

## The Effect of *Lactobacillus buchneri* and *Lactobacillus plantarum* on the Fermentation, Aerobic Stability, and Ruminant Degradability of Low Dry Matter Corn and Sorghum Silages

I. Filya

Department of Animal Science, Faculty of Agriculture,  
Uludag University, 16059 Bursa, Turkey

### ABSTRACT

The effect of *Lactobacillus buchneri*, alone or in combination with *Lactobacillus plantarum*, on the fermentation, aerobic stability, and ruminant degradability of low dry matter corn and sorghum silages was studied under laboratory conditions. The inoculants were applied at  $1 \times 10^6$  cfu/g. Silages with no additives served as control. After treatment, the chopped forages were ensiled in 1.5-L anaerobic jars. Three jars per treatment were sampled on d 2, 4, 8, 15, and 90. After 90 d of storage, the silages were subjected to an aerobic stability test lasting 5 d, in which CO<sub>2</sub> production, as well as chemical and microbiological parameters, was measured to determine the extent of aerobic deterioration. At the end of the ensiling period (d 90), the *L. buchneri*- and *L. buchneri* + *L. plantarum*-inoculated silages had significantly higher levels of acetic acid than the control and *L. plantarum*-inoculated silages. Therefore, yeast activity was impaired in the *L. buchneri*- and *L. buchneri* + *L. plantarum*-inoculated silages. As a result, *L. buchneri*, alone or in combination with *L. plantarum*, improved aerobic stability of the low dry matter corn and sorghum silages. The combination of *L. buchneri* and *L. plantarum* reduced ammonia N concentrations and fermentation losses in the silages compared with *L. buchneri* alone. However, *L. buchneri*, *L. plantarum*, and a combination of *L. buchneri* + *L. plantarum* did not effect in situ rumen dry matter, organic matters, or neutral detergent fiber degradability of the silages. The *L. buchneri* was very effective in protecting the low dry matter corn and sorghum silages exposed to air under laboratory conditions. The use of *L. buchneri*, alone or in combination with *L. plantarum*, as a silage inoculant can improve the aerobic stability of low dry matter corn and sorghum silages by inhibition of yeast activity.

(**Key words:** aerobic stability, *Lactobacillus buchneri*, *Lactobacillus plantarum*, silage)

**Abbreviation key:** LAB = lactic acid bacteria, WSC = water-soluble carbohydrates.

### INTRODUCTION

Ensiling is a preservation method for moist forage crops. It is based on lactic acid bacteria (LAB) converting water-soluble carbohydrates (WSC) 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). Air is detrimental to silage quality because it enables aerobic spoilage microorganisms, such as yeasts and molds, to become active (Woolford, 1990). During exposure to air during the feedout phase, silage also might undergo increases in temperature and pH and losses of WSC and fermentation end products, which reduce silage quality and digestibility (Pitt et al., 1991). Susceptibility to spoilage is especially a problem in warm climates and is a very important factor in determining silage quality and value (Ashbell et al., 2002). Therefore, under warm conditions, additives that protect the silage upon exposure to air might be very useful.

It is possible to apply bacterial inoculants at ensiling in order to promote adequate fermentation patterns. Inoculants, comprising homofermentative LAB such as *Lactobacillus plantarum*, *Enterococcus faecium*, and *Pediococcus* species, are often used to control the ensiling fermentation by rapid production of lactic acid and the consequent decrease in pH. However, such inoculants enhance the aerobic spoilage of wheat, sorghum, and corn silages (Weinberg et al., 1993; Filya et al., 2000; 2002a,b) because in these fermentations, not enough VFA are produced to protect the silage against aerobic yeasts and molds (Moon, 1983). Aerobic deterioration of silage is not only associated with high DM losses, but also with a risk of mycotoxin production in the feed by aerobic fungi; such mycotoxins are detrimental to animal health. Weinberg et al. (1993) hypoth-

Received May 28, 2003.

Accepted July 14, 2003.

Corresponding Author: I. Filya; e-mail: ifilya@uludag.edu.tr.

esized that high levels of residual WSC, combined with high lactic acid concentrations and a lack of sufficient concentrations of protective VFA in the silages inoculated with homofermentative LAB were associated with aerobic spoilage. This is because both WSC and lactic acid are substrates for fungi, and VFA often inhibit these organisms. These findings stimulated the search for bacterial strains that might be suitable as silage inoculants and might also protect the silage upon aerobic exposure.

A heterofermentative lactic acid bacterium, *Lactobacillus buchneri*, has been studied as an additive to improve the aerobic stability of silages (Muck, 1996). *L. buchneri* produces high levels of acetic acid in silage. Experiments in laboratory silos indicated that its application at ensiling improved the aerobic stability of the silages (Driehuis et al., 1999a,b; Filya, 2001, 2003; Kung and Ranjit, 2001; Filya et al., 2002; Ranjit et al., 2002; Weinberg et al., 2002). Oude Elferink et al. (2001) reported that *L. buchneri* improved aerobic stability by fermenting lactic acid to acetic acid and 1,2-propanediol. Although extensive heterolactic fermentation is usually deemed undesirable compared with a homolactic fermentation (McDonald et al., 1991), improvements in aerobic stability during prolonged storage and feeding may be beneficial, thus making small losses of DM incurred from heterofermentation less important (Kung and Ranjit, 2001).

