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ORIGINAL ARTICLE

Fermentative profile of maize silage inoculated with *Lactobacillus buchneri*

Perfil fermentativo de silagem de milho inoculada com Lactobacillus buchneri

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ABSTRACT: Biological silage additives can assist in making silages by promoting a rapid reduction in silage pH and preventing aerobic deterioration. The current *Lactobacillus buchneri* on the market produces acetic acid slowly and identifying strains that would improve aerobic stability earlier in the ensiling process would be helpful. This study aimed to investigate the changes in microbial population, dry matter (DM) recovery and fermentation profile of maize silage with or without inoculation with *L. buchneri* after 45 days of ensiling. The wild *L. buchneri* strains were isolated from tropical maize silage in a previous study. Four strains of *L. buchneri* (56.22, 56.27, 56.28 and 56.29) were used as inoculants. Data from the silo openings were analyzed as a completely randomized design, with four replicates per treatment (inoculants). Selected strains did not affect the DM content, yeast and mould population, DM recovery, water-soluble carbohydrates (WSC), lactic acid and butyric acid of maize silage after 45 days of ensiling ($p > 0.05$). The pH, lactic acid bacteria (LAB) population and concentrations of acetic and propionic acids and ethanol were affected by inoculants ($p < 0.05$). The strains 56.22, 56.27 and 56.28 showed lower pH than the untreated control silage, but lower acetic acid concentration.

RESUMO: *Os aditivos biológicos podem auxiliar na produção de silagens promovendo uma rápida redução no pH da silagem e evitando a deterioração aeróbia. O atual* Lactobacillus buchneri *no mercado produz ácido acético lentamente, e identificar cepas que melhorariam a estabilidade aeróbica mais rápido no processo de ensilagem seria vantajoso. Este trabalho tem objetivo avaliar as mudanças na população microbiana, a recuperação de matéria seca (MS) e o perfil fermentativo que ocorrem em silagem de milho com ou sem inoculação de* Lactobacillus buchneri *após 45 dias de ensilagem. As cepas selvagens de* L. buchneri *foram isoladas de silagem de milho tropical em estudo anterior. Quatro cepas de* L. buchneri *(56.22, 56.27, 56.28 e 56.29) foram usadas como inoculantes. Os dados das aberturas do silo foram analisados em delineamento inteiramente casualizado, com quatro repetições por tratamento (inoculantes). As linhagens selecionadas não afetaram o teor de MS, a população de leveduras e bolores, a recuperação de MS, os carboidratos solúveis, o ácido láctico e o ácido butírico da silagem de milho após 45 dias de ensilagem ($p > 0,050$). O pH, a população de bactérias do ácido láctico (BAL) e as concentrações de ácidos acético e propiônico e etanol foram afetados pelos inoculantes ($p < 0,050$). As linhagens 56.22, 56.27 e 56.28 apresentaram menor pH e menor concentração de ácido acético que a testemunha não tratada.*

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1 Introduction

Grazing is the most common and economical way to feed cattle, however it cannot be done over the entire year, due the climatic conditions that limit the grasses growth. In the dry season, for example, there is no forage production enough to feed the animals (Doonan et al., 2004). The choice of suitable forage conservation process to provide constantly feed, essentially depends of the climatic conditions at harvest. In hot areas with dry seasons, probably the haymaking is the best choice for forage preservation, because it is a simple technology. However, in tropical regions with hot and humid climates, it is difficult to produce high quality hay, due to high humidity and frequent rainfall at the optimum stage of maturity for crop with better nutritional value. In this context, ensiling is an important method of forage preservation because it is not too dependent on weather as the haymaking. In addition, in many parts of world the silage is the major source of energy in the total mixed rations in ruminant diets (Chiba et al., 2005).

Properly made and managed silage is an excellent feed that poses no health risks to humans or livestock. Biological silage additives can assist in making silages by promoting a rapid reduction in silage pH and preventing aerobic deterioration (Driehuis et al., 2018). Studies have shown that the *Lactobacillus buchneri* application in silages can reduce losses and increasing the aerobic stability, degradability rate and animal performance (Rabelo et al., 2017; Schmidt et al., 2014). This obligate heterolactic acid bacterium increasing acetic acid concentration and decreasing yeast and mould of silage; however, the effects are strain-specific and dose-dependent (Muck et al., 2018).

