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Short Communication

Fermentation and aerobic stability of high-moisture corn silages inoculated with different levels of *Lactobacillus buchneri*

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ABSTRACT - Fermentation and aerobic stability were evaluated in high-moisture corn (HMC) silage inoculated with different levels of *Lactobacillus buchneri*. The HMC composed of 654 g/kg dry matter (DM) was ensiled in quadruplicate laboratory silos (7 L) per treatment. *L. buchneri* 40788 was applied at 5×10^4; 1×10^5; 5×10^5; and 1×10^6 cfu/g to the ground corn. Silages with no additive were used as controls. After 140 d of ensiling, the silages were subjected to an aerobic stability evaluation for 12 days in which the chemical parameters, microbiological parameters and silage temperature were measured to determine the aerobic deterioration. The lactic acid, acetic acid and propionic acid concentrations did not differ between silages. The fermentation parameters of HMC were not affected by *L. buchneri*. The HMC containing *L. buchneri* had a low number of yeast and mould colonies and a more stable pH until in the eighth measurement, which improved the aerobic stability without affecting gas loss. Doses of *L. buchneri* greater than or equal to 5×10^5 cfu/g applied to the HMC were the most efficient in control of aerobic deterioration.

Key Words: aerobic deterioration, mold, yeasts

Introduction

The fermentation of high-moisture corn (HMC) is often restricted because of its relatively moisture low and fermentable sugar contents. The accumulation of total acids produced is quite low in HMC, and the concentration of starch is high in HMC (Kung Jr. et al., 2007). Starch is an energy source for yeasts in anaerobic conditions and is fermented into ethanol. When air penetrates a silage, it has negative effects on feed quality (Woolford, 1990) because yeasts assimilate lactic acid, causing the pH increase. Thus, the microorganisms that were inhibited by low pH begin to proliferate and spoil the silage.

Lactobacillus buchneri, an obligate heterofermentative lactic acid bacterium, has been suggested as an additive to improve the aerobic stability of silages (Driehuis et al., 1999; Ranjit et al., 2002; Taylor & Kung Jr., 2002; Filya et al., 2006; Kleinschmit & Kung Jr., 2006; Tabacco et al., 2009). *L. buchneri* converts glucose and fructose to lactic acid, acetic acid and other end products (McDonald et al., 1991). *L. buchneri* also converts lactic acid to the following end products: acetic acid; 1,2-propanediol; and small amounts of ethanol (Oude Elferink et al., 2001). The presence of organic acids protects the silage against spoilage by aerobic microorganisms (Moon, 1983). Heterolactic fermentation is usually undesirable when compared with homolactic fermentation because the loss of dry matter (DM) is greater with heterolactic fermentation (McDonald et al., 1991). However, improvements in aerobic stability during the prolonged exposition and feeding phase may be beneficial. Thus, small losses of DM caused by heterofermentation may become less important (Kung & Ranjit, 2001).

High-moisture grain and whole-plant corn silage are more susceptible to aerobic deterioration than legume and grass silages. Therefore, inoculation with *L. buchneri* may provide great benefits to preserving HMC silage (Combs & Hoffman, 2001). In Brazil, high temperatures result in large losses on the surfaces of the silos (Bernardes et al., 2009) and low doses of *L. buchneri* may be insufficient to control aerobic deterioration of silages. Therefore, the objective of this study to evaluate the effects of *L. buchneri* at different doses on the fermentation parameters and aerobic stability of HMC in laboratory silos.

Material and Methods

The HMC from the Maxymus cultivar (Syngenta) was harvested at approximately 654 g/kg of dry matter (DM)

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and ground through a roller mill. The following treatments were applied to the corn: control (untreated); *L. buchneri* 40778 (Lallemand Animal Nutrition) at rates of 5×10^4; 1×10^5; 5×10^5; and 1×10^6 cfu/g. The application rate of the inoculants was determined in accordance with the instructions from the manufacturer. The correct amount of inoculants for each treatment was weighed to achieve the desired application rates. The manufacturer recommends the application of 6×10^5 cfu/g of high moisture corn. However, high doses can be economically unviable.

