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New inoculants on maize silage fermentation

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ABSTRACT - The objective of this study was to evaluate the effect of bacterial inoculants at two inoculation rates on chemical and biological characteristics of maize silage. The treatments consisted of two inoculating rates (5 and 6 log cfu g^{-1} of forage) for each strain of lactic acid bacteria (LAB) identified as *Lactobacillus buchneri, L. hilgardii*, or *L. plantarum*. The maize was ensiled in experimental PVC silos. Samples were taken for the determination of the contents of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), water-soluble carbohydrates (WSC), organic acids and alcohols, for the evaluation of the populations of lactic acid bacteria, yeasts, filamentous fungi, and for the determination of pH values during ensilage and after 30 or 90 days of fermentation. The doses of inoculants did not promote significant differences on the evaluated characteristics. There was effect of inoculants on acetic acid, 1.2-propanediol, LAB population, filamentous fungi, and pH value. No significant influence of the treatments with inoculants was observed in the variables DM, WSC, CP, lactic acid concentrations, or ethanol. The maximum temperature, i.e., the time to achieve the maximum temperature (TMT) and aerobic stability (AS), was not influencied by treatments. However, a decrease in maximum temperature, an increase in TMT, and improvement in the AS were observed after 90 days of fermentation. These results proved the advantage of microbial inoculation. The treatments influenced LAB populations and filamentous fungi, but no effect was observed on the yeast population. The best inoculation dose is 6 cfu g^{-1} of forage because it provides higher reduction of filamentous fungi in maize silage, thereby decreasing the aerobic deterioration by these microorganisms.

Key Words: filamentous fungi, inoculation, lactic acid bacteria, volatile fatty acids, yeasts

Introduction

Maize presents adequate characteristics for the production of a good-quality silage with dry matter (DM) content between 28 and 35%, high production of DM per area (16 to 21 t ha^{-1}) and low buffering capacity (Neumann, 2013). However, maize silages are susceptible to nutrient losses and aerobic deterioration (Siqueira et al., 2005).

The lactic acid bacteria (LAB) are the main microorganism group responsible for the conservation process of the ensiled mass. Bacterial inoculants comprise the most used additive class (Henderson, 1993). In the current market, there are inoculants containing different species and strains of bacteria and the effect of the inclusion of these inoculants has been variable. However, in most studies, their inclusion has provided positive effects, whether on chemical or microbiological composition of the silage (Wilkinson et al., 2003) or on animal performance (Contreras-Gouveia et al., 2010). For an inoculant to be effective, the plant and the selected microorganism must be compatible.

Ávila et al. (2014) isolated and selected 14 strains of LAB from sugarcane silages to be used as starter cultures in these silages. Carvalho et al. (2014) studied the fermentation profile of these strains and observed that the UFLA SIL 32 and 35 (*L. plantarum*) and UFLA SIL 51 and 52 (*L. hilgardii*) strains provided an increase in the aerobic stability of the sugarcane silage. In a similar way, Santos et al. (2013) selected LAB strains for the maize ensilage and among nine tested strains, they observed that UFLA SIL 103, and 108 (*L. buchneri*) and UFLA SIL 41 (*L. plantarum*) showed the best results.

The recommended inoculation rate is 5 to 6 log cfu g^{-1} of fresh forage (Kung Jr. and Shaver, 2001). This population, however, varies among different bacterial species present in the inoculant and according to the amount of substrate in the fermentation process (Jones et al., 1992). Thus, the selection of new bacterial strains and the adequate inoculation rate for initiating cultures in silages are of great importance to improve the quality and/or aerobic stability.

The objective of this research was to study the effects of the inclusion of new LAB strains and a commercial strain of the *Lactobacillus buchneri* species in two doses

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on the chemical and microbiological characteristics and on the aerobic stability of maize silages.

Material and Methods

The strains of lactic acid bacteria (LAB) used in this trial were isolated from sugarcane silage belonging to Culture Collection of the Microbiology Laboratory of Universidade Federal de Lavras. Three strains of *Lactobacillus buchneri* (UFLA SIL 09, 103 and 108), three strains of *L. plantarum* (UFLA SIL 32, 35 and 41) and two strains of *L. hilgardii* (UFLA SIL 51 and 52) were used. Additionally, a commercial strain of *L. buchneri* species was also tested.

The maize, approximately 102 to 119 days old, was harvested using a self-propelled harvester with the particle size set to 10 mm. Inoculants were previously prepared according to Ávila et al. (2009), mixed with 80 mL of distilled water and homogenized in 3 kg of forage to be ensiled. Then, the experimental inoculants were inoculated at the rate of 5 or 6 log cfu g^{-1} of forage.

Forage was ensiled in experimental PVC silos (10 cm in diameter and 60 cm in height) adapted with Bunsen valves and with an average forage compaction density of 628 kg/m^3. Forage was manually compacted in the silos, which were sealed, weighed and stored in a covered area. After 30 and 90 days of ensiling, the silos were weighed and opened. The loss of DM was calculated using weights of the DM content of the fresh forage and silage according to the equation:

$$\text{DM loss (\%)} = 100 - (WO \times DMO)/(WC \times DMC) \times 100,$$

in which WO = weight of forage at opening; DMO = DM content at opening; WC = weight of forage at closure; and DMC = DM content at closure.

Samples were removed from fresh forage and from silages after 30 and 90 days of fermentation. One sample was weighed and dried in a fan-assisted oven at 55 °C for 72 h; another sample was used to make a water extract to determine the pH value, microbial population, and fermentation end products. The dried samples were ground in a Wiley mill using a 30-mesh screen and stored in labelled plastic containers. The samples were analyzed for the DM and crude protein (CP) contents (AOAC 1990), water-soluble carbohydrates (WSC) by the phenol method (Dubois et al., 1956), and neutral detergent fiber (NDF) was analyzed according to Pell and Schofield (1993).

