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Short Communication

Fermentation and aerobic stability of corn silage inoculated with *Lactobacillus buchneri*

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ABSTRACT - The characteristics of fermentation and aerobic stability were evaluated in corn silage inoculated with different doses of *Lactobacillus buchneri*. The whole corn plant (300 g/kg DM) was ensiled in quadruplicate laboratory silos (7L). *L. buchneri* 40788 was applied at 5×10^4, 1×10^5, 5×10^5 and 1×10^6 cfu/g of fresh forage. Silages with no additive were used as controls. After 130 d of ensiling, the silages were subjected to an aerobic stability evaluation for 12 days, in which chemical and microbiological parameters as well as the temperature of the silage were measured to determine the aerobic deterioration. The addition of *L. buchneri* resulted in increased acetic acid concentrations. The number of yeast colonies was low in all treated silages. The pH, lactic and propionic acid concentrations did not differ between silages. Under aerobic conditions, all the treated silages showed a low number of yeasts and a great aerobic stability. Therefore, *L. buchneri* is effective against yeasts and improves the aerobic stability of corn silage in laboratory silos. However, doses equal or superior to 1×10^5 cfu/g of fresh forage were more efficient in the control of aerobic spoilage.

Key Words: acetic acid, mold, pH values, yeasts

Introduction

Corn silage is susceptible to aerobic deterioration, primarily in warm weather, because yeasts utilise the lactic acid, produced by lactic acid bacteria, as a source of energy. Thus, silages become a favourable environment for the growth of mold and bacteria, resulting in lower-quality silages.

Lactobacillus buchneri, a 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; Filya et al., 2006; Kleinschmit & Kung, 2006; Tabacco et al., 2009). This bacteria converts glucose and fructose to lactic acid, acetic acid and other end products (McDonald et al., 1991). *Lactobacillus buchneri* can also convert lactic acid to acetic acid, 1, 2-propanediol and small amounts of ethanol (Oude Elferink et al., 2001). The presence of volatile fatty acids protects the silage against spoilage by aerobic microorganisms (Moon, 1983). Usually, heterolactic fermentation is deemed as undesirable compared with homolactic fermentation because the loss of dry matter is greater with heterolactic fermentation (McDonald et al., 1991). However, improvements in the aerobic stability during the prolonged exposure and feeding phase may be beneficial; thus, small losses of dry matter caused by heterofermentation become less important (Kung Jr. & Ranjit, 2001).

Lactobacillus buchneri is used in grass, legumes, sorghum, corn and high-moisture silage. However, corn silage is more susceptible to aerobic deterioration than legume and grass silages. Basso et al. (unpublished data) found 52% of dry matter loss on the upper area of corn silage in a stack silo in south eastern Brazil. Therefore, the inoculation with *L. buchneri* can provide great benefits to preserving corn silage.

In the United States of America and Europe, traditional inoculants are applied to the crop in order to achieve a concentration of at least 1×10^5 bacteria/g of forage. In Brazil, these concentrations are unlikely to reach 1×10^5 bacteria/g of forage at the time of ensiling. Usually, only 5×10^4 bacteria/g of forage are applied because, according to manufacturers, it is too expensive to inoculate with higher doses in the current Brazilian's economic conditions.

Therefore, this study aimed to evaluate the effects of *Lactobacillus buchneri* on the fermentation parameters and aerobic stability of corn silage in laboratory silos at different doses.

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Material and Methods

Whole corn plants of the cultivar Maximus (Syngenta) were harvested at the one-half milk line stage (300 g DM/kg fresh weight) and chopped by a conventional forage harvester to 0.5 cm. The following treatments were applied to fresh forage: 1) control (untreated); 2) *L. buchneri* 40778 (3×10^{10} colony forming units (cfu) of *L. buchneri*/g of product - Lallemand Animal Nutrition) at a rate of 5×10^{4} cfu/g fresh forage (LB1); 3) *L. buchneri* 40778 at a rate of 1×10^{5} cfu/g fresh forage (LB2); 4) *L. buchneri* 40778 at a rate of 5×10^{5} cfu/g fresh forage (LB3); and 5) *L. buchneri* 40778 at a rate of 1×10^{6} cfu/g fresh forage (LB4). The application rate of the inoculants was determined in accordance with the instructions of the manufacturer. The correct amount of inoculants for each treatment was weighed to achieve the desired application rates. The inoculants were diluted with distilled water at the rate of 5 mL/kg of fresh forage and then applied in a uniform manner with a spray on the fresh forage with a constant mixer. The control received 5 mL/kg of distilled water. Immediately after the inoculation, samples of fresh corn of all treatments were obtained to determine the dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) contents, pH values and microorganism counts (yeasts and mold).

