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# The Effect of Bacterial Inoculants and a Chemical Preservative on the Fermentation and Aerobic Stability of Whole-crop Cereal Silages

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**ABSTRACT :** Three microorganisms and one chemical preservative were tested for their effects on the fermentation and aerobic stability of whole-crop wheat, sorghum and maize silages. Wheat at the early dough stage, sorghum at the late milk stage and maize at the one-third milk line stage were harvested and ensiled in 1.5-l anaerobic jars untreated or after the following treatments: control (no additives); *Lactobacillus plantarum* (LP) at $1.0\times10^6$ colony-forming units (CFU)/g of fresh forage; *L. buchneri* (LB) at $1.0\times10^6$ CFU/g; *Propionibacterium acidipropionici* (PA) at $1.0\times10^6$ CFU/g; and a formic acid-based preservative (FAP) at 3 ml/kg of fresh forage weight. Three jars per treatment were sampled on d 90 after ensiling, for chemical and microbiological analysis. At the end of the ensiling period, 90 d, the silages were subjected to an aerobic stability test lasting 5 d. In this test, $CO_2$ produced during aerobic exposure was measured along with chemical and microbiological parameters which serve as spoilage indicators. The silages inoculated with LP had higher concentration of lactic acid compared with the controls and the other treated silages ($p<0.05$). The controls and LP-inoculated silages spoiled upon aerobic exposure faster than LB, PA and FAP-treated silages. The controls and LP-inoculated silages spoiled upon aerobic exposure faster than LB, PA and FAP-treated silages due to more $CO_2$ production ($p<0.05$) in these two groups and development of yeasts unlike the other groups. In the experiment, the silages treated with LB, PA and FAP were stable under aerobic conditions. However, the numbers of yeasts was higher in the LP-inoculated wheat, sorghum and maize silages compared with the LB, PA and FAP-treated silages. The LB, PA and FAP improved the aerobic stability of the silages by causing more extensive heterolactic fermentation that resulted in the silages with high levels of acetic and propionic acid. The use of LB, PA and FAP as silage additives can improve the aerobic stability of whole-crop wheat, sorghum and maize silages by inhibition of yeast activity. (**Key Words :** Silage, Whole-crop Cereals, Additives, Fermentation, Aerobic Stability)

## INTRODUCTION

Ensiling is a preservation method for moist forage crops. It is based on lactic acid bacteria (LAB) converting water-soluble carbohydrates (WSCs) into organic acids, mainly lactic acid, under anaerobic conditions. As a result, pH decreases and the moist forage is preserved from spoilage microorganisms (McDonald et al., 1991). Oxygen is detrimental to silage quality because it enables aerobic spoilage microorganisms such as yeasts, moulds and aerobic bacteria to become active (Woolford, 1990). When the silo is opened, oxygen enters the silo face, and aerobic microorganisms begin to multiply. Aerobic microorganisms respire primarily the preserving organic acids and other soluble compounds. This results in losses of highly digestible dry matter (DM), possible production of mycotoxins or growth of pathogenic species and the production of heat. These factors can make the silage unpalatable and induce browning reactions which reduce digestibility (O'Kiely et al., 1986).

Aerobic deterioration of sensitive silages is still a big problem in the ensiling process. Spoilage microorganisms in aerobically deteriorated silages include lactate-assimilating yeasts and moulds (Pahlow, 1991). Whole-crop cereal silages, such as wheat, sorghum and maize are susceptible to aerobic deterioration. Susceptibility to spoilage is a very important factor determining silage quality and digestibility (Ashbell et al., 2002). Therefore, it is very important to find suitable additives that inhibit fungi and protect the silage upon aerobic exposure.