A 25 g sample of fresh forage or maize silage was blended in 225 mL of 0.1% sterile peptone water and homogenized in an orbital mixer for 20 min at 150 rpm. The pH of each sample was then determined. Water extracts (2 mL) were acidified with 10 µL of 50% (vol/vol) H_2SO_4 and frozen prior to analysis for fermentation and products (Canale et al., 1984). Acidified water extracts were analyzed for lactic acid, acetic acid, propionic acid, butyric acid, ethanol and 1,2-propanediol using high-performance liquid chromatography (Shimadzu model LC-10Ai; Shimadzu Corp., Tokyo, Japan) (Carvalho et al., 2012).

The other portion of water extracts was used for enumeration of microorganisms according to Ávila et al. (2014). Sequential ten-fold dilutions were prepared to quantify the microbial groups. Yeasts and filamentous fungi were enumerated on Dichloran Rose Bengal Chloramphenicol Medium (DRBC, Difco; Becton Dickinson, Sparks, MD, USA). The Petri dishes were incubated at 28 ºC for 72 h. Yeasts were distinguished from filamentous fungi by colony appearance and cell morphology. For enumeration of LAB, plating onto DeMan Rogosa Sharpe agar (M641I, Himedia; Mumbai, India) plus nystatin (4 mL L^{-1}) was performed. The plates were incubated at 30 ºC for 72 h. Colonies were counted on plates containing a minimum of 30 and a maximum of 300 cfu.

After opening the silos, samples of approximately 3 kg were collected from each silo and placed in plastic buckets to assess the aerobic stability. Temperatures were measured at every 30 minutes using Data Loggers (Impac, model MI-IN-D-2-L; São Paulo, Brazil) inserted into the silage mass to 10 cm depth. The aerobic stability was defined as the number of hours the silage remained stable before rising more than 2 °C above the ambient temperature (25.6 °C) (Kung Jr. et al., 2003).

The experiment was carried out in a randomized-block design in a 9 × 2 × 2 factorial arrangement, with nine inoculants, two inoculation rates and two times of fermentation, with three replicates. The statistical analysis was performed using the SISVAR® (Lavras, Brazil) software (version 4.5).

Results

Freshly chopped maize presented DM of 303.8 g kg^{-1}, pH of 4.78 and concentrations of WSC, crude protein and NDF of 11.57, 71.97, and 537.4 g kg^{-1} of dry matter, respectively. The populations of lactic acid bacteria (LAB), filamentous fungi, and yeasts were 5.79, 2.74, and 2.09 log cfu g^{-1} of forage, respectively (Table 1).

No significant effect ($P>0.05$) of the inoculants, inoculation rate, and fermentation time, or their interaction was observed on the rates of DM, soluble carbohydrates, NDF and crude protein (Table 2). The average content of DM in the silages was 287.5 g kg^{-1}, regardless of the

Table 1 - Microbiological and chemical composition of maize before ensiling

Item	Mean	Standard deviation
Dry matter (g kg^{-1} of fresh matter)	303.8	0.82
Content (g kg^{-1} of dry matter)		
Water-soluble carbohydrates	11.57	1.67
Neutral detergent fiber	537.4	2.82
Crude protein	71.97	1.69
Population (log cfu g^{-1} of forrage)		
Lactic acid bacteria	5.79	1.13
Yeast	5.87	0.80
Mold	4.00	0.30
pH	4.78	0.42

fermentation time, inoculant, or inoculation rate used. There was a trend for effect of fermentation time (Table 2) on DM losses, averaging an increase of 6% of dry matter in the silages at 30 days of fermentation and 7.4% of dry matter in the silages at 90 days of fermentation.

Inoculation rates had no effect of the on the production of any of the studied end products (P>0.05) (Table 3). The lactic acid content was not significantly influenced (P>0.05) by the studied factors, whose average concentration was 48.5 g kg^{-1} of dry matter (Table 4). The rates of acetic acid were affected (P<0.001) by inoculats and fermentation periods, without interaction between them. Thus, on average, the silages inoculated with UFLA SIL 32 (*L. plantarum*), UFLA SIL 51 and 52 (*L. hilgardii*), and UFLA SIL 09; 103 and 108 (*L. buchneri*) strains presented similar concentrations of acetic acid. However, these concentrations were higher in these silages than in the silages inoculated with the other strains (Table 4). The concentration of acetic acid increased by 1.4 g kg^{-1} dry matter from 30 to 90 days of fermentation.

The alcohols detected in this study were ethanol and 1,2-propanediol. There was only effect of the fermentation period (P<0.001) on the ethanol contents of the maize silages. The ethanol concentration was 10.3 and 17.1 g kg^{-1} of dry matter at 30 and 90 days, respectively, regardless of the inoculant used. Regarding the 1,2-propanediol content, a significant interaction was observed between the inoculants and the fermentation period (Table 3). At 30 days of fermentation, the silages showed similar concentrations of 1,2-propanediol (average 4.4 g kg^{-1} of dry matter) (Table 4). However, at the 90 days of fermentation, the silage inoculated with the UFLA SIL 09 (*L. buchneri*) strain presented a higher rate of 1,2-propanediol (12.4 g kg^{-1} of dry matter) than the other silages, whose rates were similar, averaging 5.4 g kg^{-1} of dry matter. Butyric acid was not detected in any of the silages.

The inoculation rate (P<0.05) and fermentation period (P<0.001) showed a significant effect (P<005) on the pH values of the silages (Table 6). The silages inoculated with UFLA SIL 09 and 103 (*L. buchneri*) differentiated from the others for having lower pH values. The highest dose of inoculation (6 log cfu g^{-1} silage) resulted in silages with a higher pH, which were superior at 90 days of fermentation.

