statistical analysis of the study findings. Variance analysis was applied to determine to investigate binary interactions of experiment groups by time and Duncan test was made to find the difference between groups (Moore and McCabe, 1993). For this purpose, SAS statistical packet program was used (SAS, 2009).

RESULTS AND DISCUSSION

Crude protein contents of silages in control ensiled at early milk stage, milk stage and dough stages on the 60th day of ensiling were 8.78, 7.25 and 7.68%, respectively and metabolisable energy values were 8.42, 9.03 and 9.89 MJ kg^{-1} DM, respectively. Crude protein contents of silages in treatment groups ensiled at the same stage of vegetation on the 60th day of ensiling were 7.63, 7.14 and 7.88%, respectively and metabolisable energy values were 7.73, 8.44 and 9.16 MJ kg^{-1} DM, respectively (Table 1).

The ammonia nitrogen concentrations of silage samples in control and treatment groups were shown in Table 2. The pH values and organic acid concentrations of silages in control and treatment groups ensiled at early milk stage, milk stage and dough stage on 15, 30, 45 and 60th days of the study for were shown in Table 3-5,

Table 1: Metabolisable energy (MJ kg^{-1} DM) and crude protein (DM%) of corn silages in control and treatment groups

	Early milk stage				Milk stage				Dough stage			
	Control		LAB		Control		LAB		Control		LAB	
Ensiling day	CP	ME	CP	ME	CP	ME	CP	ME	CP	ME	CP	ME
30	8.91	9.19	7.70	7.97	7.31	8.64	7.44	8.89	7.87	10.09	7.90	10.11
60	8.78	8.42	7.63	7.73	7.25	9.03	7.14	8.44	7.68	9.89	7.88	9.16

CP: Crude Protein, ME: Metabolisable Energy, DM: Dry Matter

Table 2: Ammonia nitrogen levels (mg L^{-1}) of corn silages in control and treatment groups (n = 8)

	Early milk stage			Milk stage			Dough stage		
Ensiling day	Control	LAB	p-values	Control	Control	p-values	LAB	Control	p-values
15	65.00±23.90	53.75±2.27	<0.05	56.38±5.42	60.63±2.75	NS	58.13±1.62	58.14±1.62	NS
30	56.25±22.27	56.88±1.62	NS	57.50±1.89	54.38±1.99	NS	55.00±1.34	56.88±1.62	NS
45	51.88±21.32	51.88±0.91	NS	50.63±1.13	48.13±0.92	NS	63.75±0.82	65.63±1.48	NS
60	45.00±21.34	46.88±0.91	NS	45.00±0.01	45.63±0.63	NS	63.75±2.27	63.75±1.83	NS

NS: Not Significant, LAB: Lactic Acid Bacteria (treatment group)

Table 3: Organic acid (g kg^{-1} DM) and pH values of corn silages in control and treatment groups ensiled at early milk stage (n = 8)

	pH			Lactic acid			Acetic acid			Propionic acid			Butyric acid		
Ensiling day	Control	LAB	p-values	Control	LAB	p-values	Control	LAB	p-values	Control	LAB	p-values	Control	LAB	p-value
15	3.99±0.01	3.99±0.03	NS	25.23±2.39	30.60±4.18	NS	5.26±0.82	6.37±1.30	NS	0.20±0.02	0.15±0.02	NS	3.58±0.91	16.83±6.48	<0.05
30	3.80±0.02	3.84±0.01	NS	40.24±5.05	38.81±1.99	NS	13.51±2.22	10.95±0.52	NS	1.06±0.13	0.20±0.08	NS	29.37±4.20	22.40±1.47	NS
45	3.83±0.01	3.78±0.01	<0.05	44.96±4.34	44.49±3.05	NS	15.55±2.02	15.26±1.10	NS	1.66±0.16	3.22±1.57	<0.05	30.84±4.89	33.46±6.31	NS
60	3.82±0.02	3.77±0.01	<0.05	42.91±6.94	41.51±5.89	NS	13.98±2.22	11.77±2.43	NS	2.44±0.62	2.08±0.30	NS	24.95±4.82	26.69±5.74	NS