The purpose of the present work was to study the effects of *L. buchneri*, alone or in combination with homofermentative LAB, on the fermentation, aerobic stability, and ruminal degradability of low-DM corn and sorghum silages. This was done by application of the microorganisms to laboratory silages.

## MATERIALS AND METHODS

Corn (*Zea mays* L.) at dent stage and sorghum (*Sorghum bicolor* L.) at flowering stage of maturity were harvested and chopped using a conventional forage harvester (Sezer, Bandirma, Turkey) to approximately 1.5 cm and ensiled in 1.5-L anaerobic jars (Weck, Wher-Oftlingen, Germany) equipped with a lid that enabled gas release only. Each jar was filled with approximately 850 g (wet weight) of chopped forage, without a headspace. The packing density was 133.2 and 125.8 kg of DM/m<sup>3</sup> in corn and sorghum, respectively. There were 60 jars per crop, and they were stored at ambient temperature (24 to 28°C). Fresh and ensiled materials (on d 2, 4, 8, 15, and 90 after ensiling; 3 jars for each time) were sampled for chemical and microbiological analysis. At the end of the ensiling period (d 90), the silages were subjected to an aerobic stability test at room temperature (28°C), which lasted 5 d, in a "bottle" system

developed by Ashbell et al. (1991). In this system, CO<sub>2</sub> production, change in pH, and numbers of yeast and molds serve as spoilage indicators.

The following treatments were applied to fresh forages: 1) Control (no additives); 2) *L. buchneri* (Pioneer brand 11A44, Des Moines, IA; final application rate of  $1 \times 10^6$  cfu/g of fresh forage); 3) an inoculant containing *L. plantarum* ( $1 \times 10^6$  cfu/g); and 4) a combination of *L. buchneri* ( $1 \times 10^6$  cfu/g) and *L. plantarum* ( $1 \times 10^6$  cfu/g). The application rate determined by manufacturers stated the level of LAB in the products. The inoculants were applied as follows: on the day of the experiment, inoculants were suspended in 20 ml of deionized water and the whole suspension was sprayed over 10 kg (wet weight) of the chopped forage spread over a  $1 \times 4$  m area. All inoculants were applied to the forages in a uniform manner with constant mixing.

## Analyses

Chemical analyses were performed in triplicate and presented on DM basis. The DM content of the fresh materials and silages was determined by oven drying for 48 h at 60°C. Ash was obtained after 3 h at 550°C. Crude protein was determined by the Kjeldahl method (AOAC, 1990). Neutral detergent fiber was analyzed by using sulfite and amylase (Van Soest et al. 1991). Wet samples stored at -20°C were extracted for 3 min in a blender in water or in ethyl acetate (1:9) for WSC and fermentation product analysis, respectively. Water-soluble carbohydrates were determined by the phenol-sulfuric acid method (Dubois et al., 1956). Lactic acid, ethanol, and volatile fermentation end products were determined in aqueous extracts by means of a GLC with a semi-capillary FFAP (nitroterephthalic acid-modified polyethylene glycol) column (Hewlett-Packard, Wardbronn, Germany), over a temperature range of 45 to 230°C. Ammonia N was determined in the silages by extraction of 40-g frozen samples with 360 ml of distilled water for 3 min in a Stomacher blender (IUL, Barcelona, Spain). The extract was filtered through Whatman No. 1 paper (Whatman, Maidstone, U.K.), and 100 ml of the extract was used for distillation in a Kjeltex auto analyzer (Gerhardt, Bonn, Germany) without a digestion step. Gas losses were evaluated by weight loss. Rumen degradability of the silages was measured by the in situ procedure of Mehrez and Orskov (1977). Air-dried forage samples were ground through a 1-mm screen using a Laboratory 3303 Mill (Hundunge, Sweden). The milled samples were placed in  $9 \times 14$  cm Dacron bags (pore size = 10 to 40  $\mu$ m), which were inserted into the rumen of three fistulated Merino sheep fed a concentrate and alfalfa hay diet. The Dacron bags were incubated in the rumen for 48 h.

**Table 1.** Chemical (DM basis) and microbiological analysis of the fresh forages.

Forage type	pH	%					log <sub>10</sub> cfu/g		
		DM	WSC <sup>1</sup>	Ash	CP	NDF	Lactobacilli	Yeasts	Molds
Corn	5.86	23.5	9.25	8.1	7.6	51.6	3.86	4.06	2.58
Sorghum	5.93	22.2	25.63	8.7	6.6	64.4	3.15	3.74	2.96

<sup>1</sup>Water-soluble carbohydrates.

Microbiological analysis was performed on pooled samples of the three replicate silos per treatment, per time point, except for replicate samples, which differed considerably in their appearance. Microbiological evaluation included enumeration of lactobacilli on pour-plate Rogosa agar (Oxoid CM627, Oxoid, Basingstoke, U.K.), and yeast and molds on spread-plate malt extract agar (Difco, Detroit, MI) acidified with lactic acid to pH 4.0. Plates were incubated for 3 d 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<sub>10</sub>.

The other data were analyzed as a completely randomized design and subjected to ANOVA by the GLM procedure of SAS (SAS, Inst., Inc., Cary, NC). Differences among means were tested using Tukey's test (Snedecor and Cochran, 1980) and significance was declared at  $P < 0.05$ .