A significant interaction (P<0.001) was observed between the inoculatnts and fermentation period for the LAB population (Table 3). The silages with the UFLA SIL 32 (*L. plantarum*), UFLA SIL 51 (*L. hilgardii*), UFLA SIL 103 and commercial (*L. buchneri*) strains presented the highest average populations of LAB at 30 days of ensilage (Table 4). However, after 30 days, the silages with the

Table 2 - Probability of effects (P-value) and mean values for the chemical composition of maize silage

Item	Mean	P-value						
		Inoculant (I)	Dose (D)	Time (T)	I × D	I × T	D × T	I × D × T
Dry matter (g kg^{-1} fresh matter)	287.5	NS	NS	NS	NS	NS	NS	NS
Dry matter loss (%)	6.75	NS	NS	0.08	NS	NS	NS	NS
Water-soluble carbohydrates (g kg^{-1} dry matter)	12.34	NS	NS	NS	NS	NS	NS	NS
Neutral detergent fiber (g kg^{-1} dry matter)	523.7	NS	NS	NS	NS	NS	NS	NS
Crude protein (g kg^{-1} dry matter)	76.90	NS	NS	NS	NS	NS	NS	NS

Effects of interactions: inoculant and dose (I × D); inoculant and time (I × T); dose and time (D × T); inoculant and dose and time (I × D × T).
NS - not significant.

Table 3 - Probability of effects (P-value) and mean values for metabolites and population of lactic acid bacteria and yeasts of maize silage

Item (g kg^{-1} dry matter)	Mean	P-value						
		Inoculant (I)	Dose (D)	Time (T)	I × D	I × T	D × T	I × D × T
Latic acid	48.55	NS	NS	NS	NS	NS	NS	NS
Acetic acid	3.04	0.001	NS	<0.001	NS	NS	NS	NS
1,2-propanediol	5.32	<0.001	NS	<0.001	NS	0.01	NS	NS
Ethanol	13.7	NS	NS	<0.001	NS	NS	NS	NS
Lactic acid bacteria (log cfu g^{-1})	8.03	<0.001	NS	<0.001	NS	<0.001	NS	NS

Effects of interactions: inoculant and dose (I × D); inoculant and time (I × T); dose and time (D × T); inoculant and dose and time (I × D × T).
NS - not significant.

UFLA SIL 32, 35 and 41 (*L. plantarum*), UFLA SIL 09; 103 and 108 (*L. buchneri*) strains presented the highest populations of LAB. All other silages showed lower LAB populations during fermentation from 30 to 90 days.

The yeast population which was initially high in the fresh forage (5.87 log cfu g^{-1} forage) reduced after fermentation, where it was possible to count yeasts only in some silages and, for that reason, that variable was not subjected to statistical analysis. At 30 days of fermentation, the yeast populations in the silages inoculated with UFLA SIL 09 and 32 and 51 were of 2.3 and 2.2 and 2.3 log cfu g^{-1} silage, respectively, at the inoculation rate of 6 log cfu g^{-1} of forage. At 90 days of fermentation, it was possible to count the yeast population in the silages inoculated with 5 log cfu g^{-1} of forage for UFLA SIL 09 (2.4 log cfu g^{-1} silage), 35 (2.6 log cfu g^{-1} silage), 41 (2.3 log cfu g^{-1} silage) and 52 (2.7 log cfu g^{-1} silage). At 90 days of fermentation, it was possible to count the yeast population in the silages inoculated with 6 log cfu g^{-1} of forage for the UFLA SIL 32 (2.3 log cfu g^{-1} silage), 35 (2.5 log cfu g^{-1} silage), 41 (2.3 log cfu g^{-1} silage), 51 (2.3 log cfu g^{-1} silage), 103 (2.4 log cfu g^{-1} silage) and 108 (2.2 log cfu g^{-1} silage) and commercial (2.3 log cfu g^{-1} silage) strains. In the other silages, the yeast population was below the minimum limit of detection by the dilution technique, which is 2 log cfu g^{-1} of silage.

The filamentous fungi population was the most affected variable by the studied factors (P<0.001) (Table 5). When 5 log cfu g^{-1} of forage was added, silages with the UFLA SIL 108 and commercial inoculants presented counts lower than the minimum detectable (2 log cfu g^{-1} silage) and for the other silages the values were superior and similar

Table 4 - Metabolites and population of lactic acid bacteria (LAB) in maize silage

Item (g kg^{-1} dry matter)	Time	Mean of inoculant	Inoculant (mean)								
			09	32	35	41	51	52	103	108	Commercial
Latic acid	30	48.0	33.6	56.2	56.0	45.5	46.1	53.9	52.9	45.0	47.6
	90	49.1									
Acetic acid	30	2.3B	3.8a	3.1a	1.9b	2.3b	3.3a	3.3a	4.2a	3.3a	2.2b
	90	3.7A									
1,2-propanediol	30	4.4B	5.1aB	4.2a	3.7a	3.3a	5.1a	5.9a	6.1a	3.1aA	3.3a
	90	6.2A	12.4aA	4.9b	3.6b	4.1b	6.1b	6.7b	7.3b	5.9bB	4.7b
Ethanol	30	10.3B	12.5	13.7	12.6	14.7	13.3	12.8	13.1	15.6	15.2
	90	17.1A									
LAB (log cfu g^{-1} silage)	30	8.6A	8.3bA	9.0aA	8.4bA	8.3bA	8.9aA	8.5bA	8.9aA	8.6bA	8.9aA
	90	7.4B	7.8aB	7.9aA	7.6aB	7.3aB	7.1bB	7.1bB	7.7aB	7.5aB	6.5cB

Means in columns with uppercase letters and in rows with lowercase letters differ (P<0.05) by Scott-Knott's test.
Inoculants: UFLA SIL 09, 103, 108 and comercial correspond to strains of *L. buchneri*; UFLA SIL 32, 35 and 41, strains of *L. plantarum*; UFLA SIL 51 and 52, strains of *L. hilgardii*.