NS: Not Significant, LAB: Lactic Acid Bacteria (treatment group)

Table 4: Organic acid (g kg^{-1} DM) and pH values of corn silages in control and treatment groups ensiled at milk stage (n = 8)

	pH			Lactic acid			Acetic acid			Propionic acid			Butyric acid		
Ensiling day	Control	LAB	p-values	Control	LAB	p-values	Control	LAB	p-values	Control	LAB	p-values	Control	LAB	p-values
15	4.04±0.02	4.08±0.03	<0.05	24.18±3.41	13.71±1.85	<0.05	5.79±1.72	6.28±1.52	NS	0.13±0.02	0.10±0.02	NS	10.35±4.79	6.94±2.20	NS
30	3.90±0.02	3.89±0.01	NS	30.52±2.11	23.47±0.89	NS	8.38±1.39	9.06±0.90	NS	0.51±0.02	0.09±0.01	NS	13.78±3.08	6.85±0.36	NS
45	3.84±0.01	3.88±0.01	NS	32.85±1.66	23.44±4.01	NS	9.00±0.55	10.61±0.71	NS	0.76±0.05	0.07±0.02	NS	16.77±1.98	9.32±1.33	NS
60	3.80±0.01	3.82±0.01	NS	36.18±1.15	32.82±1.30	NS	9.01±0.57	11.27±0.86	NS	1.18±0.04	0.09±0.01	NS	15.56±1.32	8.96±1.09	NS

NS: Not Significant, LAB: Lactic Acid Bacteria (treatment group)

Table 5: Organic acid (g kg^{-1} DM) and pH values of corn silage in control and treatment groups ensiled at dough stage (n = 8)

	pH			Lactic acid			Acetic acid			Propionic acid			Butyric acid		
Ensiling day	Control	LAB	p-values	Control	LAB	p-values	Control	LAB	p-values	Control	LAB	p-values	Control	LAB	p-values
15	4.02±0.01	4.03±0.01	NS	11.36±1.90	17.18±1.30	NS	2.63± 0.48	2.62±0.28	NS	0.36±0.05	0.58±0.04	NS	0.42±0.16	0.48±0.17	NS
30	3.93±0.01	3.93±0.01	NS	30.99±2.64	30.36±2.07	NS	9.15±1.32	9.31±0.95	NS	0.93±0.09	1.15±0.09	NS	13.95±2.74	16.23±2.38	NS
45	3.92±0.01	3.90±0.01	NS	34.53±1.53	35.56±1.24	NS	10.04±0.79	10.69±0.70	NS	1.45±0.07	1.52±0.06	NS	15.12±1.99	15.22±2.12	NS
60	3.84±0.01	3.83±0.01	NS	31.09±0.36	30.89±0.85	NS	7.51±0.37	8.41±0.48	NS	1.07±0.12	1.20±0.09	NS	9.99±0.77	13.73±1.73	NS

NS: Not Significant, LAB: Lactic Acid Bacteria (treatment group)

respectively. Ammonia nitrogen (mg L^{-1}), pH and organic acid (g kg^{-1} DM) values of silages for control and treatment groups were shown in Table 6 and 7, respectively. Crude protein and metabolisable energy values of silages in control and treatment groups for harvested at the different stage of vegetation were generally similar. There was no negative result in color, mixture properties and odour of corn silages in the study. The rapid decrease of pH value is the most important factor during fermentation of silage material to avoid increasing in undesirable microorganisms. The decline in pH reflects the concentration of lactic acid that is responsible for the fermentation. The pH value of control and treatment groups were <4.2 on day 15 of ensiling in the study. The pH value which was acceptable for silage was even <4.0 on days of 30, 45 and 60th of ensiling in the study. The pH value of on days 45th and 60th of ensiling in treatment group applied LAB to corn crop harvested at early milk stage was lower ($p<0.05$) than that of control. The result for pH of silage sample ensiled at dough stage (36.9% DM content) was similar to that of Bolsen (1995) for corn silage (33% DM content in fresh silage material).