An amount of chopped corn (5 kg) from each treatment was packed into 7 L silos in quadruplicate, sealed with lid and adhesive tape and stored at ambient temperature (average 24 ºC). Experimental silos (plastic pail) were weighed after filling and at the end of the ensiling period (130 days) to determine the gas loss. The DM recovery of the silages was calculated by subtracting the initial weights from the final weights of the silos, considering the DM content of the ensiled material.

After the fermentation period, the silos were opened, the spoiled forage was discarded, and the remainder was homogenised and sampled to determine the DM content, pH values, ammonia nitrogen in relation total nitrogen (NH_3-N), lactic acid, acetic acid, propionic acid, butyric acid, yeast and mold counts, CP, NDF and ADF.

To determine the aerobic stability, three kg of silage were placed in plastic buckets and maintained in a closed place at room temperature (average 23 ºC). The temperature of the silage was measured every half hour by a datalogger placed in the centre of the mass during the aerobic exposure (0, 4, 8 and 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 pH values, lactic acid levels, and acetic acid levels as well as yeast and mold counts. 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 neutral detergent fibre (NDF) was analysed using amylase without sulphite (Mertens, 2002), and the acid detergent fibre (ADF) was analysed using the procedure of Van Soest & Robertson (1985). A water extract was made out of the wet samples according to Kung Jr. et al. (1984), and its pH was determined. A portion of the water extract was used to determine the lactic acid concentration by the spectrophotometry method (Silva & Queiroz, 2002), the volatile fatty acid concentration was determined by gas chromatography (Wilson, 1971) and the NH_3-N relation was determined by distillation with potassium hydroxide (KOH) 2N according Fenner (1965) adapted by Vieira (1980). Twenty-five grams of sample (fresh forage and silage) from each replicate were homogenised with 225 mL of sterile water. The counting of yeasts and mold were done on a spread-plate of potato dextrose agar acidified with lactic acid (85%). The plates were incubated at 28 ºC, the couting of yeasts was done at 48 hours and of the counting mold was done at 96 h. All the microbiological data were log-transformed.

The experimental design was completely randomized with four replicates. In aerobic conditions, the data were analyzed as repeated measures in time. The data were subjected to ANOVA using SAS software (Statistical Analysis System, 1991). 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. In this study, to improve the aerobic stability of corn silage, different doses of *L. buchneri* 40778, a heterolactic acid bacterium, were applied to whole corn plants, which were ensiled in laboratory silos.

The DM content of corn plant was 300 g/kg. The NDF and ADF content were 518 and 221 g/kg DM, respectively. These values are lower than found by Valadares et al. (2006) of 610 and 317 g/kg DM, respectively in a data collection conducted in throughout Brazil. The PB content verified in the corn forage in this research was higher (113 g/kg DM)

than reported by authors referred (72.5 g/kg DM). This result could be due the caracteristics of hybrid (Maximus – Syngenta), which has big ears and low fibrous components, according to manufacturers. The pH value of corn plant was 6.08. The number of yeasts and mold was 6.9 and 5.3 Log_{10} cfu/g of corn plant.

After 130 days of ensilage, the DM content was lower in the LB2, LB3 and LB4-treated silages when compared with the control and LB1-treated silage (P<0.05), because of intense microbial fermentation. However, the DM recovery and the gas loss did not differ between the treatments. All silages were well-preserved, and the pH did not differ between the silages (Table 1).

At the end of ensiling period, the silages treated with *L. buchneri* showed a higher ammonia-N concentration compared with the untreated ones (P<0.05). According to Driehuis et al. (2001), the *L. buchneri*-treated corn silage is associated with a relative increase in the pH during the storage phase because of the high metabolic activity of *L. buchneri* in these silages. Although the final pH values were similar between silages in the present study, in the treated silages, the decrease in pH may have been less pronounced than in the untreated ones, which provided greater proteolysis, resulting in higher concentrations of ammonia in treated silages.