Currently there is a need for improvement of inoculants based on *L. buchneri* over the rapidity of acetic acid production and its effects on aerobic stability, because producers often must feed forages within weeks or even days of ensiling. The current *L. buchneri* on the market take approximately 45 to 60 d before substantially improving aerobic stability. Identifying strains that would improve aerobic stability earlier in the ensiling process would be helpful (Muck et al., 2018).

This study aimed to investigate the changes in microbial population, DM recovery and fermentation profile that occur in maize silage with or without inoculation with *L. buchneri* after 45 days of ensiling.

2 Materials and Methods

The experiment was conducted at the Department of Animal Science of the Federal University of Viçosa (Universidade Federal de Viçosa – UFV, Viçosa, Minas Gerais, Brazil) between March and April 2015. Viçosa is located at 20°45' South latitude, 42°51'West longitude and 657 meters above sea level.

Maize plants were harvested with the kernels at hard dough stage of maturity. Whole plants were manually harvested and chopped at 2 cm approximately of cut length using a JF-92 Z10 forage harvester (JF Agricultural Machinery, SP, Brazil). The plants characteristics before ensiling are shown in Table 1.

Table 1. Plant characterization before ensiling

Tabela 1. Caracterização da planta antes da ensilagem

Item	Whole maize plant
Dry matter (% of FM[1])	35.5
Water-soluble carbohydrates (% of DM[2])	9.41
pH	5.76
Lactic acid bacteria (log cfu/g of FM)	6.87
Yeasts and molds (log cfu/g of FM)	5.86

[1]Fresh matter.

[2]Dry matter.

The wild *Lactobacillus buchneri* strains isolated from tropical maize silage were identified according to Silva et al. (2018). Four strains of facultative heterofermentative *L. buchneri* (56.22, 56.27, 56.28 and 56.29) were used as inoculants. For all treatments, the theoretical application rate was 1.0×10^6 colony-forming units (cfu) per g of fresh weight, applied through 70 ml of cooled distilled water in 8 kg of chopped fresh forage. Maize silage without inoculant were used and applied just 70 ml of cooled-distilled water (control).

Inoculants were cultured in de Man, Rogosa and Sharpe (MRS, Difco, São Paulo, Brazil) broth for 16 h, and then the inoculum was standardized using a spectrophotometer (630 nm) at an optical density of 0.05, into 20 ml of MRS broth and cultured for 12 h. This schedule was obtained after the growth rate evaluation, which showed the maximum number of cells after incubation of 12 h. With this, the amount of inoculum needed to reach 8.0×10^9 cfu/g was obtained. The amount of inoculum was centrifuged at 1,000 g × 10 min and the supernatant discarded. Cells were resuspended with 70 ml distilled water and applied to achieve the final concentration of 1.0×10^6 cfu/g of fresh forage. Cells number was checked by cell counting using drop plate.

Chopped forage was mixed either with the inoculants or with cooled water (control) and approximately 500 g of treated material were conditioned in nylon-polyethylene bags and vacuum sealed (25 × 35 cm; Doug Care Equipment Inc., Springville, CA; Eco vacuum 1040, Orved, Italy). Four mini-silos (replicates) were prepared for each treatment. Mini-silos were stored at room temperature (25 ± 2°C) and opened after 45 d.

The pre- and post-ensiling samples were used to determination of DM (at 105°C for 18 hr), and the water extract was prepared for chemical analysis and microbial count. Water extracts from the silages and fresh forage samples were prepared by homogenizing 25 g of sample in 225 ml of sterile Ringer's solution (Oxoid, Hampshire, England) in an industrial blender for 1 min, and divided in two portions. One portion was subjected to serial dilutions ranging from 10^{-1} to 10^{-10} for microbial analysis. Pour plates were prepared with MRS (Difco, São Paulo, Brazil) agar for LAB, and Potato Dextrose Agar (PDA; Difco, São Paulo, Brazil) containing 1.5% of tartaric acid solution (10% w./v.) for yeast and mould (Y&M). The MRS plates were incubated at 37°C for 48 h in the anaerobic jars (Permution, Curitiba, PR, Brazil). The PDA plates were incubated aerobically at 25°C for 5 d. All colonies were counted on plates with 25–250 well-isolated colony-forming units.

