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The Effect of Bacteria+Enzyme Mixture Silage Inoculant on the Fermentation Characteristic, Cell Wall Contents and Aerobic Stabilities of Maize Silage

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Abstract: This research was carried out to determine the effects of Bacteria+Enzyme mixture inoculant using as silage additives on the fermentation characteristics, cell wall contents and aerobic stabilities of maize silages. Maize silage was harvested at milk blood stage. Maize-All (Alltech, UK) was used as additive which contains *Lactobacillus plantarum, Pediococcus acidilactici* and amylase in its biological composition. Maize was ensiled in 1.0 liter special glass jars equipped with a lid that enables gas release only. The jars were stored at 16±2.5°C under laboratory conditions. Three jars from each group were sampled for chemical and microbiological analyses on the days 3, 7, 14, 21 and 45 after ensiling. All silages were opened at the end of the ensiling period (45 days) and subjected to an aerobic stability test for 5 days. As a result, bacteria+enzyme improved fermentation characteristics, decreased cell wall contents and aerobic stability of maize silages.

Key words: Aerobic stability, fermentation characteristics, enzyme, lactic acid bacterize, maize silage

Introduction

Ensiling is a conservation method for moist forage crops. It is based on natural fermentation under anaerobe epiphytic lactic acid bacteria (LAB) convert water- soluble carbohydrates (WSC) into organic acids, As a result, pH decreases and the forage is preserved. Air is detrimental to the ensiling process a aerobic spoilage micro-organisms (Woolford, 1999).

In order to improve the ensiling process, various chemical and biological additives have been developed. The biological additives are advantageous because they are safe and easy to use, non corrosive to machinery, do not pollute the environment, and are regarded as natural products. Bacterial inoculants are added to silage in order to stimuli LA fermentation, accelerating the decrease in pH, and thus improving silage preservation. Most available inoculants consist of selected strains of homofermentative LAB, such as *Lactobacillus plantarum, Pediococcus, and Enterococcus species* (Weinberg and Muck, 1996). Many studies have shown advantages of such LAB inoculants (Lindgren, 1983; Weinberg *et al*., 1993) indicated that addition of homofermantative LAB inoculant impaired the aerobic stability of silages of mature cereal crops (wheat, sorghum, maize). This was suggested by rise in pH, visible mould 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 (Rust et al., 1989; Kennedy, 1990). Earlier observations had resulted in the opposite that LAB inoculants improved aerobic stability of silages (Ohyama *et al*., 1975; Pahlow, 1982). The likely explanation fort his phenomena is that under aerobic conditions, the homofermantative LAB inoculants produce mainly LA, which can serve as a substrate for lactate-assimilating yeasts upon aerobic exposure. Thus, only small amounts of short-chain VFAs (volatile fatty acids) such as acetic, propionic, and butyric acids produced. These short-chain aliphatic acids can inhibit yeasts and moulds (Moon, 1983), making silages treated with homofermantative LAB inoculants deteorate faster upon exposure to air.

The study was to study the effect of Bacteria+Enzyme mixture using as silage additives on the fermentation characteristics, cell wall contents and aerobic stabilities of maize silage.

Materials and Methods

Maize forage was harvested at the milk stage (229g kg^{-1} DM chopped to about 1.5 cm, treated with inoculant and ensiled in 60 glass jars. The jars were stored at ambient temperature (16±2.5°C). Three jars from each treatment were sampled for chemical and microbiological analyses on days 3, 7, 14, 21 and 45 after ensiling. Silages were exposed to air for 5 days after opening (45^{th} days) and temperature was monitored daily. In this test, numbers of yeast and mould, change in pH, DM content of silages were used as the indicators of aerobic deterioration.

The four treatments were Group 1; Control (no additive) and, Group 2; Inoculation level was $5.0x10^5$ cfu g^{-1} FM, Group 3; Inoculation level was $1.0x10^6$cfu g^{-1} FM, Group 4; Inoculation level was $2.0x10^6$ cfu g^{-1} FM.