The concentrations of lactic, propionic and butyric acid in silages were not affected by the different doses of *L. buchneri* (Table 1). The concentration of acetic acid was the highest in the treatments with the highest doses of *L. buchneri* (P<0.05). The results of our study showed that adding doses of $\geq 1\times10^5$ cfu of *L. buchneri* 40778 g^{-1} of corn at the time of ensiling increased the concentration of acetic acid in corn silage. However, the concentration of lactic acid did not decrease. There are two fermentation pathways by *L. buchneri*. First, according McDonald et al. (1991), *L. buchneri* converts glucose and fructose into lactic acid, acetic acid, mannitol, CO_2 and water. Secondly, *L. buchneri* is also able to convert lactic acid anaerobically into acetic acid, 1, 2 propanediol and small amounts of ethanol (Oude Elferink et al., 2001). In the current study, it is likely that the first fermentation pathway occurred. Although the butyric acid concentrations were not significantly affected by doses of *L. buchneri*, the silages treated showed higher concentrations of this acid. Ranjit et al. (2002) also had higher concentrations of butyric acid in corn silages inoculated with *L. buchneri* 40788 than untreated silage; these authors suggest that 1,2 propanediol elutes at a time that is very similar to that of butyric acid on the gas chromatograph column used for analyses of volatile fatty acids.

All the treated silages had low (P<0.05) number of yeasts colonies compared with untreated (Table 1). The mold counts did not differ between silages.The acetic acid is highly antimycotic (Woolford, 1975), and it has been shown to inhibit yeasts (Moon, 1983). Yeasts that assimilate lactate are primarily responsible for the spoilage of silages when exposed to air (Moon, 1983). Filya et al. (2006) reported higher acetic acid concentration and a decreasing number of yeast counts in corn silage inoculatec with 1×10^5, 5×10^5, 1×10^6 cfu of *L. buchneri* 40778/g compared to the untreated control. Ranjit & Kung Jr. (2000) found a lower number of yeast (2.01 log_{10} cfu/g) and twice the concentration of acetate (from 18.2 to 36.0 g/kg of DM) in corn silage inoculated with 1×10^6 cfu of *L. buchneri* 40778/g compared to the control silage.

Silages treated with *L. buchneri* were more stable (P<0.05) than the control (Table 1). The aerobic stability was improved in all treated silages due to the low number of yeast in these silages, as reported by Muck (2010) when *L. buchneri* was used. Driehuis et al. (1999), Ranjit & Kung Jr. (2000), Nishino et al. (2003) and Kleinschmit et al. (2005) reported an improvement in the aerobic stability cof corn silage inoculated with $\geq 1\times10^6$ cfu of *L. buchneri* 40778/g. Ranjit et al. (2002) found improved aerobic stability in corn silage inoculated with 1×10^5, 2.5×10^5, 5×10^5 or 1×10^6 cfu of *L. buchneri* 40778/g, but in silages with higher doses (5×10^5 and 1×10^6 cfu/g), the temperature did not increase for 572 hours.

The concentration of CP was lower in LB4-treated silage (P<0.05) presumably due to a high proteolysis in this silage evinced by the increase in ammonia-N concentration (Table 1). The NDF and ADF concentrations were high in treated silages, mainly in the LB4-treated silage (P<0.05). Increases in the fiber concentration can be due to differences in the amount of DM loss from fermentation. However, the differences between the untreated NDF or ADF and the corresponding fiber values of the treated silages are at least twice as great as can be explained by the differences in DM recovery (Table 1). There are differences in NDF and ADF in the unensiled corn compared with values of silage. The ADF of the control silage is similar to the unensiled value. In contrast, the NDF of the control silage is much lower than the fresh forage value. Apparently, considerable loss of hemicellulose occurred in the ensiling process, particularly in the control. This loss can be due to a combination of enzymatic and acid hydrolysis. A slower fermentation should increase enzymatic hydrolysis. A lower pH will increase acid hydrolysis. Both factors would favor more hydrolysis, and consequenly a lower NDF content in the control. Thus, the higher fiber values are caused by a combination of lower DM

recovery and reduced hydrolysis of hemicellulose in the treated silages.

Silages are often exposed to air for prolonged periods. Sometimes silos are disproportionately large in relation to the size of the herd being fed, and considerable quantities of silage may not be removed from the silos between feedings (Ranjit & Kung Jr., 2000). Therefore, to evaluate the chemical and microbiological changes that might occur when silages were exposed to air, the silages were sampled and analysed.