## RESULTS

The chemical and microbiological compositions of the fresh corn and sorghum are given in Table 1. The effects of *L. buchneri* and *L. plantarum* on the fermentation characteristics of the corn and sorghum silages are shown in Table 2. After 2 d of ensiling, concentrations of acetic acid were higher in *L. buchneri*- or *L. buchneri* + *L. plantarum*-inoculated silages compared with other silages ( $P < 0.05$ ). The same trend was shown in the 4, 8, and 15 d of ensiling. During fermentation, the pH and WSC levels of the silages were reduced, and concentrations of lactic and acetic acid, ethanol, and ammonia N increased. After 90 d of ensiling, *L. buchneri* inoculated silages had higher pH than the control, *L. plantarum*-, and *L. buchneri* + *L. plantarum*-inoculated silages ( $P < 0.05$ ). Silages inoculated with *L. plantarum* or *L. buchneri* + *L. plantarum* had higher content of lactic acid than the control and silages inoculated with *L. buchneri* alone ( $P < 0.05$ ). The control and *L. plantarum*-inoculated silages contained more residual WSC than the silages inoculated with *L. buchneri* or *L. buchneri* + *L. plantarum* ( $P < 0.05$ ). The latter had higher levels of acetic acid ( $P < 0.05$ ). Ammonia N concentrations were lower in the *L. plantarum*- and *L. buchneri* + *L. plantarum*-inoculated silages than in the silages

inoculated with *L. buchneri* ( $P < 0.05$ ). Weight losses were higher in the *L. buchneri* inoculated silages than the control, *L. plantarum*-, and *L. buchneri* + *L. plantarum*-inoculated silages ( $P < 0.05$ ).

The microbiological compositions of the corn and sorghum silages are given in Table 3. Lactobacilli, yeasts, and molds numbers of the silages increased during the fermentation. After 90 d of ensiling, yeasts and molds were not detected ( $< 2.0$  cfu/g) in silages inoculated with *L. buchneri* and *L. buchneri* + *L. plantarum*, whereas appreciable numbers of yeasts and molds were detected in the control and the *L. plantarum*-inoculated silages.

Table 4 gives the results of the aerobic exposure test of the corn and sorghum silages. Silage deterioration indicators are pH change, CO<sub>2</sub> production, and an increase in yeast and mold numbers. *L. plantarum*-inoculated silages were unstable in the aerobic conditions. This was evident from intensive CO<sub>2</sub> production and development of yeasts. A high level of lactic acid and yeasts impaired the aerobic stability of *L. plantarum*-inoculated silages. However, *L. buchneri*- and *L. buchneri* + *L. plantarum*-inoculated silages inhibited yeast growth and reduced CO<sub>2</sub> production ( $P < 0.05$ ).

Values for in situ rumen DM, OM, and NDF degradabilities of low-DM corn and sorghum silages after 48 h of incubation are given in Table 5. Inoculation with the *L. buchneri*, *L. plantarum*, and *L. buchneri* + *L. plantarum* did not affect rumen degradability of the silages.

## DISCUSSION

Whole-crop cereal silages, such as corn and sorghum, are susceptible to aerobic deterioration, especially in warm climates. This is because aerobic yeasts are the most active at 20 to 30°C (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, various types of 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 natural products. Until now bacterial inoculants have been added to silage in order