Table 5 - Filamentous fungi population and pH values of maize silage treated with different inoculants at different fermentation times

	Mold (log cfu g^{-1} of silage)										
	Inoculant (I)		Dose (D)		Time (T)		I × D		I × T		D × T
P-value	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001
Inoculant		09	32	35	41	51	52	103	108	Commercial	Mean
Dose	5 log	7.8aA	4.8aA	4.4aA	4.2aA	4.6aA	4.8aA	4.1aA	<2bA	<2bA	3.7
	6 log	2.8aB	<2bB	<2bB	<2bB	<2bB	<2bB	<2bB	<2bA	<2bA	<2
Time	30	4.7aA	3.6bA	3.5bA	3.5bA	4.8aA	3.6bA	3.4bA	<2cB	<2cB	3.4
	90	2.9aB	2.6aA	<2aA	<2bB	<2bB	2.2aB	<2bB	2.1aA	2.4aA	2.0
	Mean	3.8	3.1	2.9	2.1	3.2	2.9	2.7	<2log	<2log	

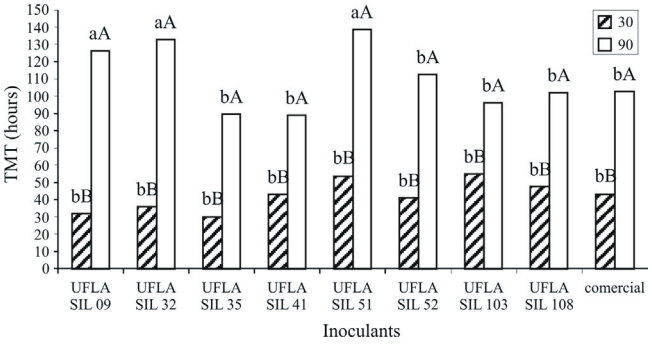
	pH values									
	Inoculant (I)	Dose (D)	Time (T)	I × D	I × T	D × T				
P-value	<0.042	<0.041	<0.001	NS	NS	NS				
Inoculant	09	32	35	41	51	52	103	108	Commercial	Mean
	3.99a	3.88b	3.88b	3.88b	3.88b	3.90b	3.95a	3.91b	3.90b	3.9
Dose	5 log	6log					Time		30	60
	3.89b	3.93a							3.86b	3.95a

NS - not significant.
Means in colunms with uppercase letters and in rows with lowercase letters differ (P>0.05) by Scott-Knott's test.
Inoculants: UFLA SIL 09, 103, 108 and comercial correspond to strains of *L. buchneri*; UFLA SIL 32, 35 and 41, strains of *L. plantarum*; UFLA SIL 51 and 52, strains of *L. hilgardii*.

between them. When 6 log cfu g^{-1} of forage were added, with the exception of the UFLA SIL 09 (*L. buchneri*) strain, which presented an average count of 2.8 log cfu g^{-1} of silage, all the other silages presented counts lower than 2 log cfu g^{-1} of silage. There was a reduction in the count of filamentous fungi with an increase in the inoculation rate from 5 to 6 log cfu g^{-1} of forage for all other strains tested, except for UFLA SIL 108 and the commercial strain. The silages inoculated with these two strains presented an increase in the population of filamentous fungi from 30 to 90 days of fermentation.

The different inoculants and the two studied doses did not significantly modify the maximum temperature or the aerobic stability of the silages. There was an effect only in the period of ensilaging (P<0.001) on these variables. The aerobic stability was greater and the maximum temperature was lower at 90 days of fermentation (Table 6).

A significant interaction of the effects of the inoculants and periods of fermentation (P<0.01) was observed on the time to reach the maximum temperature after the opening of the silos (TMT). At 30 days of fermentation, the values of TMT were similar between the silages with different inoculants (Figure 1). However, at 90 days of fermentation, the silages with the UFLA SIL 09 (*L. buchneri*), 32 (*L. plantarum*) and 51 (*L. hilgardii*) inoculants took a longer time to reach the maximum temperature.



Means followed by the same uppercase letters differ statistically (P<0.05) between fermentation days for each inoculant.
Means followed by the same lowercase letters do not differ statistically (P<0.05) between inoculants for each fermentation time.
Both applied for Scott-Knott's test at 5% probability.

Figure 1- Time necessary to reach maximum temperature (TMT) in maize silage treated with different inoculants at two fermentation times.

Discussion

The dry matter content of the fresh maize (303.8 g kg^{-1} of dry matter) at the moment of ensilage was adequate, within the interval of 300 to 350 g kg^{-1} of dry matter, suggested by Neumann (2013) as adequate for the ensilage process. The concentrations of NDF (537.4 g kg^{-1} of dry matter) and crude protein were compatible with the values found in the literature (Neumann, 2013; Santos et al., 2013). The concentration of WSC in the maize plant was, on average, 11.17 g kg^{-1} of dry matter. This value was lower than the 60 g kg^{-1} of dry matter recommended for an efficient fermentation (Jaakkola et al., 1991; Rooke & Hatfield, 2003). The interval between the maize chopping and the analyses was of approximately six hours. The fermentation may have started in this period, leading to the intake of carbohydrates present in the plant and thus to the drop of pH.

The absence of effect of inoculation of LAB strains on bromatological characteristics of silages has been observed by other authors (Gimenez et al., 2006). The effect of the use of inoculants was more evident in the microbiological characteristics and metabolites produced during the fermentation. Comparing the rates of NDF and crude protein of the maize plant before and after the ensiling, a small variation was observed. The increase in CP with the fermentation could be related to the synthesis of the microbial protein (Bolsen, 1995).