Muck (1993) reported that using of two different type inoculants for corn crop with 17.3, 24.6 and 26.3% dry matter contents did not affect on pH values of silages in control or treatment group on day 60 of ensiling. This result is compatible with pH values of silages ensiled at all stages on day of 60 of ensiling in the study.

Ammonia nitrogen concentrations of silages ensiled at early milk stage, milk stage and dough stage without inoculant (control) were similar to those of treatment groups (with inoculant). This result was compatible with that of Khorvash *et al.* (2005).

There was no difference between control and treatment groups for lactic acid, acetic acid and butyric acid contents on day of 60 of ensiling of corn silages ensiled at the each stage. This result was compatible with those of Kleinschmit *et al.* (2005) and Sucu and Filya (2006).

Jones and Gogerddan (1994) reported that the effect of LAB inoculant on silage fermentation may be suppressed by initial lactic acid bacteria population of fresh corn crops. Muck (1990) mentioned that the effect of lactic acid bacteria on silage fermentation was related to initial amount of lactic acid bacteria in silage material and if initial lactic acid bacteria in silage material were high, the effect of LAB inoculant on silage fermentation decreased. Some researcher (Patterson *et al.*, 1997) reported that enzyme+inoculant complex decreased lactic acid content of sorghum silage compared to control. In the present study, it was determined that a lactic acid bacteria inoculant had no considerable effect on corn silage fermentation. This result may be attributed to the fact that LAB inoculants could be suppressed by initial LAB population in silage material. It may be difficult for inoculants to provide important contributions for silage fermentation because low pH and typically high lactic acid formation in natural fermentation of crops with high carbohydrate content as corn.

The lactic acid concentrations of controls (without inoculant) on days of 30, 45 and 60th of ensiling were

Table 6: Ammonia nitrogen (mg L^{-1}), pH and organic acid (g kg^{-1} DM) values of control group (n = 8)

		Ensiling day			
Parameters	Harvest time	15	30	45	60
Ammonia nitrogen	Early milk stage	65.00±23.90^{a}	56.25±22.27	51.88±21.320^{b}	45.00±21.34^{b}
	Milk stage	56.38±5.420^{b}	57.50±1.890	50.63±1.130^{b}	45.00±0.010^{b}
	Dough stage	58.13±1.620^{b}	55.00±1.340	63.75±0.820^{a}	63.75±2.270^{a}
pH	Early milk stage	3.99±0.010^{b}	3.80±0.020^{b}	3.83±0.010^{b}	3.82±0.020
	Milk stage	4.04±0.020^{a}	3.90±0.020^{a}	3.84±0.010^{b}	3.80±0.010
	Dough stage	4.02±0.010^{ab}	3.93±0.010^{a}	3.92±0.010^{a}	3.84±0.010
Lactic acid	Early milk stage	25.23±2.390^{a}	40.24±5.050^{a}	44.96±4.340^{a}	42.91±6.940^{a}
	Milk stage	24.18±3.410^{a}	30.52±2.110^{b}	32.85±1.660^{b}	36.18±1.150^{b}
	Dough stage	11.36±1.900^{b}	30.99±2.640^{b}	34.53±1.530^{b}	31.09±0.360^{b}
Acetic acid	Early milk stage	5.26±0.820	13.51±2.220^{a}	15.55±2.020^{a}	13.98±2.220^{a}
	Milk stage	5.79±1.720	8.38±1.390^{b}	9.00±0.550^{b}	9.01±0.570^{b}
	Dough stage	2.63±0.480	9.15±1.320^{b}	10.04±0.790^{b}	7.51±0.370^{b}
Propionic acid	Early milk stage	0.20±0.020	1.06±0.130	1.66±0.160	2.44±0.620^{a}
	Milk stage	0.13±0.020	0.51±0.020	0.76±0.050	1.18±0.040^{ab}
	Dough stage	0.36±0.050	0.93±0.090	1.45±0.070	1.07±0.120^{b}
Butyric acid	Early milk stage	3.58±0.910	29.37±4.200^{a}	30.84±4.890^{a}	24.95±4.820^{a}
	Milk stage	10.35±4.790	13.78±3.080^{b}	16.77±1.980^{b}	15.56±1.320^{b}
	Dough stage	0.42±0.160	13.95±2.740^{b}	15.12±1.990^{b}	9.99±0.770^{b}