In order to improve the ensiling process, many biological and chemical additives have been developed. Inoculants, comprising homofermentative LAB such as *Lactobacillus plantarum*, *Enterococcus faecium* and *Pediococcus* species, are often used to control the ensiling

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fermentation by rapid production of lactic acid and the consequent decrease in pH. However, such inoculants enhance the aerobic deterioration of silages (Sanderson, 1993; Filya, 2002a; Ando et al., 2006) because in these fermentations, not enough volatile fatty acids (VFAs) are produced to protect the silage against aerobic spoilage microorganisms (Moon, 1983). In recent years, a heterofermentative lactic acid bacterium, *L. buchneri*, has been studied as an additive to improve the aerobic stability of silages. *Lactobacillus buchneri* produces high levels of acetic acid in silage. Experiments in laboratory silos indicated that its application at ensiling improved the aerobic stability of silages (Driehuis et al., 1999; Kung and Ranjit, 2001; Weinberg et al., 2002; Filya, 2003a, b). Oude Elferink et al. (2001) reported that *L. buchneri* improved aerobic stability of silage by fermenting lactic acid to acetic acid and 1,2 propanediol. However, some studies under laboratory conditions showed that propionic acid bacteria (PAB), such as *Propionibacterium acidipropionici* and *P. shermanii*, improved the aerobic stability of mainly mature and dry cereal crops (Weinberg et al., 1995a; Filya et al., 2004). It would be expected that PAB would produce in the silage substances, which have antimycotic properties and which would, therefore, inhibit the development of yeasts and moulds upon aerobic exposure (Weinberg et al., 1995b). Chemical additives are also alternatives to improve the aerobic stability of silages. Application of chemical additives results in rapid acidification of the crop and partial inhibition of microbial growth (Woolford, 1984). Applying formic acid-based products have enhanced the aerobic stability of silages (Driehuis and Van Wikselaar, 1996; Salawu et al., 2001).

The objective of this study was to evaluate the effects of inoculation with homofermentative or heterofermentative LAB, PAB or treatment with a formic acid-based preservative (FAP) on the fermentation characteristics, microbial flora and aerobic stability of whole-crop cereal silages, such as wheat, sorghum and maize.

## MATERIALS AND METHODS

### Experimental

The following whole forage crops were used in these experiments: wheat (*Triticum aestivum* L.), sorghum (*Sorghum bicolor* L.) and maize (*Zea mays* L.). All forage crops were grown in different years in the Experimental Station (40°14' N, 28º50' E) of Agricultural Faculty of Uludag University, Bursa, Turkey. The sampling area is located at an altitude of 105 m above sea level. The mean annual rainfall and temperature are 729 mm and 15°C. Wheat was harvested on 26 May 2002 at the early dough stage, sorghum was harvested on 8 September 2003 at the late milk stage and maize was harvested on 24 August 2004 the one-third milk line stage. Forages were harvested by hand and chopped with a laboratory type chopper to about 1.5 cm and ensiled in 1.5 L anaerobic jars (Weck®, Wher-Oflingen, Germany) equipped with a lid that enables gas release only. Each jar was filled with about 800 g (wet weight) of chopped forage, without a headspace. The packing density was 189.5, 146.8 and 193.6 kg of DM/$m^3$ wheat, sorghum and maize, respectively. Each experiment had five treatments (untreated control and four additives), three jars per treatment. There were 15 jars per crop and they were stored at ambient temperature (22-28°C). Fresh and ensiled materials (on d 90 after ensiling, three jars per treatment) were sampled for chemical and microbiological analysis. At the end of the ensiling period, 90 d, the silages were subjected to an aerobic stability test at room temperature (25°C), which lasted for 5 d, in a "bottle" system developed by Ashbell et al. (1991). The system was constructed in two parts from recycled soft drink bottles (polyethylene terepthalate): the upper part (1 L) was filled with about 250 g (wet weight) of loosely packed silage, and the lower part with 100 ml of 20% KOH. Gas was exchanged through 1 cm holes in the upper part. Carbon dioxide produced during aerobic exposure was absorbed in the base and determined by titration with 1 N HCL. In addition, change in pH, yeast and mould counts served as indicators of aerobic spoilage. Chemical and microbiological analyses were carried out on the silage samples, initially and after 5 d exposure to air.