Inoculant included two species of homofermentative lactic acid bacteria, *Lactobacillus plantarum* ve *Pediococcus acidilactici,* and an Amylase enzyme (Maize-All, Alltech, UK). 0.15,0.30,0.60 g of the inoculum powder, respectively, for groups 2, 3 and 4 was

Table 1: Chemical analysis of the maize silage (DM %)

Days	Treat-ment	pH	DM, g kg^{-1}	WSC g kg^{-1}	NH_3-N, g/kg	CP, g kg^{-1} DM	LA, % FM	AA, % FM
0	FM	4.61	229.2	71.58	-	62.9	-	-
3	C	3.64±0.02	220.8±0.45	60.03±0.04^{c}	-	59.5±0.05^{b}	1.10±0.09	0.61±0.02
	I_1	3.60±0.03	227.3±0.42	60.11±0.03^{b}	-	55.3±0.06^{c}	1.12±0.12	0.61±0.04
	I_2	3.60±0.01	223.4±0.04	60.97±0.01^{ab}	-	64.1±0.04^{a}	1.13±0.04	0.60±0.02
	I_3	3.51±0.06	229.1±0.22	61.58±0.02^{a}	-	64.4±0.02^{a}	1.16±0.80	0.58±0.01
7	C	3.96±0.01^{a}	238.4±0.11^{b}	49.02±0.02^{c}	-	56.3±0.03^{d}	1.21±0.03	0.60±0.05^{a}
	I_1	3.91±0.02^{ab}	218.2±0.48^{c}	51.36±0.03^{b}	-	64.3±0.00^{c}	1.25±0.07	0.59±0.02^{ab}
	I_2	3.86±0.02^{b}	228.4±0.08^{b}	52.12±0.05^{a}	-	65.8±0.01^{b}	1.28±0.02	0.56±0.02^{c}
	I_3	3.86±0.01^{b}	254.9±0.11^{a}	52.13±0.01^{a}	-	67.4±0.04^{a}	1.32±0.09	0.55±0.04^{c}
14	C	3.69±0.03	222.1±0.16^{b}	34.96±0.01	-	59.2±0.00^{c}	1.43±0.12^{c}	0.74±0.02^{a}
	I_1	3.68±0.01	224.7±0.24^{b}	34.99±0.03	-	62.0±0.03^{b}	1.54±0.05^{b}	0.69±0.03^{b}
	I_2	3.65±0.01	206.9±0.38^{c}	35.03±0.04	-	64.1±0.02^{a}	1.60±0.02^{a}	0.64±0.05^{c}
	I_3	3.63±0.02	248.7±0.18^{a}	35.98±0.02	-	65.4±0.01^{a}	1.61±0.05^{a}	0.62±0.06^{c}
21	C	3.80±0.01^{a}	224.7±1.12	25.86±0.01	-	58.7±0.00^{b}	1.59±0.02^{a}	0.70±0.02
	I_1	3.76±0.02^{a}	225.0±0.57	25.98±0.05	-	53.4±0.09^{c}	1.60±0.07^{a}	0.68±0.03
	I_2	3.70±0.01^{b}	217.9±1.41	26.00±0.06	-	59.2±0.02^{b}	1.63±0.05^{a}	0.67±0.06
	I_3	3.67±0.02^{b}	238.9±0.42	26.01±0.02	-	63.4±0.04^{a}	1.68±0.02^{b}	0.67±0.04
45	C	3.59±0.01^{a}	222.6±0.80^{b}	8.54±0.03^{b}	0.39±0.01	58.6±0.03^{b}	1.82±0.13^{a}	0.66±0.01
	I_1	3.56±0.25^{ab}	206.7±0.07^{b}	12.38±0.02^{a}	0.40±0.02	53.0±0.04^{c}	1.87±0.06^{a}	0.64±0.02
	I_2	3.55±0.00^{b}	211.6±0.19^{a}	13.09±0.01^{a}	0.35±0.04	54.2±0.02^{c}	1.96±0.07^{ab}	0.63±0.03
	I_3	3.54±0.01^{b}	234.0±0.36^{a}	14.28±0.02^{a}	0.37±0.06	61.8±0.02^{a}	2.06±0.04^{b}	0.63±0.02

DM: Dry Matter; CP: Crude Protein; NH3-NAmmonia nitrogen; FM: Fresh Matter; WSC: Water Soluble Carbohydrate; LA: Lactic acid, AA: Acedic acid, $^{a-d}$Means, within a colomn with no common supercript differ significantly, $p<0.05$.