Table 6 - Probability of effects (P-value) for the data on aerobic stability of silages

Item	Inoculant (I)	Dose (D)	Time (T)	I × D	I × T	D × T
maxT (ºC)	NS	NS	<0.001	NS	NS	NS
Time (hours)		30			90	
		39.2a			32.3b	
AS	NS	NS	<0.001	NS	NS	NS
Time (hours)		30			90	
		25.41b			58.21a	
TMT	<0.01	NS	<0.01	NS	<0.01	NS
Time (hours)		30			90	
		42.34b			109.87a	

maxT - maximum temperature; AS - aerobic stability; TMT - hours to reach maximum temperature; NS - not significant.
Means followed by different letters in the same row are statistically different (P>0.05) by Scott-Knott's test.
Interactions: I × D - inoculant and dose; I × T - inoculant and time; D × T - dose and time.

The measurement of the dry matter losses during the ensilage is a characteristic of great importance for the evaluation of the fermentation quality. The DM losses in general are greater in the beginning of the fermentation process, when the activity of the microorganisms is more intense. In this study, the occurrence of a greater loss was observed until the 30th day of fermentation. From the 30th to the 90th day of fermentation, the losses still occurred, but more slowly, increasing from 6 to 7.4% of fresh matter.

Lactic acid was the only end product which was not significantly influenced by the studied factors. The strains used in this study are of diferent species and with diferent abilities to produce metabolites during the ensilage (Santos et al., 2013; Ávila et al., 2014). Therefore, it was expected that there would be a difference in lactic acid production among the treatments. However, many factors might interfere with the lactic acid content of the silage. Strains of the same species might show differences in the metabolism and in the ability to survive in the silage environment. Another factor to be considered is the use of lactic acid by other microorganisms like yeasts (McDonald et al., 1991) or heterofermentative LAB (Axelsson, 2004) in the final stages of fermentation, which makes an increase in the rates of that acid unnoticed over time. Another important factor to be considered is the time of evaluation. Thus, the 30-day silages should already be stable. That is common, mainly when the concentrations of soluble carbohydrates are low and the ensilage conditions are ideal. The mean values of acids observed are adequate, since the ideal concentration of lactic acid from maize silages varies from 0.04 to 0.07 mL kg^{-1} of dry matter (Kung Jr. and Shaver, 2001).

Among the inoculants that produced silages with the highest acetic-acid contents, only the UFLA SIL 32 (*L. plantarum*) strain is facultative heterofermentative. The other strains are classified as obrigatory heterofermentative. The increase in the concentration of acetic acid as a final product of the fermentation is justified by the fact that heterfermentative species, specially *L. Buchneri*, do not have the acetaldehyde dehydrogenase enzyme, responsible for the reduction of the acetaldehyde to ethanol (McDonald et al., 1991; Axelsson, 2004), and thus synthesize higher concentrations of acetic acid (Mari et al., 2009). This evidence was also reported by Ranjit and Kung Jr. (2000), who observed an increase in the concentration of acetic acid when maize silages were inoculated with *L. buchneri*. The *L. buchneri* (Oude-Elferink et al., 2001) and *L. hilgardii* (Heinl et al., 2012) species have the ability to degrade lactic acid into acetic acid at the final step of fermentation.

The silage inoculated with the UFLA SIL 09 (*L. buchneri*) strain showed a much higher content of 1,2-propanediol (12.4 g kg^{-1} of dry matter) compared with the other silages, whose rates were similar, averaging 5.4 g kg^{-1} of dry matter. According to Oude-Elferink et al. (2001), microorganisms of these species can degrade lactic acid into acetic acid and 1,2-propanediol. Other strains also identified as *L. buchneri* (UFLA SIL 7, UFLA SIL 8 and Lalsil) did not present that effect, demonstrating that strains of the same species present different characteristics. According to Krooneman et al., (2002) bacteria of the *L. diolivorans* species may degrade 1,2-propanediol into propionic acid in silages. In the silages evaluated in this study, that conversion probably did not occur, as the propionic acid content was not changed in the silages. Nishino et al. (2003), evaluating the effects of inoculations with *Lactobacillus buchneri* on the fermentation characteristics and the aerobic stability of maize silage, found accumulation of 1,2-propanediol without increase in the concentration of propionic acid.

The higher production of ethanol is normally associated with fermentation of sugars and organic acids by yeast. However, it is noteworthy that ethanol traces may be present as a result of the fermentation caused by the obrigatory heterofermentative LAB such as *L. buchneri* (Freitas et al., 2006). The etanol contents of silages in this study surpassed the values found by Li and Nishino (2011) and are in the interval of values considered acceptable for ethanol (10 to 30 g kg^{-1} of dry matter) in maize silages (Kung Jr. and Shaver, 2001).

The silages inoculated with the UFLA SIL 09 and 103 strains presented a higher average value of pH in relation to other strains of the same species. However, the average values of pH obtained in the present study for all silages fit in the pH interval (3.7 to 4.2) considered adequate for the maize silage (Kung Jr. and Shaver, 2001). The increase in the pH values at 90 days of fermentation may be justified by the increase in the concentration of acetic acid, reducing the lactic:acetic acid ratio since the lactic acid did not increase in this period. It is worth noting that obrigatory heterofermentative lactic bacteria such as *L. buchneri* and *L. hilgardii* are able to degrade lactic acid into acetic acid, thus raising the pH (Oude-Elferink et al., 2001).

The highest dose of inoculant used in this study increased the pH because of the inclusion of obrigatory heterofermentative bacteria, since those bacteria lead to higher values of pH in maize and alfalfa silages (Driehuis et al., 1999; Kung Jr. et al, 2003). Other authors have observed an increase in pH values with an increase in the rates of inoculation with *L. buchneri* (Driehuis et al., 1999; Reis et al., 2008).