### Treatments

The following microbial and chemical additives were applied to fresh forages:

- Control (no additives);
- Homofermentative LAB inoculant, containing *L. plantarum* ((LP) Biomax5; Chr. Hansen Biosystems, USA; final application rate of $1.0\times10^6$ colony-forming units (CFU)/g of fresh forage weight);
- Heterofermentative LAB inoculant, containing *L. buchneri* ((LB) Pioneer® brand 11A44, USA; $1.0\times10^6$ CFU/g);
- PAB inoculant, containing *P. acidipropionici* ((PA) MA26/4U, Lallemand, France; $1.0\times10^6$ CFU/g);
- FAP, liquid formulation containing 860 g/kg active ingredients (formic acid, ammoniumformiate, propionic acid, benzoic acid and ester of benzoic acid; Kemisile® 2000, Kemira Chemicals, Finland; final application rate of 3 ml/kg of the fresh forage weight).

All information pertaining to additives is derived from the manufacturers' statements. The inoculants were diluted in deionised water and applied at the rate of 1 g/100 g of the fresh forage. The control received 1 g of deionised water/100 g fresh forage. Forages treated with FAP at the

**Table 1.** Chemical and microbiological compositions of experimental fresh forages prior to ensiling

| Forage type | pH | DM (g/kg) | DM (g/kg) | | | | log CFU/g | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | WSC | Ash | CP | NDF | Lactobacilli | Yeasts | Moulds |
| Wheat | 6.52[a] | 355.3[a] | 108.4[b] | 63.2[b] | 70.0[a] | 536.7[b] | 3.05[c] | 3.64[c] | 3.35[b] |
| Sorghum | 6.14[b] | 275.2[b] | 164.1[a] | 66.0[a] | 53.6[c] | 594.9[a] | 3.47[b] | 4.23[b] | 3.06[c] |
| Maize | 6.05[b] | 363.0[a] | 71.6[c] | 54.1[c] | 65.5[b] | 503.3[c] | 3.76[a] | 4.54[a] | 3.67[a] |
| SEM | 0.03 | 3.49 | 1.82 | 0.50 | 0.52 | 2.17 | 0.02 | 0.03 | 0.03 |

DM = Dry matter, CFU = Colony-forming units, WSC = Water-soluble carbohydrates.
CP = Crude protein, NDF = Neural detergent fibre, SEM = Standard error mean.