Inoculants were diluted with distilled water (5 mL kg/corn) and then applied uniformily by ground sprayers with a constant mixer. The control treatment received only water. Immediately after the inoculation, samples of fresh ground corn were obtained to determine the DM and crude protein content, pH and microorganism count (yeast and mould counts) of each sample to characterize the fresh material (DM: 654 g/kg; CP: 117 g/kg of DM; pH: 5.45; yeasts: 6.84 Log_{10} cfu/g of corn and moulds: 4.90 Log_{10} cfu/g of corn).

An amount of ground corn (6 kg) from each treatment was packed into 7 L silos in quadruplicate, sealed with adhesive tape and stored at ambient temperature (average 24 ºC). Experimental silos were weighed after filling and at the end of the ensiling period (140 d) to determine the gas loss.

After the fermentation period, the silos were opened, the spoiled forage was discarded and the remaining forage was homogenized. After homogenization, the forage was sampled to determine the levels of DM, pH, lactic acid, acetic acid, propionic acid, yeast, mould, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and ammonia nitrogen in relation to total nitrogen (NH_3-N).

To determine the aerobic stability, 3 kg of silage were placed in plastic buckets and maintained in a closed space at room temperature (average 28 ºC). The temperature of the silage was measured every half hour by a datalogger placed in the center of the mass during the aerobic exposure by 12 days. The ambient temperature was measured by a datalogger distributed near the experimental silos. The aerobic stability was defined as the number of hours that the temperature of the silage remained stable before rising more than 2 ºC above the ambient temperature (Taylor & Kung Jr., 2002). During the aerobic exposure (4, 8 and 12 days), the silages were sampled to determine the levels of pH, lactic acid, acetic acid, yeast and mould. These wet samples were stored at -20 ºC, except for the samples used to determine the microorganisms, which were evaluated immediately.

The DM content of the samples was determined at 55 ºC in a forced-ventilation oven for 72 h. The total nitrogen (TN) was determined by the Kjeldahl method, and the CP was calculated by multiplying TN by the factor of 6.25. The NDF was analyzed using amylase without sulfite (Mertens, 2002), and the ADF was analyzed using the procedure of Van Soest & Robertson (1985). A water extract was made from the wet samples according to Kung Jr. et al., (1984), and the pH of the water extract was determined. A portion of the water extract was used to determine the lactic acid concentration by a spectrophotometry method (Silva & Queiroz, 2002), the volatile fatty acid concentration was determined by gas chromatography (Wilson, 1971) and the NH_3-N rate was determined by distillation with potassium hydroxide (KOH) 2N according Fenner (1965) adapted by Vieira (1980). A sample (25 g) of fresh ground corn and silage from each replicate was homogenized with 225 mL of sterile water. The yeast and mould counts were done on a spread-plate of potato dextrose agar acidified with lactic acid. The plates were incubated for 4 d at 28 ºC. All the microbiological data were log-transformed.

The experimental design was completely randomized with four replicates. In aerobic conditions, the data were analysed as repeated measures in time. When there was no interaction between treatments and time, the data were analysed to each time. The data were subjected to ANOVA using software SAS (Statistical Analysis System, version 9.0.). The means were separated by Tukey´s test, and the significance level was $P<0.05$.

Results and Discussion

Several bacterial inoculants have been used to improve the aerobic stability of silages. Aerobic deterioration is a larger problem in countries with warm climates. Therefore, to improve the aerobic stability of HMC, different doses of *L. buchneri* 40778, a heterolactic acid bacterium, were applied to the ground corn that was ensiled in laboratory silos.

After 140 d of ensiling, the DM content, DM recovery and gas loss, the concentration of NH_3-N, lactic, acetic and propionic acids, CP, NDF and ADF were not affected by the different doses of *L. buchneri* (Table 1). The contents of NH_3-N and lactic, acetic acid and propionic acids found in this study are closer to what Kung & Shaver (2001) reported to HMC. The difference found between the CP content of HMC before and after ensilage is due to the proteolysis that occurs during the process of fermentation (McDonald et al., 1991).

The pH values were lowest in the HMC treated with doses greater than or equal to 1×10^5 cfu/g when compared with the untreated HMC (Table 1). Moreover, the fact that the concentration of acetic acid and propionic acid did

not increase with the addition of the inoculants, and the lactic acid concentration did not decrease in response to the different doses of inoculants are in contrast to what has been observed in corn silages treated with *L. buchneri*, a difference that may be due to restriction of *L. buchneri* in the fermentation, because in the HMC there is low humidity (Kung Jr. et al., 2007).