**Table 2.** Chemical (DM basis) analyses of the silages.

Days of ensiling	Forage type	Treatment	pH	%						
				DM	WSC <sup>1</sup>	Lactic acid	Acetic acid	Ethanol	NH <sub>3</sub> -N	Weight loss
2	Corn	Control	5.54 <sup>a,b</sup>	23.3	5.88 <sup>a</sup>	2.28 <sup>b</sup>	0.65 <sup>b</sup>	0.16	0.107 <sup>b</sup>	0.30 <sup>a</sup>
		<i>Lactobacillus buchneri</i>	5.67 <sup>a</sup>	23.3	5.41 <sup>a</sup>	1.75 <sup>b</sup>	1.96 <sup>a</sup>	0.18	0.122 <sup>a</sup>	0.35 <sup>a</sup>
		<i>L. plantarum</i>	5.30 <sup>c</sup>	23.4	5.17 <sup>a</sup>	3.87 <sup>a</sup>	0.11 <sup>c</sup>	0.13	0.080 <sup>c</sup>	0.26 <sup>a</sup>
		<i>L. buchneri</i> + <i>L. plantarum</i>	5.44 <sup>b,c</sup>	23.2	5.39 <sup>a</sup>	2.52 <sup>b</sup>	1.50 <sup>a</sup>	0.15	0.087 <sup>c</sup>	0.33 <sup>a</sup>
		SE	0.038	1.412	1.287	0.433	0.293	1.448	0.015	1.035
	Sorghum	Control	5.76 <sup>a</sup>	22.0	14.27 <sup>a</sup>	2.43 <sup>b</sup>	0.46 <sup>b</sup>	0.16	0.115 <sup>b</sup>	0.41 <sup>a</sup>
		<i>L. buchneri</i>	5.75 <sup>a</sup>	22.1	13.63 <sup>a</sup>	1.67 <sup>b</sup>	2.29 <sup>a</sup>	0.20	0.136 <sup>a</sup>	0.44 <sup>a</sup>
		<i>L. plantarum</i>	5.52 <sup>b</sup>	21.9	13.32 <sup>a</sup>	4.03 <sup>a</sup>	0.12 <sup>c</sup>	0.14	0.092 <sup>c</sup>	0.40 <sup>a</sup>
		<i>L. buchneri</i> + <i>L. plantarum</i>	5.63 <sup>a,b</sup>	22.0	13.56 <sup>a</sup>	2.84 <sup>b</sup>	1.55 <sup>a</sup>	0.15	0.095 <sup>c</sup>	0.42 <sup>a</sup>
		SE	0.041	1.377	1.131	0.369	0.275	1.417	0.017	1.074
4	Corn	Control	5.28 <sup>a</sup>	22.8	5.43 <sup>a</sup>	2.97 <sup>b,c</sup>	0.79 <sup>b</sup>	0.21	0.134 <sup>b</sup>	0.54 <sup>a</sup>
		<i>L. buchneri</i>	5.37 <sup>a</sup>	22.7	5.01 <sup>a</sup>	2.11 <sup>c</sup>	2.47 <sup>a</sup>	0.24	0.151 <sup>a</sup>	0.76 <sup>a</sup>
		<i>L. plantarum</i>	4.80 <sup>c</sup>	22.8	4.63 <sup>a</sup>	5.79 <sup>a</sup>	0.14 <sup>c</sup>	0.19	0.102 <sup>c</sup>	0.37 <sup>a</sup>
		<i>L. buchneri</i> + <i>L. plantarum</i>	5.04 <sup>b</sup>	22.5	4.80 <sup>a</sup>	3.57 <sup>b</sup>	1.97 <sup>a</sup>	0.20	0.109 <sup>c</sup>	0.45 <sup>a</sup>
		SE	0.039	1.284	1.245	0.396	0.258	1.532	0.017	0.967
	Sorghum	Control	5.47 <sup>a</sup>	21.5	13.05 <sup>a</sup>	3.26 <sup>b</sup>	0.57 <sup>b</sup>	0.24	0.143 <sup>b</sup>	0.76 <sup>a</sup>
		<i>L. buchneri</i>	5.49 <sup>a</sup>	21.5	12.48 <sup>a</sup>	2.10 <sup>c</sup>	2.76 <sup>a</sup>	0.27	0.164 <sup>a</sup>	0.83 <sup>a</sup>
		<i>L. plantarum</i>	5.13 <sup>b</sup>	21.4	11.99 <sup>a</sup>	5.83 <sup>a</sup>	0.27 <sup>c</sup>	0.20	0.115 <sup>c</sup>	0.58 <sup>a</sup>
		<i>L. buchneri</i> + <i>L. plantarum</i>	5.36 <sup>a</sup>	21.6	12.36 <sup>a</sup>	3.99 <sup>b</sup>	2.08 <sup>a</sup>	0.23	0.121 <sup>c</sup>	0.70 <sup>a</sup>
		SE	0.043	1.330	1.124	0.378	0.236	1.356	0.018	0.985
8	Corn	Control	4.80 <sup>a</sup>	22.2	4.69 <sup>a</sup>	4.06 <sup>b</sup>	1.06 <sup>b</sup>	0.30	0.160 <sup>b</sup>	0.94 <sup>b</sup>
		<i>L. buchneri</i>	4.96 <sup>a</sup>	22.1	4.27 <sup>a</sup>	2.57 <sup>c</sup>	3.23 <sup>a</sup>	0.32	0.186 <sup>a</sup>	1.58 <sup>a</sup>
		<i>L. plantarum</i>	4.41 <sup>c</sup>	22.0	3.85 <sup>a</sup>	8.04 <sup>a</sup>	0.24 <sup>c</sup>	0.25	0.129 <sup>c</sup>	0.43 <sup>b</sup>
		<i>L. buchneri</i> + <i>L. plantarum</i>	4.64 <sup>b</sup>	22.3	4.10 <sup>a</sup>	4.89 <sup>b</sup>	2.48 <sup>a</sup>	0.28	0.137 <sup>c</sup>	0.64 <sup>b</sup>