**Table 2.** Fermentation profiles of experimental silages after 90 days of fermentation

| Forage type | Treatment | pH | DM (g/kg) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | WSC | Lactic acid | Acetic acid | Propionic acid | Butyric acid | Ethanol | $NH_3$-N | Weight loss |
| Wheat | Control | 4.22[b] | 59.5[a] | 49.6[b] | 9.3[c] | 0.2[c] | 0.7 | 2.1 | 0.230[b] | 0.16[b] |
| | LP | 3.96[c] | 54.3[a] | 81.4[a] | 5.6[c] | 0.1[c] | 0.2 | 1.6 | 0.194[c] | 0.15[b] |
| | LB | 4.67[a] | 20.7[b] | 36.3[c] | 27.4[a] | 0.1[c] | 0.1 | 1.7 | 0.259[a] | 0.32[a] |
| | PA | 4.55[a] | 57.9[a] | 51.5[b] | 18.3[b] | 7.8[a] | 0.3 | 1.4 | 0.246[a] | 0.29[a] |
| | FAP | 3.94[c] | 58.8[a] | 56.5[b] | 14.9[b] | 2.4[b] | 0.2 | 1.5 | 0.155[d] | 0.17[b] |
| | SEM | 0.03 | 0.04 | 0.05 | 0.03 | 0.02 | 0.10 | 0.18 | 0.011 | 0.03 |
| Sorghum | Control | 3.92[b] | 73.2[a] | 58.6[b] | 11.6[c] | 0.3[c] | 0.3 | 1.9 | 0.190[b] | 0.14[b] |
| | LP | 3.83[b] | 66.5[a] | 97.1[a] | 8.0[c] | 0.1[c] | 0.2 | 1.5 | 0.147[c] | 0.12[b] |
| | LB | 4.41[a] | 25.0[b] | 40.4[c] | 34.5[a] | 0.1[c] | 0.1 | 1.7 | 0.231[a] | 0.30[a] |
| | PA | 4.34[a] | 77.7[a] | 55.9[b] | 22.0[b] | 6.9[a] | 0.2 | 1.3 | 0.220[a] | 0.31[a] |
| | FAP | 3.78[b] | 74.6[a] | 63.7[b] | 18.2[b] | 2.8[b] | 0.1 | 1.3 | 0.124[d] | 0.14[b] |
| | SEM | 0.04 | 0.02 | 0.03 | 0.02 | 0.03 | 0.11 | 0.21 | 0.012 | 0.02 |
| Maize | Control | 3.96[b] | 26.0[a] | 54.6[b] | 10.5[c] | 0.1[c] | 0.2 | 2.0 | 0.156[b] | 0.11[b] |
| | LP | 3.78[bc] | 21.7[a] | 92.3[a] | 7.5[c] | 0.1[c] | 0.1 | 1.6 | 0.133[c] | 0.11[b] |
| | LB | 4.55[a] | 4.3[b] | 39.0[c] | 32.0[a] | 0.1[c] | 0.1 | 1.7 | 0.188[a] | 0.27[a] |
| | PA | 4.48[a] | 25.8[a] | 53.7[b] | 21.1[b] | 7.4[a] | 0.1 | 1.3 | 0.184[a] | 0.25[a] |
| | FAP | 3.66[c] | 23.7[a] | 60.6[b] | 18.7[b] | 2.2[b] | 0.1 | 1.4 | 0.101[d] | 0.10[b] |
| | SEM | 0.02 | 0.05 | 0.04 | 0.03 | 0.02 | 0.14 | 0.17 | 0.013 | 0.03 |

Within a column and forage type means followed by different letter differ significantly ($p<0.05$).
DM = Dry matter, WSC = Water-soluble carbohydrates, $NH_3$-N = Ammonia-nitrogen, LP = *Lactobacillus plantarum.*
LB = *Lactobacillus buchneri,* PA = *Propionibacterium acidipropionici,* FAP = Formic acid-based preservative, SEM = Standard error mean.

rate of 3 ml/kg of the fresh forage weight. The amount of chopped forage for a given jar was weighed out, sprayed with the appropriate additives using a plant sprayer (one sprayer for each treatment), mixed by hand, and then placed into the jar by hand with periodic tamping. Equipment coming in contact with treated forage was washed and wiped with ethanol between treatments to prevent cross-contamination. Silos were weighed before and after filling to determine the actual amount ensiled. Over the course of filling the jars for all treatments, three samples of untreated chopped forage were taken for analysis of initial characteristics, and all additives were analyzed for LAB counts.

**Analytical procedures**

Chemical analyses of fresh forages and silages were performed in triplicate and presented on DM basis. The silage pH was measured directly from the silage juice using a pH meter (Sartorius PB-20, Germany). The DM content of the fresh forages and silages was determined by drying at 60°C for 48 h in a fan-assisted oven. Ash was obtained after 3 h at 550°C. Crude protein (CP) was determined by a Kjeldahl method (AOAC, 1990). Neutral detergent fibre (NDF) assayed without a heat stable amylase and expressed inclusive of residual ash (Van Soest et al., 1991). Wet samples stored at -20°C were extracted for 3 min in a blender with water, or ethyl acetate (1:9), for WSCs and fermentation product analysis, respectively. Water-soluble carbohydrates were determined by the phenol sulphuric acid method (Dubois et al., 1956). Lactic acid, ethanol, and volatile fermentation end-products were determined in aqueous extracts using a gas chromatograph with FID detector and a semi-capillary FFAP column (Hewlett Packard, Germany), over a temperature range of 45-230°C. Ammonia in the silages was determined by extraction of 40 g frozen samples with 360 ml distilled water for 3 min in a Stomacher blender; 100 ml of the extract were used for distillation in the Kjeldahl unit without a digestion step but with the addition of base. Gas losses during storage were estimated by weight loss, calculated separately for each silo