The populations of lactic acid bacteria (LAB), yeasts and filamentous fungi in the fresh forage were smaller

than the values reported in the study by Filya (2003). That variation in the epiphytic population of microorganisms in the forage may be attributed to several factors, such as the temperature, ultraviolet radiation, and factors associated with the forage itself (Merry et al., 2008). In most of the studies, the number of epiphytic lactic bacteria is usually low at the moment that precedes the ensilage, but the population is elevated during the process (Lin et al., 1992). At 30 days, the silages with the UFLA SIL 32 (*L. plantarum*), UFLA SIL 51 (*L. hilgardii*), UFLA SIL 103 and the commercial (*L. buchneri*) strains presented the highest populations of LAB, whereas at 90 days this happened with the UFLA SIL 32, 35 and 41 (*L. plantarum*); UFLA SIL 09, 103 and 108 (*L. buchneri*) strains.

Except for the silage with the UFLA SIL 32 (*L. plantarum*) strain, there was a reduction in the population of LAB during the fermentation from 30 to 90 days in all others. Reduction in the number of LAB during the fermentation process has also been reported by other authors (Li and Nishino, 2011) and it is related to resistance to acidic conditions. During the process, even the LAB which belong to the *Lactobacillus* genus may lose viability, and some specialized microorganisms, such as *L. buchneri*, continue active (Oude-Elferink et al., 2000). Despite the reduction of the LAB population at 90 days of fermentation, this population may be considered high, showing that these microorganisms dominate the fermentation process compared with the yeasts and the filamentous fungi. In this study, the viability of the species inoculated during fermentation was not monitored. However, the increased population of LAB is a positive characteristic.

An explanation for the dose of inoculant not having effect on the number of LAB may be the period in which the evaluation took place. Larger inoculant populations may lead to differences in the beginning of the process, but at 30 days of fermentation this population may have equaled.

The population of yeast of the silages decreased during the fermentation. The reduction in the yeast population during fermentation might occur due to susbtrate limitation and/or the production of metabolites that can inhibit these microorganisms. Yeasts are facultative aerobic microorganisms thus are able to survive in environments with low oxygen concentration; however, they change their metabolism prioritizing fermentation and as a consequence there is no increase in cell number. It is important to note that even in the silages where it was possible to count yeast population, their numbers were close to the minimum limit of detection. Therefore, the total count of yeasts only was not a sufficient parameter to compare the effect of inoculants.

The UFLA SIL 108 and commercial inoculants were the most efficient in the inhibition of filamentous fungi at lower rates of inoculation. For all other treatments, the highest inoculation rate (6 log cfu g^{-1}) was necessary to reduce the population of those microorganisms. These results showed that the inoculation rate depends on the type of inoculant used. The influence of the dosage of the inoculant in the maize silage on the population of filamentous fungi has also been reported by Schmidt and Kung Jr. (2010). These authors found a lower population of these microorganisms when LAB strains were inoculated at a dose of 5.6 log cfu g^{-1} of forage in relation to a dose of 5 log cfu g^{-1} of forage. Similar results were found by Reis et al. (2008), who obtained a lower number of filamentous fungi when a dose of inoculant equal to 5 log cfu g^{-1} of forage was applied in relation to the dosage of 4.7 log cfu g^{-1} of forage for *L. buchneri* in silages of maize grain.

The population of filamentous fungi underwent variations which depended on the type of inoculant used. The lower counts of filamentous fungi after 90 days of the fermentation process may be related to the concentrations of acetic acid observed during the fermentation process. At 90 days of fermentation, there was an increase in the concentration of that acid, which possibly reflected directly in the population of filamentous fungi, thereby leading to a reduction of the number of those microorganisms at the end of the fermentation (90 days). Ranjit and Kung Jr. (2000) also highlighted the effect of the inoculation of LAB in maize silage leading to lower counts of filamentous fungi compared with the non-treated silages. The average number of filamentous fungi in the present study was similar to the 1.18 log cfu g^{-1} reported by Muck (2004) at 90 days of fermentation in silage treated with different inoculants, including *L. buchneri*.

The improvement in the aerobic stability of the silages at 90 days of fermentation may have occurred due to the higher rate of acetic acid, lower count of filamentous fungi, or even the lower environment temperature (28 °C) in this evaluation compared with the evaluation at 30 days. These results indicated that the silages in tropical environments tend to have a lower stability (Driehuis et al., 2001; Ashbell et al., 2002; Bernardes et al., 2007). This stability value was higher than that found by Gimenez et al. (2006) for maize silage treated with bacterial inoculant (33 hours) after 74 days of fermentation and lower than those found by Schmidt and Kung Jr. (2010) (123 hours).

At 90 days of fermentation, the highest values of TMT were found in the UFLA SIL 09 (*L. buchneri*), 32 (*L. plantarum*) and 51 (*L. hilgardii*) treatments. Ranjit and Kung Jr. (2000) relate the higher aerobic stability of

the silage lead by heterofermentative LAB species to the larger population of acetic acid which, in turn, inhibits the deteriorating microorganisms responsible for the decrease in the aerobic stability of silage.

The filamentous fungi and yeasts are the most responsible microorganisms for the aerobic deterioration of silages with consequent increase in temperature. Silages inoculated with facultative heterofermentative LAB were expected, along with the *L. plantarum* strain employed in this study, to be more conducive to the action of those deteriorating microorganisms, since the lactic acid produced in high concentration by that LAB group is substrate for the growth of such undesirable microorganisms. On the contrary, silages inoculated with obrigatory heterofermentative LAB strains, such as *L. buchneri* and *L. hilgardii*, are less susceptible to deterioration due to the higher production of acetic acid normally observed in these silages. The data of this study show that an increase in the stability is more closely related to the LAB strain in the inoculant than to the bacterial species.