		SE	0.041	1.546	1.009	0.381	0.264	1.319	0.016	0.435
	Sorghum	Control	5.06 <sup>a</sup>	20.9	10.84 <sup>a</sup>	4.67 <sup>b</sup>	0.72 <sup>b</sup>	0.35	0.176 <sup>b</sup>	1.13 <sup>b</sup>
		<i>L. buchneri</i>	5.10 <sup>a</sup>	21.0	10.35 <sup>a</sup>	2.44 <sup>c</sup>	3.50 <sup>a</sup>	0.38	0.205 <sup>a</sup>	1.88 <sup>a</sup>
		<i>L. plantarum</i>	4.70 <sup>b</sup>	20.8	9.88 <sup>a</sup>	8.61 <sup>a</sup>	0.45 <sup>c</sup>	0.34	0.142 <sup>c</sup>	0.65 <sup>b</sup>
		<i>L. buchneri</i> + <i>L. plantarum</i>	4.95 <sup>a</sup>	20.9	10.06 <sup>a</sup>	5.37 <sup>b</sup>	2.71 <sup>a</sup>	0.34	0.150 <sup>c</sup>	0.93 <sup>b</sup>
		SE	0.045	1.343	0.976	0.342	0.244	1.408	0.015	0.421
15	Corn	Control	4.48 <sup>a</sup>	22.0	3.99 <sup>a</sup>	5.29 <sup>c</sup>	1.13 <sup>b</sup>	0.39	0.215 <sup>b</sup>	1.46 <sup>b</sup>
		<i>L. buchneri</i>	4.57 <sup>a</sup>	22.2	3.36 <sup>a</sup>	3.13 <sup>d</sup>	3.45 <sup>a</sup>	0.42	0.240 <sup>a</sup>	2.57 <sup>a</sup>
		<i>L. plantarum</i>	4.02 <sup>c</sup>	22.1	3.17 <sup>a</sup>	11.33 <sup>a</sup>	0.27 <sup>c</sup>	0.37	0.171 <sup>c</sup>	0.55 <sup>c</sup>
		<i>L. buchneri</i> + <i>L. plantarum</i>	4.26 <sup>b</sup>	22.2	3.28 <sup>a</sup>	6.95 <sup>b</sup>	2.80 <sup>a</sup>	0.38	0.178 <sup>c</sup>	0.97 <sup>b,c</sup>
		SE	0.044	1.451	1.144	0.324	0.246	1.368	0.013	0.398
	Sorghum	Control	4.73 <sup>a</sup>	20.6	9.07 <sup>a</sup>	6.01 <sup>c</sup>	0.83 <sup>b</sup>	0.44	0.228 <sup>b</sup>	1.64 <sup>b</sup>
		<i>L. buchneri</i>	4.78 <sup>a</sup>	20.7	8.07 <sup>a</sup>	3.09 <sup>d</sup>	3.93 <sup>a</sup>	0.46	0.257 <sup>a</sup>	2.96 <sup>a</sup>
		<i>L. plantarum</i>	4.26 <sup>c</sup>	20.5	7.56 <sup>a</sup>	12.46 <sup>a</sup>	0.56 <sup>c</sup>	0.40	0.189 <sup>c</sup>	0.83 <sup>c</sup>
		<i>L. buchneri</i> + <i>L. plantarum</i>	4.48 <sup>b</sup>	20.5	7.79 <sup>a</sup>	7.50 <sup>b</sup>	3.15 <sup>a</sup>	0.43	0.196 <sup>c</sup>	1.22 <sup>b,c</sup>
		SE	0.047	1.347	0.955	0.316	0.211	1.381	0.011	0.386
90	Corn	Control	3.72 <sup>b</sup>	21.6	3.15 <sup>a</sup>	4.04 <sup>c</sup>	1.27 <sup>b</sup>	0.47	0.262 <sup>b</sup>	1.65 <sup>b</sup>
		<i>L. buchneri</i>	4.13 <sup>a</sup>	21.4	0.64 <sup>b</sup>	2.76 <sup>d</sup>	3.89 <sup>a</sup>	0.49	0.285 <sup>a</sup>	3.26 <sup>a</sup>
		<i>L. plantarum</i>	3.64 <sup>b</sup>	21.7	2.54 <sup>a</sup>	7.94 <sup>a</sup>	0.33 <sup>c</sup>	0.42	0.211 <sup>c</sup>	0.75 <sup>c</sup>
		<i>L. buchneri</i> + <i>L. plantarum</i>	3.80 <sup>b</sup>	21.5	1.03 <sup>b</sup>	5.55 <sup>b</sup>	3.17 <sup>a</sup>	0.45	0.220 <sup>c</sup>	1.14 <sup>b,c</sup>
		SE	0.037	1.309	0.048	0.342	0.222	1.320	0.010	0.370
	Sorghum	Control	3.87 <sup>b</sup>	20.3	6.75 <sup>a</sup>	4.86 <sup>c</sup>	0.96 <sup>b</sup>	0.50	0.281 <sup>b</sup>	1.97 <sup>b</sup>
		<i>L. buchneri</i>	4.26 <sup>a</sup>	20.4	1.36 <sup>b</sup>	2.54 <sup>d</sup>	4.30 <sup>a</sup>	0.53	0.308 <sup>a</sup>	3.49 <sup>a</sup>
		<i>L. plantarum</i>	3.75 <sup>b</sup>	20.1	5.96 <sup>a</sup>	9.39 <sup>a</sup>	0.62 <sup>c</sup>	0.47	0.234 <sup>c</sup>	0.94 <sup>c</sup>
		<i>L. buchneri</i> + <i>L. plantarum</i>	3.88 <sup>b</sup>	20.3	2.02 <sup>b</sup>	6.18 <sup>b</sup>	3.49 <sup>a</sup>	0.49	0.244 <sup>c</sup>	1.45 <sup>b,c</sup>
		SE	0.040	1.411	0.043	0.310	0.202	1.305	0.009	0.357