**Table 3.** Microbiological composition of experimental silages after 90 days of fermentation

| Forage type | Treatment | log CFU/g | | |
|---|---|---|---|---|
| | | Lactobacilli | Yeasts | Moulds |
| Wheat | Control | 4.28[b] | 3.37[b] | 1.50 |
| | LP | 6.96[a] | 4.63[a] | 1.42 |
| | LB | 3.97[b] | 2.04[c] | 1.38 |
| | PA | 4.15[b] | 2.12[c] | 1.45 |
| | FAP | 4.03[b] | 1.81[c] | 1.23 |
| | SEM | 0.06 | 0.18 | 0.43 |
| Sorghum | Control | 5.58[b] | 4.07[b] | 1.45 |
| | LP | 7.80[a] | 5.10[a] | 1.37 |
| | LB | 5.06[b] | 2.16[c] | 1.29 |
| | PA | 5.79[b] | 2.08[c] | 1.33 |
| | FAP | 5.65[b] | 1.95[c] | 1.15 |
| | SEM | 0.07 | 0.13 | 0.37 |
| Maize | Control | 4.97[b] | 3.49[b] | 1.47 |
| | LP | 8.61[a] | 4.87[a] | 1.40 |
| | LB | 5.30[b] | 1.98[c] | 1.34 |
| | PA | 4.84[b] | 2.06[c] | 1.32 |
| | FAP | 5.13[b] | 1.79[c] | 1.16 |
| | SEM | 0.04 | 0.15 | 0.34 |

Within a column and forage type means followed by different letter differ significantly ($p<0.05$).
CFU = Colony-forming units, LP = *Lactobacillus plantarum*.
LB = *Lactobacillus buchneri*. PA = *Propionibacterium acidipropionici*.
FAP = Formic acid-based preservative, SEM = Standard error mean.

the difference in the weight at the beginning and end of the ensiling period.

Microbiological analyses of fresh forage and silages were performed in triplicate and presented on fresh and wet silage basis. Microbial evaluation included enumeration of lactobacilli on pour-plate Rogosa agar (Oxoid CM627, Oxoid, Basingstoke, UK), and yeast and moulds on spread-plate malt extract agar (Difco, Detroit, MI, USA) acidified with lactic acid to pH 4.0. Plates were incubated for 3 d at 30°C. All microbiological data were transformed to $\log_{10}$.

The data were analyzed as a completely randomized design and subjected to ANOVA by the general linear model procedure of Statistical Analysis System (SAS, 1989). Differences in silage characteristics between additive treatments within a forage were tested using Duncan's Multiple Range test and significance was declared at $p<0.05$.

## RESULTS AND DISCUSSION

The chemical and microbiological compositions of fresh wheat, sorghum and maize prior to ensiling are given in Table 1. With these forages a wide range of chemical compositions and ensiling characteristics was obtained. All fresh forages were high pH value. Fresh sorghum had higher WSCs, ash and NDF and lower content of CP than wheat and maize ($p<0.05$). The numbers of lactobacilli, yeasts and moulds in fresh maize were higher than wheat and sorghum ($p<0.05$).