However, the present study demonstrated that the addition of *L. buchneri* 40778 with doses greater than or equal to 1×10^5 cfu/g to the corn at the time of ensiling decreased the number of yeast colonies found in HMC after 140 days of fermentation (Table 1). In contrast, Taylor & Kung Jr. (2002) and Kung Jr. et al. (2007) did not find a lower number of yeast colonies in HMC inoculated with *L. buchneri* when compared with untreated HMC. The mould counts did not differ between the various treatment groups of HMC after ensilage.

To evaluate the chemical and microbiological changes that occurred when the silages were exposed to air, the HMC groups were sampled and analyzed in time (0, 4, 8 and 12 days).

The pH values and the concentrations of volatile fatty acids (VFA) were evaluated during aerobic exposure. However, the VFA content did not differ between treatments (data not published).

The number of yeast colonies was low in HMC treated with *L. buchneri* ($\geq 5 \times 10^5$ cfu/g) until the fourth measurement ($P<0.05$; Table 2). In the eighth measurement, the number of yeast colonies was low in the HMC with 1×10^6 cfu of *L. buchneri*/g, although without significant difference. Mould count was lower in the HMC with 1×10^6 cfu of *L. buchneri*/g when compared with the other treatments in the fourth measurement of aerobic exposure ($P<0.05$). The mould count did not differ between the different treatments of HMC in the eighth and twelfth measurements. Reis et al. (2008) verified lower count of moulds and yeasts in HMC inoculated with *L. buchneri* after 5 days of aerobic exposure.

In the present study, the yeasts and moulds in all HMC increased on the days of aerobic exposure. Yeasts that assimilate lactate are primarily responsible for the spoilage of silages when they are exposed to air (Moon, 1983). Yeasts can utilize lactate as carbon and energy source, promoting a favorable environment for the growth of moulds (McDonald et al., 1991).

The pH level is an indicator of aerobic deterioration in the silage because the lactic acid is consumed by yeasts during aerobic exposure, allowing the growth of other undesirable microorganisms such as moulds and bacteria.

Regarding the pH values of HMC treated with different doses of *L. buchneri* sampled at different times, no interaction was observed between treatment and time (Table 3). Therefore, the data were evaluated at each time.

At the fourth time of aerobic exposure, the pH value of all HMC still was near the ideal range to HMC (4.0 – 4.5) according Kung & Shaver (2001). The pH value was low in the HMC treated with 1×10^6 cfu/g in the eighth measurement ($P<0.05$), this is correlated with low yeast count in this treatment and time (Table 2). In the twelfth measurements, the pH values did not differ between the different groups of HMC.

According to McDonald et al. (1991), the increase of silage temperature is generated by the metabolic activity of microorganisms. The aerobic stability was improved in all treated groups of HMC (Figure 1; CV: 4.83% and $P<0.0001$). The result from this study is accordance with Kung Jr. et al. (2007), who found improvement of aerobic stability of ground and whole HMC inoculated with *L. buchneri*. Reis et al. (2008) also found improvement of aerobic stability of 58 and 197 hours in HMC inoculated with 5×10^4 and 4×10^5 cfu of *L. buchneri*/g compared with control.

Table 1 - Chemical and microbial composition of high-moisture corn silage treated with doses of *L. buchneri* after 140 d of ensiling

Item	Control	5×10^4	1×10^5	5×10^5	1×10^6	CV (%)	P value
Dry matter (g/kg)	662.8	666.6	660.7	666.1	664.2	0.77	0.4900
Gas losses (g/kg)	18.5	19.0	14.4	12.7	12.1	61.73	0.5567
pH	3.98a	3.98a	3.90b	3.90b	3.90b	0.80	0.0026
Ammonia/total nitrogen (g/kg)	2.38	2.65	3.24	2.86	2.60	15.11	0.0915
Lactic acid (g/kg of DM)	39.0	37.0	38.4	31.6	36.1	11.66	0.1612
Acetic acid (g/kg of DM)	3.6	3.4	3.7	3.5	4.2	14.24	0.3304
Propionic acid (g/kg of DM)	0.1	0.2	0.1	0.2	0.3	1.04	0.2592
Yeasts (log10 cfu/g of silage)	6.70a	4.38ab	2.33b	2.0b	1.34b	33.16	0.0038
Moulds (log10 cfu/g of silage)	3.68	3.83	1.71	2.14	2.85	57.83	0.4741
Crude protein (g/kg of DM)	109.6	107.0	101.7	102.9	106.9	10.56	0.1060
Neutral detergent fiber (g/kg of DM)	55.3	59.7	57.9	62.4	64.0	14.28	0.6332
Acid detergent fiber (g/kg of DM)	11.9	10.4	13.4	16.4	14.3	19.21	0.0446

Means in rows with unlike letters differ ($P<0.05$).
CV - coefficient of variation; DM - dry matter.