Table 2: Effect of adding LAB+Enzyme mixture on the structual composition maize silage, DM%

Days	Treatment	NDF, gkg^{-1} DM	ADF, gkg^{-1} DM	ADL, gkg^{-1} DM	Hemicellulose, gkg^{-1} DM	Cellulose, gkg^{-1} DM
0	FM	569.5	331.2	82.8	238.3	248.4
45	C	527.2±0.03^{a}	285.4±0.01^{a}	76.3±0.02^{a}	241.8±0.25^{a}	209.1±0.06^{c}
	I_1	518.1±0.01^{b}	284.1±0.01^{a}	71.4±0.03^{b}	234.0±.0.15^{a}	212.7±0.03^{b}
	I_2	500.9±0.09^{c}	281.7±0.01^{b}	68.4±0.01^{c}	219.2±0.21^{b}	213.3±0.04^{b}
	I_3	485.1±0.03^{d}	279.3±0.03^{c}	62.3±0.02^{d}	207.4±0.26^{c}	216.9±0.10^{a}

NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL, acid detergent lignin; Hemisellüloz: NDF-ADF; Selüloz: ADF-ADL
$^{a-d}$Means, within a colomn with no common supercript differ significantly, $p<0.05$.

suspended in 60 ml tap water and sprayed over 30 kg (wet weight) of the chopped forage spread over 1x 4m^2 area, followed by through mixing. Thus, about 5.0x10^5, 1.0x10^6, 2.0x10^6 colony forming units (cfu) g^{-1} wet forage were applied.

The control silage was treated with an equivalent amount of water.

DM was determined by oven drying for 48 h at 60°C. Crude protein (CP) was determined by a Kjeldahl method (AOAC, 1980). The pH values of both fresh material and silage samples were obtained using the methods reported by Chen *et al.* (1994). The ammonia-N and water soluble carbohydrate contents of silages was determined, according to ADAS (1980). For the analysis of silo acids (lactic, acetic and butyric) the shortened version of Lepper's method (Akyildiz, 1984) was employed. Fibre analysis (neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) was performed according to Goering and Van Soest (1983). Lactic acid bacteria (LAB), yeast and mould counts were obtained according to the methods reported by Seale *et al.* (1986). Accordingly, as the incubation medium; MRS agar was used for LAB and malt extract was used for mould and yeast. LAB, mould and yeast counts of the samples were obtained at 30°C degrees following 3 days incubation period. The LAB, mould and yeast counts of the samples were converted into logarithmic coli-form unit (cfu/g).

The statistical analysis was a one-way analysis of variance with Duncan's multiple range test, performed with the Statistical Analysis System (SAS, 1988).

Results

The chemical composition of the fresh and ensiled maize silages were given Table 1. All silages were well preserved. In the experiment, LAB+Enzyme inoculant improved the fermentation parameters of maize silage. The pH of all silages decreased faster and to a greater extent. During fermentation, significant difference was shown between the pH values of control and LAB+Enzyme inoculated silages ($p<0.05$). In the experiment, the WSC in all silages decreased with the decrease in pH. LAB+Enzyme inoculated maize silages had significantly lower WSC compared with control

Table 3: Microbiological analysis of the fresh material, after 45 ensiling (means±.SD)

Days	Treatment	LAB log_{10} cfu/g FM	Mould and yeast log_{10} cfu / g FM
0		2.52	0
3	C	2.69±0.03	-
	I_1	2.69±.04	-
	I_2	2.71±.04	-
	I_3	2.73±.010	-
7	C	2.78±.03^{c}	-
	I_1	2.83±.02^{b}	-
	I_2	2.87±.02^{a}	-
	I_3	2.94±.01^{B}	-
14	C	3.83±0.03^{b}	-
	I_1	3.83±0.04^{b}	-
	I_2	3.86±0.03^{aB}	-
	I_3	3.88±0.02^{a}	-
21	C	4.31±0.01^{c}	-
	I_1	4.14±0.02^{bc}	-
	I_2	4.44±0.03^{b}	-
	I_3	4.56±0.04^{a}	-
45	C	4.19±0.02^{c}	-
	I_1	4.21±0.03^{bc}	-
	I_2	4.23±0.01^{b}	-
	I_3	4.28±0.02^{a}	-

LAB: lactic acid bacteria, cfu: colony forming unit; FM: fresh matterial, $^{a-d}$Means, within a colomn with no common supercript differ significantly, $p<0.05$.