The effect of additives on the fermentation profiles of the wheat, sorghum and maize silages after 90 d of fermentation are shown in Table 2. All silages were well preserved, as would be expected with carbohydrate-rich crops. After 90 d of ensiling, the pH and residual WSCs levels of the silages were reduced, and concentrations of lactic acid, VFAs and ethanol increased. The major fermentation product in all silages was lactic acid. The silages inoculated with LP had higher lactic acid than the control and the silages treated with LB, PA and FAP ($p<0.05$). *Lactobacillus buchneri* and PA-inoculated silages had higher pH than the control, LP and FAP-treated silages ($p<0.05$). The pH was the highest in the LB-inoculated silages. *Lactobacillus buchneri*-inoculated silages had higher levels of acetic acid and lower levels of residual WSCs and lactic acid than the control, LP, PA and FAP-treated silages ($p<0.05$). *Propionibacterium acidipropionici*-inoculated silages increased propionic acid levels of the silages compare with the control, LP, LB and FAP-treated silages ($p<0.05$). The highest propionic acid was found in the PA-inoculated silages. High levels of acetic and propionic acid in the LB and PA-inoculated silages were evident from acetic and propionic acid production. *Propionibacterium acidipropionici*-inoculated silages had higher levels of acetic acid than the control and LP-inoculated silages ($p<0.05$). However, additives did not affect levels of butyric acid and ethanol of the silages. *Lactobacillus buchneri* and PA-inoculated silages increased ammonia-N concentrations of the silages compared to the control, LP and FAP-treated silages ($p<0.05$). Ammonia-N concentrations were the lowest in the FAP-treated silages. Weight losses were higher in the LB and PA-inoculated silages than in the control, LP and FAP-treated silages ($p<0.05$).

The microbiological composition of the wheat, sorghum and maize silages after 90 days of fermentation is given in Table 3. Lactobacilli number of the silages was increased during the fermentation. After 90 d of ensiling, numbers of lactobacilli were higher in the LP-inoculated silages compared with the control, LB, PA and FAP-treated silages ($p<0.05$). In the experiment, LP increased numbers of yeasts of the silages. Yeasts numbers were the highest in the LP-inoculated silages. However, additives did not affect moulds numbers of the silages.

The effect of additives on the aerobic stability of the wheat, sorghum and maize silages after exposure to air for 5 d is given in Table 4. Silage deterioration indicators were pH change, $CO_2$ production and an increase in yeast and mould numbers. In the aerobic stability test, LP-inoculated silages produced more $CO_2$ than the control, LB, PA and FAP-treated silages ($p<0.05$). *Lactobacillus plantarum*-inoculated silages had higher numbers of yeasts and moulds than the controls, LB, PA and FAP-treated silages during

**Table 4.** Aerobic stability of experimental silages after exposure to air for 5 days

| Forage type | Treatment | pH | $CO_2$ (g/kg DM) | log CFU/g | |
|---|---|---|---|---|---|
| | | | | Yeasts | Moulds |
| Wheat | Control | 5.24[a] | 26.62[b] | 4.40[b] | 4.83[b] |
| | LP | 5.45[a] | 38.15[a] | 6.68[a] | 6.90[a] |
| | LB | 4.42[b] | 6.54[c] | 2.61[c] | 2.35[c] |
| | PA | 5.24[a] | 7.10[c] | 2.85[c] | 2.31[c] |
| | FAP | 4.06[c] | 5.93[c] | 2.24[c] | 1.93[c] |
| | SEM | 0.03 | 0.05 | 0.15 | 0.11 |
| Sorghum | Control | 5.87[a] | 21.48[b] | 4.10[b] | 4.97[b] |
| | LP | 5.75[a] | 40.06[a] | 7.09[a] | 7.12[a] |
| | LB | 4.37[b] | 5.69[c] | 2.47[c] | 2.34[c] |
| | PA | 5.56[a] | 6.36[c] | 2.66[c] | 2.18[c] |
| | FAP | 4.18[b] | 5.28[c] | 2.38[c] | 1.80[c] |
| | SEM | 0.01 | 0.04 | 0.13 | 0.08 |
| Maize | Control | 5.51[a] | 17.85[b] | 3.81[b] | 4.52[b] |
| | LP | 5.60[a] | 33.93[a] | 5.94[a] | 6.73[a] |
| | LB | 4.34[c] | 4.88[c] | 2.38[c] | 2.05[c] |
| | PA | 5.06[b] | 5.26[c] | 2.45[c] | 2.01[c] |
| | FAP | 4.03[c] | 4.59[c] | 2.20[c] | 1.67[c] |
| | SEM | 0.03 | 0.06 | 0.18 | 0.09 |