<sup>a,b,c,d</sup>Within a column and forage type means followed by different letter differ significantly ( $P < 0.05$ ).

<sup>1</sup>Water-soluble carbohydrates.

to stimulate lactic acid fermentation, accelerating the decrease in pH, and thus improving silage preservation. Most available inoculants consist of selected strains of homofermentative LAB strains. They produce large amounts of lactic acid in the silage in a short time

and so stabilize it with minimal losses. However, these homofermentative LAB strains enhance aerobic deterioration of whole-crop cereal silages, probably because not enough VFA are produced to inhibit fungi (Weinberg et al., 1993). This agrees with our previous experi-

**Table 3.** Microbiological analysis of the silages.<sup>1</sup>

Days of ensiling	Forage type	Treatment	log <sub>10</sub> cfu/g		
			Lactobacilli	Yeasts	Molds
2	Corn	Control	5.25	3.95	2.74
		<i>Lactobacillus buchneri</i>	4.88	3.83	2.50
		<i>L. plantarum</i>	6.77	4.17	2.66
		<i>L. buchneri</i> + <i>L. plantarum</i>	5.03	3.92	2.61
	Sorghum	Control	4.84	3.87	3.10
		<i>L. buchneri</i>	4.28	3.70	2.88
		<i>L. plantarum</i>	5.23	3.90	3.03
		<i>L. buchneri</i> + <i>L. plantarum</i>	4.40	3.72	2.86
4	Corn	Control	6.44	3.91	2.87
		<i>L. buchneri</i>	6.10	3.16	2.36
		<i>L. plantarum</i>	7.96	4.24	2.78
		<i>L. buchneri</i> + <i>L. plantarum</i>	6.31	3.55	2.50
	Sorghum	Control	5.77	3.93	3.23
		<i>L. buchneri</i>	4.91	3.42	2.71
		<i>L. plantarum</i>	6.67	4.08	3.11
		<i>L. buchneri</i> + <i>L. plantarum</i>	5.16	3.58	2.74
8	Corn	Control	6.98	3.96	2.95
		<i>L. buchneri</i>	6.57	2.61	2.25
		<i>L. plantarum</i>	8.63	4.33	2.87
		<i>L. buchneri</i> + <i>L. plantarum</i>	6.70	2.88	2.37
	Sorghum	Control	6.29	4.02	3.41
		<i>L. buchneri</i>	5.43	2.96	2.49
		<i>L. plantarum</i>	7.54	4.21	3.20
		<i>L. buchneri</i> + <i>L. plantarum</i>	5.58	3.20	2.40
15	Corn	Control	7.80	3.90	3.16
		<i>L. buchneri</i>	6.85	2.25	2.12
		<i>L. plantarum</i>	9.69	4.40	2.98
		<i>L. buchneri</i> + <i>L. plantarum</i>	7.57	2.42	2.26
	Sorghum	Control	7.15	4.11	3.54
		<i>L. buchneri</i>	5.86	2.33	2.22
		<i>L. plantarum</i>	8.10	4.50	3.27
		<i>L. buchneri</i> + <i>L. plantarum</i>	6.17	2.57	2.26
90	Corn	Control	8.35	3.86	3.26
		<i>L. buchneri</i>	7.03	<2.00	<2.00
		<i>L. plantarum</i>	10.40	4.45	3.08
		<i>L. buchneri</i> + <i>L. plantarum</i>	8.66	<2.00	<2.00
	Sorghum	Control	7.92	4.18	3.65
		<i>L. buchneri</i>	6.13	<2.00	<2.00
		<i>L. plantarum</i>	8.75	4.73	3.34
		<i>L. buchneri</i> + <i>L. plantarum</i>	6.67	<2.00	<2.00

<sup>1</sup>Microbiological analysis was performed on a single sample each time. Therefore, no statistical analyses are available.

ments (Filya et al., 2000; Filya, 2002a,b) with wheat, sorghum, and corn silages.

*Lactobacillus buchneri* is a heterofermentative LAB, which produces high levels of acetic acid in silage. Results with this microorganism in laboratory studies were promising with regard to aerobic stability (Driehuis et al., 1999a,b; Filya, 2001, 2003; Kung and Ranjit, 2001; Filya et al., 2002; Ranjit et al., 2002; Weinberg et al., 2002).

The results in the current study indicate clearly that inoculation with *L. buchneri* alone or in combination with a homofermentative LAB improved the aerobic stability of low-DM corn and sorghum silages. *Lactoba-*

*cillus buchneri* or *L. buchneri* + *L. plantarum* inoculated silages had higher levels of acetic acid than the control and *L. plantarum* inoculated silages ( $P < 0.05$ ). *Lactobacillus buchneri* was able to protect the aerobic stability of the low dry matter corn and sorghum silages, even in the presence of *L. plantarum*. The explanation for the aerobic stability-enhancing effect of *L. buchneri* is that the activity of yeasts is impaired. Driehuis et al. (1999a) showed that yeasts are affected in 2 ways. First, during anaerobic conditions, the survival of yeasts is reduced. As a result, yeasts counts decrease in *L. buchneri* inoculated silages. Second, during the aerobic exposure, yeast growth is inhibited. These findings are in