Conclusions

Inoculation with different strains of lactic acid bacteria strains does not affect the nutritional value of maize silages but changed their fermentation profile. The fermentation time is the essential factor to be considered for the attainment of quality of maize silages inoculated with lactic acid bacteria. The recommended dose for most inoculants tested, except for the UFLA SIL 108 and commercial inoculants, is 6 log cfu g^{-1} of forage. Among the selected strains evaluated UFLA SIL 09 and UFLA SIL 103 are the most promising.

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References

Ashbell, G.; Weinberg, Z. G.; Hen, Y. and Fylia, I. 2002. The effects of temperature on the aerobic stability of wheat and corn silages. Journal of Industrial Microbiologyand Biotechnology 28:261-263.

AOAC - Association of Official Analytical Chemistry. 1990. Official methods of analysis. 15th ed. AOAC International, Arlington, VA.

Ávila, C. L.; Carvalho, B. F.; Pinto, J. C.; Duarte, W. F. and Schwan, R. F. 2014. The use of *Lactobacillus* species as starter cultures for enhancing the quality of sugar cane silage. Journal Science Dairy 97:940-951. doi: 10.3168/jds.2013-6987.

Ávila, C. L. S.; Pinto, J. C.; Figueiredo, H. C. P. and Schwan, R. F. 2009. Effects of an indigenous and a commercial *Lactobacillus buchneri* strain on quality of sugarcane silage. Grass and Forage Science 64:384-394.

Axelsson, L. 2004. Bactérias lácticas: classificação e fisiologia. p.1-66. In: Lactic acid bacteria: microbiological and functional aspects. 3rd ed. Salminen, S.; von Wright, A. and Ouwehand, A., eds. Marcel Dekker, New York.

Bernardes, T. F.; Reis, R. A.; Siqueira, G. R.; Berchielli, T. T. and Coan, R. M. 2007. Avaliação da queima e da adição de milho desintegrado com palha e sabugo na ensilagem de cana-de-açúcar. Revista Brasileira de Zootecnia 36:269-275.

Bolsen, K. K. 1995. Silage: basic principles. p.163-176. In: Forages: The science of grassland agriculture. 5th ed. Barnet, R. F.; Miller, D. A and Nelson, C. J., eds. Iowa State University, Ames.

Canale, A.; Vaalleennttee, M. E. and Ciotti, A. 1984. Determination of volatile carboxylic acids (C1-C5i) and lactic acid in aqueous acid extracts of silage by high performance liquid chromatography. Journal of the Science of Food and Agriculture 35:1178-1182.

Carvalho, B. F.; Ávila, C. L. S.; Pinto, J. C. Pereira, M. N. and Schwan, R. F. 2012. Effects of propionic acid and *Lactobacillus buchneri* (UFLA SIL 72) addition on fermentative and microbiological characteristics of sugar cane silage treated with and without calcium oxide. Grass and Forage Science 67:589-595.

Carvalho, B. F.; Ávila, C. L. S.; Miguel, M. G. C. P.; Pinto, J. C.; Santos, M. C. and Schwan, R. F. 2014. Aerobic stability of sugar cane silage inoculated with tropical strains of lactic acid bacteria. Grass and Forage Science. doi: 10.1111/gfs.12117.

Contreras-Goveaa, F. E.; Muckb, R. E.; Mertens, D. R. and Weimer, P. J. 2010. Microbial inoculant effects on silage and in vitro ruminal fermentation, and microbial biomass estimation for alfalfa, bmr corn, and corn silages. Animal Feed Science and Technology 163:2-10.

Driehuis, F.; Elferink, S. J. and Spoelstra, S. F. 1999. Anaerobic lactic acid degradation during ensilage of whole crop maize inoculated with *Lactobacillus buchneri* inhibits yeast growth and improves aerobic stability. Journal of Applied Microbiology 87:583-594.

Driehuis, F.; Oude-Elferink, S. J. W. H. and Wikselaar, P. G. Van. 2001. Fermentation characteristics and aerobic stability of grass silage inoculated with *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria. Grass and Forage Science 56:330-343.

Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A. and Smith, F. 1956. Calorimetric method for determination of sugars and related substances. Analytical Chemistry 28:350-356.

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. Journal of Applied Microbiology 95:1080-1086.

Freitas, A. W. P.; Pereira, J. C.; Rocha, F. C.; Costa, M. G.; Leonel, F. P. and Ribeiro, M. D. 2006. Avaliação da qualidade nutricional da silagem de cana-de-açúcar com aditivos microbianos e enriquecida com resíduo da colheita de soja. Revista Brasileira de Zootecnia 35:38-47.

Gimenez, A. L. G.; Mizubuti, I. Y.; Moreira, F. B.; Pereira, E. S.; Ribeiro, E. L. A. and Mori, R. M. 2006. Composição química e estabilidade aeróbia em silagens de milho preparadas com inoculantes bacteriano e/ou enzimático. Acta Scientiarum Animal Science 28:153-158.

Heinl, S.; Wibberg, D.; Eikmeyer, F.; Szczepanowski, R.; Blom, J.; Linke, B.; Goesmann, A.; Grabherr. R.; Schwab, H.; Pühler, A.

and Schlüter, A. 2012. Insights into the completely annotated genome of *Lactobacillus buchneri* CD034, a strain isolated from stable grass silage. Journal of Biotechnology 161:153-166.

Henderson, N. Additives silage. 1993. Animal Feed Science and Technology 33:35-56.

Jaakkola, S.; Huhtanen, P. and Hissa, K. 1991. The effect of cell wall degrading enzymes or formic acid on fermentation quality and on digestion of grass silage of cattle. Grass and Forage Science 46:75-87.