Within a column and forage type means followed by different letter differ significantly ($p<0.05$).
DM = Dry matter, CFU = Colony-forming units.
LP = *Lactobacillus plantarum*, LB = *Lactobacillus buchneri*.
PA = *Propionibacterium acidipropionici*.
FAP = Formic acid-based preservative, SEM = Standard error mean.

exposure to air ($p<0.05$). *Lactobacillus plantarum*-inoculated silages were unstable under aerobic conditions. These silages deteriorated upon aerobic exposure faster than the control, LB, PA and FAP-treated silages.

In the experiment, the chemical and microbiological compositions were variable among fresh forages but within the range reported in other experiments with wheat, sorghum and maize (Weinberg et al., 1993; Filya et al., 2004). Inoculation with LP decreased pH, concentration of ammonia-N and fermentation losses of the wheat, sorghum and maize silages. In addition, LP increased numbers of lactobacilli, yeasts and concentration of lactic acid of the silages. As a result, inoculation with LP improved the homolactic fermentation and impaired the aerobic stability of the wheat, sorghum and maize silages. Weinberg et al. (1993) reported that inoculation with LP improved homolactic fermentation and impaired the aerobic stability of wheat, sorghum and maize silages. Another study Filya (2002b) showed that LP treatment improved fermentation characteristics, increased numbers of yeasts and moulds, $CO_2$ production and impaired the aerobic stability of sorghum and maize silages. An explanation for the negative responses to the addition of LAB is that, under anaerobic conditions, the homofermentative LAB inoculants produce mainly lactic acid, which can serve as a substrate for lactate-assimilating yeasts upon exposure to air (Wohlt, 1989). Lactic acid by itself is not an effective antimycotic agent (Moon, 1983). Weinberg et al. (1993) reported that high levels of residual WSCs, combined with a high lactic acid concentrations and lack of sufficient concentrations of protective VFAs in the silages inoculated with homofermentative LAB were associated with aerobic spoilage. This is because both WSCs and lactic acid are substrates for lactate assimilating yeast, and VFAs often inhibit the growth of these organisms. The use of homofermentative LAB might lead to such conditions in some sugar-rich silage. This is because the homolactic fermentation is more efficient and utilizes less WSCs than heterolactic fermentation, which results in a higher content of residual sugars and lactic acid in the silage.

In order to use biological additives to overcome the problem of aerobic deterioration of silages, it has been suggested that other types of inoculants, such as PAB (Pahlow and Honig, 1994) and heterofermentative LAB (Muck, 1996). Heterofermentative LAB and PAB can ferment sugars and lactate to acetate and propionate; these short-chain aliphatic acids inhibit the growth of all yeasts and moulds (Woolford, 1975; Moon, 1983). In the experiment, inoculation with LB and PA improved the aerobic stability of the wheat, sorghum and maize silages. The higher amount of acetic acid in the PA-inoculated silages was expected because acetic acid is a co-metabolite of the fermentation of carbohydrates and lactic acid by PA (Dawson et al., 1998). However, production of acetic and propionic acid in the LB and PA-inoculated silages decreased the numbers of yeasts and moulds. Moon (1983) and McDonald et al. (1991) reported that acetic and propionic acids were fungicidal agents. Filya et al. (2004) showed that high concentrations of acetate and propionate inhibited yeasts and moulds growth in the wheat, sorghum and maize silages. In the experiment, LB and PA were able to protect the aerobic stability of the wheat, sorghum and maize silages. Driehuis et al. (1999), Kung and Ranjit (2001) and Zahiroddini et al. (2006) reported that inoculation with LB increased acetic acid concentration and decreased yeast numbers of barley and maize silages. Another studies Weinberg et al. (2002) and Filya (2003a, b) showed that LB treatment increased acetic acid concentration, decreased yeast and mould numbers, $CO_2$ production and improved the aerobic stability of wheat, sorghum and maize silages. Findings about the effects of PA on the aerobic stability of the silages are in agreement with those of Weinberg et al. (1995a, b), Bolsen et al. (1996), Dawson et al. (1998) and our previous experiment Filya et al. (2004) with wheat, sorghum and maize silages.