**Table 4.** Results of the aerobic stability test (5 d) of the final silages after 90 d of ensiling.

Forage type	Treatment	pH	CO <sub>2</sub> , g/kg of DM	log <sub>10</sub> cfu/g of wet silage	
				Yeasts <sup>1</sup>	Molds <sup>1</sup>
Corn	Control	6.18 <sup>b</sup>	36.44 <sup>b</sup>	7.26	3.45
	<i>Lactobacillus buchneri</i>	4.56 <sup>c</sup>	7.08 <sup>c</sup>	<2.00	<2.00
	<i>L. plantarum</i>	6.57 <sup>a</sup>	68.86 <sup>a</sup>	8.87	3.76
	<i>L. buchneri</i> + <i>L. plantarum</i>	4.69 <sup>c</sup>	9.61 <sup>c</sup>	2.50	<2.00
	SE	0.228	0.123		
Sorghum	Control	6.34 <sup>b</sup>	40.67 <sup>b</sup>	7.14	3.57
	<i>L. buchneri</i>	4.60 <sup>c</sup>	8.57 <sup>c</sup>	<2.00	<2.00
	<i>L. plantarum</i>	6.68 <sup>a</sup>	77.36 <sup>a</sup>	9.38	4.19
	<i>L. buchneri</i> + <i>L. plantarum</i>	4.73 <sup>c</sup>	10.20 <sup>c</sup>	2.63	<2.00
	SE	0.211	0.107		

<sup>a,b,c</sup>Within a column and forage type means followed by different letter differ significantly ( $P < 0.05$ ).

<sup>1</sup>Microbiological analysis was performed on a single sample each time. Therefore, no statistical analyses are available.

agreement with those of Driehuis et al. (1999b), Kung and Ranjit (2001), Oude Elferink et al. (2001), Ranjit et al. (2002), our previous experiments (Filya, 2001, 2003; Filya et al., 2002), and Weinberg et al. (2002) with wheat, sorghum, and corn silages. The drop in pH with time was slower than typically observed in corn and sorghum silages made at more normal DM content. The fresh forages had low numbers of lactobacilli, which may be a reason for the slow fermentation. The fact that pH dropped slowly even in the inoculated silages suggests that a high buffer capacity occurred in both crops. At the end of the ensiling period (d 90), concentration of lactic acid was the lowest in *L. buchneri*-inoculated silages. The combination of *L. buchneri* and *L. plantarum* increased the concentrations of lactic acid and initial rate of acidification of low-DM corn and sorghum silages. *Lactobacillus buchneri*-inoculated silages had higher ammonia N concentrations and silage weight losses compared with the control or the *L. plantarum*- or *L. buchneri* + *L. plantarum*-inoculated silages ( $P < 0.05$ ). Driehuis et al. (2001) reported that the increase in ammonia N concentration in *L. buchneri*-inoculated corn 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 *L. buchneri* in these silages. This finding is in agreement with my previous experiment (Filya, 2003) with *L. buchneri* in wheat, sorghum, and corn silages. The same trend was shown in this experiment.

The inoculation with *L. buchneri*, alone or in combination with *L. plantarum*, did not affect the in situ rumen DM, OM, and NDF degradability of corn and sorghum silages. These findings agree with our previous experiments (Filya et al., 2002; Filya, 2003) with wheat, sorghum, and corn silages. Salawu et al. (2001) found that application of *L. buchneri* to pea-wheat silage increased the in situ rumen nitrogen degradability, reduced the NDF degradability, and did not affect DM degradability.

## CONCLUSIONS

The results of this study show that the *L. buchneri*, alone or in combination with *L. plantarum*, can improve the aerobic stability of low-DM corn and sorghum silages. The results also show that the combination of *L.*

**Table 5.** In situ rumen degradability (48 h) of the final silages after 90 d of ensiling.

Forage type	Treatment	Degradability, %		
		DM	OM	NDF
Corn	Control	46.37	47.80	31.82
	<i>Lactobacillus buchneri</i>	45.75	47.56	32.34
	<i>L. plantarum</i>	46.60	48.33	31.27
	<i>L. buchneri</i> + <i>L. plantarum</i>	45.08	46.79	32.70
	SE	1.256	1.209	1.234
Sorghum	Control	49.52	50.72	44.71
	<i>L. buchneri</i>	49.91	51.50	43.26
	<i>L. plantarum</i>	48.09	49.61	44.15
	<i>L. buchneri</i> + <i>L. plantarum</i>	48.70	50.28	43.82
	SE	1.190	1.226	1.205

*buchneri* and *L. plantarum* is preferable because the combination accelerates the initial lactic acid fermentation rate, reducing pH and giving lower protein degradation and fermentation losses.

### ACKNOWLEDGMENTS

The author wishes to thank R. E. Muck for reviewing the manuscript.