The acid acetic concentration decreased with time of aerobic exposure in all silages (Table 2). On the fourth measurement of aerobic exposure, the acetic acid concentration increased in all the treated silages, especially in the LB4-treated silage ($P<0.05$). On the eighth measurement of aerobic exposure, the concentration of acetic acid continued to be high in the silage treated with 5×10^5 cfu of *L. buchneri*/g. According to Ranjit & Kung Jr. (2000), the production of acetate by *L. buchneri* may continue during the aerobic exposure.

The number of yeasts was lower in silages inoculated with 1×10^5, 5×10^5 or 1×10^6 cfu of *L. buchneri*/g compared with the untreated controls in the fourth measurement of aerobic exposure ($P<0.05$). On days 8 and 12, the number of yeasts did not differ between silages. There was no interaction between the silages and the time of aerobic exposure to mold count (Table 3). The occurrence of mold increased with the time of aerobic exposure ($P<0.0001$). All silages treated showed lower count of mold compared with the untreated ($P<0.0001$). Filya et al. (2006) also found a low number of yeast in corn silage treated with *L. buchneri* (1×10^5, 5×10^5 and 1×10^6 cfu/g) on the fifth day of aerobic exposure. Ávila et al. (2009) also observed increase in yeast and mold count in all silages of mombaça grass after opening the silos (during aerobic exposure). However, the authors also verified lower increase of microorganisms in silages treated with *L. buchneri*.

The pH is an indicator of aerobic deterioration of the silage because the lactic acid is consumed by yeasts during aerobic exposure, and the silage becomes favourable

Table 1 - Chemical and microbial composition of corn silage treated with doses of *Lactobacillus buchneri* after 130 days of ensilage

Item	Control	LB1	LB2	LB3	LB4	CV (%)	P value
DM content (g/kg)	320.0a	320.0a	291.0b	295.0b	296.0b	2.67	<0.0001
DM recovery (g/kg)	992.0a	950.0a	966.0a	975.0a	943.0a	2.96	0.1555
Gas losses (g/kg)	49.3a	53.1a	55.1a	48.0a	58.8a	47.89	0.9733
pH	3.9a	4.0a	3.9a	4.0a	4.0a	1.45	0.0961
Ammonia – N concentration (g/kg TN)	33.1b	51.5a	52.1a	57.5a	59.9a	13.57	0.0006
Lactic acid concentration (g/kg DM)	66.2a	67.0a	65.5a	68.8a	64.7a	20.33	0.9923
Acetic acid concentration (g/kg DM)	8.0b	10.6ab	12.4a	11.8a	13.4a	16.72	0.0094
Propionic acid concentration (g/kg DM)	0.2a	0.3a	0.1a	0.5a	0.3a	2.20	0.5730
Butyric acid concentration (g/kg DM)	0.7a	1.3a	1.6a	1.6a	1.7a	41.15	0.1623
Yeasts (log_{10} cfu/g of silage)	4.7a	1.7b	1.7b	1.2b	2.3b	32.78	0.0018
Mold (log_{10} cfu/g of silage)	3.7a	2.9a	2.5a	2.9a	2.7a	32.43	0.5913
Aerobic stability (hours)	47.0b	172.0a	170.0a	228.0a	179.0a	8.81	0.0102
CP concentration (g/kg DM)	94.3a	92.8a	91.0ab	94.3a	88.0b	2.18	0.0020
NDF concentration (g/kg DM)	388.0b	426.0ab	436.0a	421.0ab	445.0a	4.89	0.0148
ADF concentration (g/kg DM)	226.0b	248.0ab	251.0ab	258.0ab	272.0a	7.03	0.0255

Means in rows with different superscripts differ by Tukey's test ($P<0.05$).
LB - *L. buchneri* added to 5×10^4 cfu/g of fresh forage; LB2 - *L. buchneri* added to 1×10^5 cfu/g of fresh forage; LB3 - *L. buchneri* added to 5×10^5 cfu/g of fresh forage; LB4 - *L. buchneri* added to 1×10^6 cfu/g of fresh forage; CV - coefficient of variation; CP - crude protein; NDF - neutral detergent fibre; ADF - acid detergent fibre; TN - total nitrogen.