The ammonia-N in silages shows the degree of protein degradation. The combined effects of both plant and microbial enzymes result in extensive changes to the nitrogenous fractions during ensiling. Plant protein is broken down into peptides and free amino acids by the

action of plant proteases while the breakdown of amino acids to ammonia and other non-protein nitrogen compounds is mainly by the action of clostridia and enterobacteria in the silo (Ohshima and McDonald, 1978). Driehuis et al. (2001) reported that the increase in ammonia-N in LB-inoculated maize silage was associated with the relatively large increase in pH taking place during the storage phase as a result of the high metabolic activity of LB in these silages. The same trend was shown in this experiment. High metabolic activity of LB and PA increased pH levels of the wheat, sorghum and maize silages. These findings are in agreement with Bolsen et al. (1996), Kung and Ranjit (2001) and my previous experiments (Filya 2003a, b).

Buffered and unbuffered formic acid-based products have been successfully used as silage additives to improve the aerobic stability of silages (Driehuis and Van Wikselaar, 1996; Salawu et al., 2001; Adesogan and Salawu, 2002; Filya and Sucu, 2005). In the present study, FAP improved the silage quality and aerobic stability of the wheat, sorghum and maize silages. All FAP-treated silages had higher concentrations of acetic and propionic acid than the control silage ($p<0.05$). Under aerobic conditions, FAP-treated silages had lower pH, $CO_2$ production and the numbers of yeasts and moulds than the control silage ($p<0.05$). The apparent improvement in the aerobic stability of FAP-treated silages may result from the combined effect of acetic and propionic acids. This is because acetic and propionic acids are fungicidal agents and enough concentrations of acetate and propionate inhibit the growth of yeasts and moulds in the silages (Moon, 1983; McDonald et al., 1991). Adesogan and Salawu (2002) reported that in addition to the presence of acetic and propionic acids, the superior aerobic stability of the formic acid treated bi-crops at both peas to wheat ratios resulted from the rapid reduction in pH during fermentation and possibly the maintenance of low pH during exposure to air. The same trend was shown in this experiment. Low pH and high levels of acetic and propionic acids inhibited the activity of yeasts and moulds that are responsible for aerobic deterioration of silages. However, FAP reduced concentrations of ammonia-N of the silages. Davies et al. (1998) reported that the beneficial effect of FAP on the fermentation was largely because FAP enhances pH reduction and thereby reduce plant enzyme and microbial-mediated proteolysis. These findings are in agreement with Driehuis and Van Wikselaar (1996), Salawu et al. (2001), Adesogan and Salawu (2002) and our previous experiment (Filya and Sucu, 2005).

## IMPLICATIONS

In conclusion, the results of this study showed that LP improved the silage fermentation by causing more extensive homolactic fermentation and resulted in the silages with the highest levels of lactic acid. *Lactobacillus plantarum* and FAP were very effective in protecting the silages against proteolysis. *Lactobacillus plantarum*-inoculated silages were unstable under aerobic conditions because the production of antifungal compounds, such as acetic and propionic acid, was reduced. However, LB, PA and FAP improved the aerobic stability of the silages. The silages treated with LB, PA and FAP had more acetic acid. Furthermore, PA and FAP produced propionic acid. Both VFAs were fungicidal agents and inhibited yeasts and moulds growth in the silages. The use of LB, PA and FAP as silage additives can improve the aerobic stability of whole-crop cereal silages, such as wheat, sorghum and maize, by inhibition of yeast activity.

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