### REFERENCES

- AOAC. 1990. Official Methods of Analysis. Vol. I. 15th ed. Assoc. Offic. Anal. Chem, Arlington, VA.
- Ashbell, G., Z. G. Weinberg, A. Azrieli, Y. Hen, and B. Horev. 1991. A simple system to study the aerobic deterioration of silages. *Can. Agric. Eng.* 33:171–175.
- Ashbell, G., Z. G. Weinberg, Y. Hen, and I. Filya. 2002. The effects of temperature on the aerobic stability of wheat and corn silages. *J. Ind. Microbiol. Biotechnol.* 28:261–263.
- Driehuis, F., S. J. W. H. Oude Elferink, and S. F. Spoelstra. 1999a. Anaerobic lactic acid degradation during ensilage of whole crop maize inoculated with *Lactobacillus buchneri* inhibits yeast growth and improves aerobic stability. *J. Appl. Microbiol.* 87:585–594.
- Driehuis, F., S. J. W. H. Oude Elferink, and P. G. Van Wixselaar. 1999b. *Lactobacillus buchneri* improves aerobic stability of laboratory and farm scale whole crop maize silage but does not affect feed intake and milk production of dairy cows. Pages 264–265 in *Proc. XII Int. Silage Conf.*, Uppsala, Sweden.
- Driehuis, F., S. J. W. H. Oude Elferink, and P. G. Van Wixselaar. 2001. Fermentation characteristics and aerobic stability of grass silage inoculated with *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria. *Grass Forage Sci.* 56:330–343.
- Dubois, M., K. A. Giles, J. K. Hamilton, P. A. Rebes, and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28:350–356.
- Filya, I., G. Ashbell, Y. Hen, and Z. G. Weinberg. 2000. The effect of bacterial inoculants on the fermentation and aerobic stability of whole crop wheat silage. *Anim. Feed Sci. Technol.* 88:39–46.
- Filya, I. 2001. Aerobic stability of sorghum and maize silages treated with homofermentative and heterofermentative lactic acid bacteria. Pages 24–26 in *Proc. Turkey-Israeli Workshop on Silage and Agricultural By-Products for High Lactating Cows*. Bet Dagan, Israel.
- Filya, I. 2002a. The effects of lactic acid bacteria and lactic acid bacteria + enzyme mixture silage inoculants on maize silage. *Turk. J. Vet. Anim. Sci.* 26:679–687.
- Filya, I. 2002b. The effects of lactic acid bacterial inoculants on the fermentation, aerobic stability, and in situ rumen degradability characteristics of maize and sorghum silages. *Turk. J. Vet. Anim. Sci.* 26:815–823.
- Filya, I., A. Karabulut, and E. Sucu. 2002. The effect of *Lactobacillus plantarum* and *Lactobacillus buchneri* on the fermentation, aerobic stability, and ruminal degradability of maize silage in warm climate. Pages 192–193 in *Proc. XIII Intl. Silage Conf.* Auchincruive, Ayr, Scotland.
- Filya, I. 2003. The effect of *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria, on the fermentation, aerobic stability, and ruminal degradability of wheat, sorghum, and maize silages. *J. Appl. Microbiol.* (In press)
- Kung, Jr., L., and N. K. Ranjit. 2001. The effect of *Lactobacillus buchneri* and other additives on the fermentation and aerobic stability of barley silage. *J. Dairy Sci.* 84:1149–1155.
- McDonald, P., A. R. Henderson, and S. J. E. Heron. 1991. *The Biochemistry of Silage*. 2nd ed. Chalcombe Publ., Abersywyth, U.K.
- Mehrez, A. Z., and E. R. Orskov. 1977. A study of the artificial fibre bag technique for determining the digestibility of feeds in the rumen. *J. Agric. Sci.* 88:645–650.
- Moon, N. J. 1983. Inhibition of the growth of acid tolerant yeasts by acetate, lactate and propionate and their synergistic mixtures. *J. Appl. Bacteriol.* 55:454–460.
- Muck, R. E. 1996. A lactic acid bacteria strain to improve aerobic stability of silages. Pages 42–43 in *U.S. Dairy Forage Research Center 1996 Research Summaries*. Madison, WI.
- Oude Elferink, S. J. W. H., J. Krooneman, J. C. Gottschal, S. F. Spoelstra, F. Faber, and F. Driehuis. 2001. Anaerobic conversion of lactic acid to acetic acid and 1,2 propanediol by *Lactobacillus buchneri*. *Appl. Environ. Microbiol.* 67:125–132.
- Pitt, R. E., Y. Liu, and R. E. Muck. 1991. Stimulation of the effect of additives on aerobic stability of alfalfa and corn silages. *Trans. ASAE* 34:1633–1641.
- Ranjit, N. K., C. C. Taylor, and L. Kung, Jr. 2002. Effect of *Lactobacillus buchneri* 40788 on the fermentation, aerobic stability and nutritive value of maize silage. *Grass Forage Sci.* 57:73–81.
- Salawu, M. B., E. H. Warren, and A. T. Adesogan. 2001. Fermentation characteristics, aerobic stability and ruminal degradation of ensiled pea/wheat bi-crop forages treated with two microbial inoculants, formic acid or quebracho tannins. *J. Sci. Food Agric.* 81:1263–1268.
- Snedecor, G. W., and W. G. Cochran. 1980. *Statistical Methods*. 6th ed. Iowa State Univ. Press, Ames.
- Van Soest, P. H., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- Weinberg, Z. G., G. Ashbell, Y. Hen, and A. Azrieli. 1993. The effect of applying lactic acid bacteria at ensiling on the aerobic stability of silages. *J. Appl. Bacteriol.* 75:512–518.
- Weinberg, Z. G., G. Ashbell, Y. Hen, A. Azrieli, G. Szakacs, and I. Filya. 2002. Ensiling whole-crop wheat and corn in large containers with *Lactobacillus plantarum* and *Lactobacillus buchneri*. *J. Ind. Microbiol. Biotechnol.* 28:7–11.
- Woolford, M. K. 1990. The detrimental effects of air on silage. *J. Appl. Bacteriol.* 68:101–116.