Table 2 - Acetic acid concentrations (g/kg DM) of corn silage treated with doses of *Lactobacillus buchneri* sampled in periods (days of aerobic exposure)

Periods	Control	LB1	LB2	LB3	LB4	Mean
0	8.0Ab	10.6Aab	12.4Aa	11.8Aa	13.4Aa	11.24
4	4.9Bc	11.2Ab	13.1Ab	12.3Ab	18.3Aa	11.96
8	4.9Bb	5.3Bb	5.3Bb	10.9Aa	5.5Bb	6.38
12	4.7Ba	3.9Ba	4.9Ba	4.7Ba	4.6Ba	4.56
Mean	5.62	7.75	8.93	9.93	10.45	
CV (%)						18.52
P value						<0.0001

Means in rows with lowercase and in columns with uppercase letters differ ($P<0.05$) by Tukey's test.
LB - *L. buchneri* added to 5×10^4 cfu/g of fresh forage; LB2 - *L. buchneri* added to 1×10^5 cfu/g of fresh forage; LB3 - *L. buchneri* added to 5×10^5 cfu/g of fresh forage; LB4 - *L. buchneri* added to 1×10^6 cfu/g of fresh forage; CV - coefficient of variation.

to the growth of other undesirable microorganisms such as molds and bacteria. All the treated silages had low pH values in the fourth measurement of aerobic exposure (P<0.05). The pH value was low in the LB3-treated silage in the eighth measurement. On day 12, the pH values did not differ between silages (Table 4). On the twelfth measurement of aerobic exposure, there was no difference in the acetic acid concentration, yeast and mold counts or pH values between silages, which indicates that silages were spoiled.

Conclusions

Silages containing *Lactobacillus buchneri* showed high acetic acid concentrations, low number of yeast and mold and a more stable pH, which improves the aerobic stability without affecting the dry matter recovery and gas loss during ensiling period. However, doses equal or superior to 1×10^5 cfu/g of fresh forage were more efficient in the control of aerobic spoilage.

Table 3 - Microbial composition of corn silage treated with doses of *Lactobacillus buchneri* sampled in periods (days of aerobic exposure)

Periods	Control	LB1	LB2	LB3	LB4	Mean
			Yeasts (log cfu/g of silage)			
0	4.71Aa	1.71Ab	1.70Ab	1.24Ab	2.31Ab	2.33
4	8.45Ba	6.96Bab	6.47Bb	5.53Bb	6.27Bb	6.74
8	7.82Ba	8.02Ba	7.80Ba	7.61Ca	7.68BCa	7.78
12	8.50Ba	8.74Ba	8.15Ba	8.50Ca	8.55Ca	8.49
Mean	7.37	6.36	6.03	5.72	6.20	
CV (%)						9.69
P value						0.0006
			Mold (log cfu/g of silage)			
0	3.71	2.97	2.45	2.97	2.69	2.96D
4	4.69	3.89	3.97	3.34	2.71	3.72C
8	7.10	5.17	4.53	4.77	4.38	5.19B
12	7.66	6.63	6.45	6.26	6.32	6.66A
Mean	5.79a	4.66b	4.35b	4.34b	4.03b	
CV (%)						15.97
P value						0.5861

Means in rows with lowercase and in columns with uppercase letters differ by Tukey's test (P<0.05).
LB - *L. buchneri* added to $5x10^4$ cfu/g of fresh forage; LB2 - *L. buchneri* added to $1x10^5$ cfu/g of fresh forage; LB3 - *L. buchneri* added to $5x10^5$ cfu/g of fresh forage; LB4 - *L. buchneri* added to $1x10^6$ cfu/g of fresh forage; CV - coefficient of variation.

Table 4 - Values of pH of corn silage treated with doses of *Lactobacillus buchneri* sampled in periods (days of aerobic exposure)

Period	Control	LB1	LB2	LB3	LB4	Mean
0	3.90Ba	4.00Ba	3.95Ba	4.00Ba	4.00Ba	3.95
4	5.43Aa	3.93Bb	4.02Bb	4.16Bb	4.13Bb	4.33
8	5.51Aa	5.73Aa	4.92Aa	4.00Bb	4.71ABab	4.97
12	5.56Aa	5.48Aa	5.72Aa	6.01Aa	5.66Aa	5.67
Mean	5.10	4.79	4.65	4.54	4.60	
CV (%)						5.58
P value						<0.0001

Means in rows with lowercase and in columns with uppercase letters differ (P<0.05) by Tukey's test.
LB - *L. buchneri* added to 5×10^4 cfu/g of fresh forage; LB2 - *L. buchneri* added to 1×10^5 cfu/g of fresh forage; LB3 - *L. buchneri* added to 5×10^5 cfu/g of fresh forage; LB4 - *L. buchneri* added to 1×10^6 cfu/g of fresh forage; CV - coefficient of variation.

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