Jones, B. A.; Hatfield, R. D. and Muck, R. E. 1992. Effects of fermentation and bacterial inoculation on lucerne cell walls. Journal of the Science of Food and Agriculture 60:147-155.

Krooneman, J.; Faber, F.; Alderkamp, A. C.; Elferink, S. J.; Driehuis, F.; Cleenwerck, I.; Swings, J.; Gottschal, J. C. and Vancanneyt, M. 2002. *Lactobacillus diolivorans* sp. nov., a 1,2-propanediol-degrading bacterium isolated from aerobically stable maize silage. International Journal of Systematic Evolutionary Microbiology 52:639-646.

Kung Jr., L. and Shaver, R. 2001. Interpretation and use of silage fermentation analysis reports. Focus on Forage 3:1-5.

Kung Jr., L.; Stokes, M. R. and Lin, C. J. 2003. Silage additives. p.251-304. In: Silage science and technology. Buxton, D. R.; Muck, R. E. and Harrison, J. H., eds. American Society of Agronomy, Madison.

Li, Y. and Nishino, N. 2011. Bacterial and fungal communities of wilted Italian ryegrass silage inoculated with and without *Lactobacillus rhamnosus* or *Lactobacillus buchneri*. Letters in Applied Microbiology 52:314-321.

Lin, C.; Bolsen, K. K.; Brent, B. E.; Hart, R. A.; Dickerson, J. T.; Feyerherm, A. M. and Aimutis, W. R. 1992. Epiphytic micloflora on alfafa and whole-plant corn. Journal of Dairy Science 75:2484-2493.

Mari, L. J.; Schmidt, R. J.; Nussio, L. G.; Hallada, C. M. and Kung Jr., L. 2009. Short communication: An evaluation of the effectiveness of *Lactobacillus buchneri* 40788 to alter fermentation and improve the aerobic stability of corn silage in farm silos. Journal Dairy Science 92:1174-1176.

McDonald, P.; Henderson, A. R. and Heron, S. J. E. 1991. The biochemistry of silage. 2nd ed. Chalcomb Publications, Marlow, Bucks, UK.

Merry, R. J.; Winters, A. L.; Thomas, P. I.; Müller, M. and Müller, T. 2008. Degradation of fructans by epiphytic and inoculated lactic acid bacteria and by plant enzymes during ensilage of normal and sterile hybrid ryegrass. Journal of Applied Microbiology 79:583-591.

Muck, R. E. 2004. Effects of corn silage inoculants on aerobic stability. Transactions of the ASAE (American Society of Agricultural Engineers) 47:1011-1016.

Neumann, M. Parâmetros para análise de qualidade da silagem. 2013. Available at: <http://www.iepec.com/curso/listarCapituloPopUp&idCurso=58&idCapitulo=407>. Accessed on: Feb. 10, 2013.

Nishino, N.; Yoshida, M.; Shiota, H. and Sakaguchi, E. 2003. Accumulation of 1,2-propanediol and enhancement of aerobic stability in whole crop maize silage inoculated with *Lactobacillus buchneri*. Journal of Applied Microbiology 94:800-807.

Oude-Elferink, S. J. W. H.; Driehuis, F.; Gottschal, J. C. and Spoelstra, S. F. 2000. Silage fermentation processes and their manipulation. p.17-30. In: FAO Eletronic Conference on Tropical Silage. FAO, Rome.

Oude-Elferink, S. J. W. H.; Krooneman, J.; Gottschal, J. C.; Spoelstra, S. F.; Faber, F. and Driehuis, F. 2001. Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by *Lactobacillus buchneri*. Applied and Environmental Microbiology 67:125-132.

Pell, A. N. and Schofield, P. 1993. Computerized monitoring of gas production to measure forage digestion in vitro. Journal of Dairy Science 76:1063-1073.

Ranjit, N. K. and Kung Jr., L. 2000. The effect of *Lactobacillus buchneri*, *Lactobacillus plantarum*, or a chemical preservative on the fermentation and aerobic stability of corn silage. Journal of Dairy Science 83:526-535.

Reis, R. A.; Schocken-Iturrino, R. P.; Almeida, E. O.; Schocken-Iturrino, R. P.; Janusckiewicz, E. R.; Bernardes, T. F. and Roth, A. P. T. P. 2008. Efeito de doses de *Lactobacillus buchneri* "CEPA NCIMB 40788" sobre as perdas nos períodos de fermentação e pós-abertura da silagem de grãos úmidos de milho. Ciência Animal Brasileira 9:923-934.

Rooke, J. A. and Hatfield, R. D. 2003. Biochemistry of ensiling. p.251-304. In: Silage science and technology. Buxton, D. R.; Muck, R. E.; Harrison, J. H., eds. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison.

Santos, A. O.; Ávila, C. L. S. and Schwan, R. F. 2013. Selection of tropical lactic acid bacteria for enhancing the quality of maize silage. Journal of Dairy Science 96:7777-7789.

Schmidt, R. J. and Kung Jr., L. 2010. The effects of *Lactobacillus buchneri* with or without a homolactic bacterium on the fermentation and aerobic stability of corn silages made at different locations. Journal of Dairy Science 93:1616-1624.

Siqueira, G. R.; Bernardes, T. F. and Reis, R. A. 2005. Instabilidade aeróbia de silagens: efeitos e possibilidades de prevenção. p.25-60. In: Volumosos na produção de ruminantes. 2.ed. Reis, R. A.; Siqueira, G. R. and Bertipaglia, L. M. A., eds. FUNEP, Jaboticabal.

Wilkinson, J. M.; Bolsen, K. K. and Lin, C. J. 2003. History of silage. p.1-30. In: Silage science and technology. Buxton, D. R.; Muck, R. E.; Harrison, J. H., eds. ASA- CSSA-SSSA, Madison.