$^{a-c}$The letters in the same column mean significantly difference ($p<0.05$)

Table 7: Ammonia nitrogen (mg L^{-1}), pH and organic acid (g kg^{-1} DM) values of treatment groups (n = 8)

		Ensiling day			
Parameters	Harvest time	15	30	45	60
Ammonia nitrogen	Early milk stage	53.75±2.27^{b}	56.88±1.62	51.88±0.91^{b}	46.88±0.91^{b}
	Milk stage	60.63±2.75^{a}	54.38±1.99	48.13±0.92^{b}	45.63±0.63^{b}
	Dough stage	58.14±1.62^{a}	56.88±1.62	65.63±1.48^{a}	63.75±1.83^{a}
pH	Early milk stage	3.99±0.03^{b}	3.84±0.01^{c}	3.78±0.01^{b}	3.77±0.01^{b}
	Milk stage	4.08±0.03^{a}	3.89±0.01^{b}	3.88±0.01^{a}	3.82±0.01^{a}
	Dough stage	4.03±0.01^{ab}	3.93±0.01^{a}	3.90±0.01^{a}	3.83±0.01^{a}
Lactic acid	Early milk stage	30.60±4.18^{a}	38.81±1.99^{a}	44.49±3.05^{a}	41.51±5.89^{a}
	Milk stage	13.71±1.85^{b}	23.47±0.89^{b}	20.52±4.01^{c}	32.82±1.30^{b}
	Dough stage	17.18±1.30^{b}	30.36±2.07^{b}	35.56±1.24^{b}	30.89±0.85^{b}
Acetic acid	Early milk stage	6.37±1.30^{a}	10.95±0.52	15.26±1.10^{a}	11.77±2.43
	Milk stage	6.28±1.52^{a}	9.06±0.90	10.61±0.71^{b}	11.27±0.86
	Dough stage	2.62±0.28^{b}	9.31±0.95	10.69±0.70^{b}	8.41±0.48
Propionic acid	Early milk stage	0.15±0.02	0.20±0.08	3.22±1.57^{a}	2.08±0.38^{a}
	Milk stage	0.10±0.02	0.09±0.01	0.07±0.02^{b}	0.09±0.01^{b}
	Dough stage	0.58±0.04	1.15±0.09	1.52±0.06^{b}	1.20±0.09^{ab}
Butyric acid	Early milk stage	16.83±6.48^{a}	22.40±1.47^{a}	33.46±6.31^{a}	26.69±5.74^{a}
	Milk stage	6.94±2.20^{ab}	6.85±0.36^{b}	9.32±1.33^{b}	8.96±1.10^{b}
	Dough stage	0.48±0.17^{b}	16.23±2.38^{ab}	15.22±2.12^{b}	13.73±1.73^{b}

$^{a-c}$The letters in the same column mean significantly difference ($p<0.05$)

similar to those of treatment groups (with incoulant) at the same day of ensiling. The lactic acid content in silage ensiled at early milk stage with or without inoculant during fermentation was higher ($p<0.05$) than that of ensiled with or without inoculant at the other stages. This may be attributed to the high level of water soluble carbohydrate in corn crop at early milk stage. Johnson *et al.* (1966) reported that water soluble carbohydrate level was about 20-30% in corn crop at milk stage and then decreased (about 10%) at dough stage.

There were no differences in acetic, butyric and propionic acids contents of corn silages ensiled at the each stage between control and treatment groups. Butyric acid contents of control and treatment groups at the end of the study were the lowest ($p<0.05$) in milk stage and dough stage. Acetic acid, propionic acid and butyric acid levels of silages prepared by Deniz at different stages of vegetation (tasseling, milk and dough stages) were found similar with the results of the present study.

CONCLUSION

In this study the bacterial inoculant (Biomin® BioStabil Mays) used in the study under these conditions had no positive effects on corn silage fermentation ensiled at different stages of vegetation.

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