Table 2 - Microbial composition of high-moisture corn silage treated with doses of *L. buchneri* sampled at different times

Time (days of aerobic exposure)	Control	5×10^4	1×10^5	5×10^5	1×10^6	Mean
			Yeasts (log cfu/g of silage)			
0	6.70Aa	4.38Aa	2.33Ab	2.06Bb	1.34Bb	3.36
4	7.35Aa	5.96Aa	5.16Aa	3.06Bb	2.00Bb	4.71
8	7.04Aa	5.30Aa	5.64Aa	5.00Ba	3.67Ba	5.33
12	7.28Aa	6.58Aa	5.67Aa	6.37Aa	5.65Aa	6.31
Mean	7.09	5.56	4.70	4.12	3.17	
CV						27.07
P value						0.0048
			Moulds (log cfu/g of silage)			
0	3.68Aa	3.83Aa	1.71Ba	2.14Ba	2.85Ba	2.65
4	7.19Aa	5.36Aa	5.33Aa	5.03ABa	2.84Bb	5.15
8	6.04Aa	6.55Aa	6.26Aa	6.04Aa	6.30Aa	6.24
12	6.46Aa	6.08Aa	6.40Aa	6.35Aa	6.15Aa	6.29
Mean	5.84	5.45	4.93	4.89	4.30	
CV						18.85
P value						0.0027

Means in rows with lowercase letters and in column with uppercase letters differ by the Tukey test (P<0.05).
CV - coefficient of variation

Table 3 - pH values of high-moisture corn silage treated with doses of *L. buchneri* sampled at different times

Time (days of aerobic exposure)	Control	5×10^4	1×10^5	5×10^5	1×10^6	CV (%)	P value
4	4.00a	4.10a	4.00a	3.90a	3.90a	0.78	0.2038
8	6.10a	6.30a	6.15a	5.50ab	4.76b	0.45	0.0006
12	7.41a	7.14a	7.20a	6.70a	7.30a	0.89	0.3249

Means in rows followed by unlike letters differ (P<0.05).
CV - coefficient of variation.

The low fungal activity in the HMC inoculated with *L. buchneri* resulted in more stable pH values and improvements in aerobic stability (Table 3; Figure 1). Although no increases in acetic acid concentration with the addition of *L. buchneri* doses were observed, the growth of yeasts and moulds was controlled and improved aerobic stability. Therefore, buchnericin LB produced by *Lactobacillus buchneri* may have been present in the HMC (Yildirim et al., 2002).

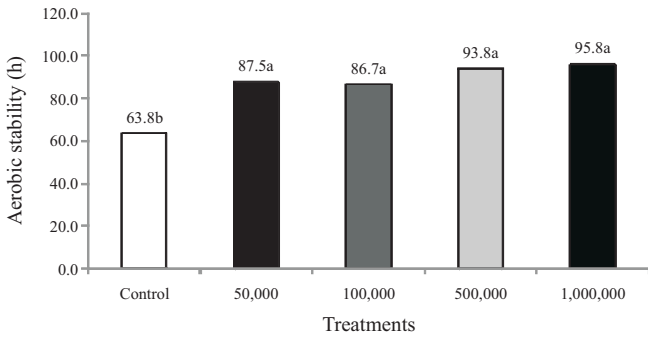

Figure 1 - Aerobic stability of high moisture corn treated with *L. buchneri*.

Conclusions

The fermentation parameters of high-moisture corn were not affected by *L. buchneri*. The high-moisture corn silage containing *L. buchneri* had a low number of yeast and mould colonies and a more stable pH until in the eighth measurement, which improved the aerobic stability without affecting gas loss. Doses of *L. buchneri* greater than or equal to 5×10^5 cfu/g applied to the high-moisture corn silage were the most efficient in the control of aerobic deterioration.

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