Table 4: Result of the aerobic stability of maize silage

	pH	DM, %	WSC g/kg^{-1} DM	Moulds and yeasts log_{10} cfu/g FM
C	6.80±0.01^{a}	32.97±0.02^{a}	-	5.76±0.17
I_1	6.41±0.01^{b}	31.59±0.03^{b}	-	6.14±0.04^{c}
I_2	5.97±0.01^{c}	28.95±0.01d	-	6.56±0.03^{b}
I_3	5.92±0.01^{d}	31.13±0.02^{c}	-	6.65±0.02^{a}

$^{a-d}$Means, within a colomn with no common supercript differ significantly, $p<0.01$.

silage ($p<0.05$). Inoculant treatments did not affect the concentration of ammonia–N of the silages. After 3 days of ensiling, the silages inoculated had significantly higher lactic acid and lower acetic acid levels than the control silages ($p<0.05$). The same trend was shown at 7,14, 21 and days ensiling. During fermentation, no butyric acid was present in the silages.

The structural composition of the fresh and ensiled maize was given Table 2. The addition of enzyme, in combination with inoculum, improve ($p<0.05$) cell wall content of silages.

The microbial composition of the maize silages were given Table 3. Lactobacilli numbers of maize silages increased during the fermentation. In the present study, LAB+Enzyme inoculants increased lactobacilli and no yeast and mold numbers of maize silages compared with the control silage, except 45. days.

Table 4 gives the results of the aerobic exposure test of maize silages. Silage deterioration indicators are pH change, WSC, DM and an increase in yeast and mold numbers. The silages inoculated had significantly low pH and DM, higher moulds and yeasts than control silage ($p<0.05$).

Discussion

The success of 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 properties of the inoculant (Henderson and Mc Donald, 1984). Until now homofermantative 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, homo-fermentative LAB inoculant improved lactic acid production of silages. Bolsen *et al.* (1989) 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 (Moon *et al.*, 1981) were similar and the present findings further confirm these earlier conclusions. Seale (1986), in this 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 WSC of the crop 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 WSC and more extensive fermentation (McDonald *et al.*, 1991).

LAB inoculant did not ($p>0.05$) affect concentrations of ammonia-N of maize silages compared with the control silage. McDonald *et al.* (1991) reported that lower pH values inhibited protein degradation in silages. Therefore, concentrations of ammonia-N of all maize silages were low in the experiment.

At the end of ensiling period, LAB inoculants improved the microbiological composition of low DM maize silages as expected. LAB inoculants increased lactobacilli numbers of maize silages compared with the control silage. These findings are agreement with those reported by Spoelstra (1991), Filya (2002a,b; 2003; 2003). Saarisola *et al.* (2002) and Filya *et al.* (2001).

The results in the present study clearly indicated that LAB inoculants showed different effects on the aerobic stability of low DM maize silages. The increase in pH and mould and yeasts during air exposure is an indication of silage deterioration. Treatments had effect on temperature variation during exposure to air. It has been reported that the homofermentative LAB inoculated silages were unstable in aerobic conditions, as compared with heterofermentative LAB inoculed silages (Weinberg *et al.*, 1993; Ranjit and Kung, 200029). Researcher's expiation for the phenemon is that heterofermentative LAB produce some volatile fatty acids such as acetic and propionic acids which inhibit yeast and moulds, especially in the silages of higher DM.

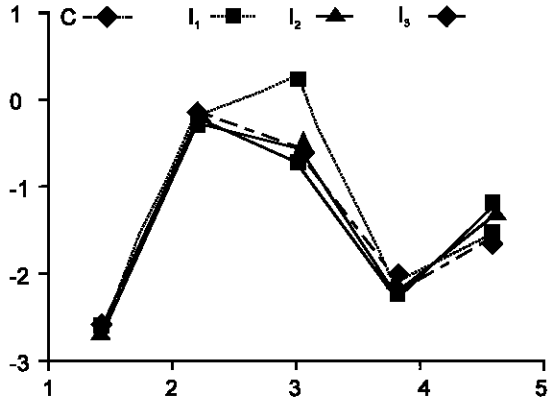

Fig. 1: Maize silage temperature differences from ambient over 5 day of air exposure.

These findings contrast with early observations improved aerobic stability of inoculated silages (Pahlow, 1982). The present study results clearly indicated that the homofermentative LAB+Enzyme affected aerobic stability of maize silage.

In conclusion, the results of the present study showed that LAB+Enzyme improved the fermentation characteristics or aerobic stability, and decreased cell wall contents of maize silages.

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