In the other water-extract portion, the pH was measured using a potentiometer (Tecnal, SP, Brazil). After this, the water extract was filtered through Whatman 54 filter paper (Whatman, Florham, NJ), and 10 ml was acidified with 1:1 H_2SO_4 diluted with distilled water for the further chemical analysis. The filtered and acidified water extracts were analyzed for water-soluble carbohydrates (WSC) using glucose (Sigma-Aldrich, São Paulo, Brazil) to make the standard curve (Nelson, 1944). One millilitre of the acidified extract was centrifuged at 10,000 g × 15 min, and subsequently analyzed for lactic acid, acetic acid, propionic acid, butyric acid and ethanol by high-performance liquid chromatography (HPLC; SPD-10 AVP, Shimadzu, OR, USA) (Siegfried et al., 1984). The HPLC apparatus was equipped with a refractive index detector and used an Aminex HPX-87H column (BIO-RAD, CA, USA) with the mobile phase containing 0.005 M sulphuric acid, and a flow rate of 0.6 ml/min for organic acids and of 1.0 ml/min for ethanol, at 50°C.

Apparent DM recovery was calculated using the weight and DM content of the fresh forage and silage (Jobim et al., 2007). The DM content was corrected for volatile compounds according to Weißbach and Strubelt (2008).

Data from the silo openings were analyzed as a completely randomized design, with four replicates per treatment (inoculants). All microbial counts were converted into the logarithmic base (log10 cfu). Variance analysis was performed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA) and the least squares means were compared by Tukey's test ($\alpha \leq 0.05$).

3 Results

Selected strains did not affect the DM content, yeast and mould population, DM recovery (Table 2), WSC, lactic acid and butyric acid (Table 3) of maize silage after 45 d of ensiling ($p > 0.050$).

The pH, LAB population and concentrations of acetic and propionic acids and ethanol were affected by inoculants ($p < 0.050$). The strains 56.22, 56.27 and 56.28 showed lower pH than the untreated control silage. The LAB population was lower for the silage treated with the strain 56.29 in comparison with the others treatments ($p < 0.001$; Table 2).

Table 2. The dry matter content (DM), pH, number of lactic acid bacteria (log cfu g^{-1} of FM), number of yeasts and molds (log cfu g^{-1} of FM), and DM recovery of maize silage treated with *Lactobacillus buchneri* strains after 45 d of ensiling

Tabela 2. Teores de matéria seca (MS), pH, número de bactérias ácido lácticas (log ufc g^{-1} de FM), número de leveduras e bolores (log ufc g^{-1} de FM) e recuperação de MS das silagens de milho tratadas com cepas de *Lactobacillus buchneri* após 45 dias de ensilagem

Item	Control	*L. buchneri* strains				SEM[1]	*P-value*
		56.22	56.27	56.28	56.29		
Dry matter (% of FM[2])	35.9	35.5	35.8	35.6	36.2	0.114	0.265
pH	3.72[a]	3.67[c]	3.68[c]	3.69[bc]	3.71[ab]	0.004	<.001
Lactic acid bacteria	6.21[ab]	6.26[a]	6.35[a]	6.09[ab]	5.70[b]	0.123	<.001
Yeast and mould	5.00	5.52	5.87	5.77	5.78	0.108	0.530
DM recovery (%)	99.4	98.9	98.7	98.4	99.8	0.217	0.464

[1] Standard error of mean.

[2] Fresh matter.

[a–c] Means with different letters within a row differ ($p < 0.050$).

All strains decreased the concentration of acetic acid in comparison with the untreated control silage ($p = 0.002$). The butyric acid concentration was lower for strains 56.28 and 56.29 ($p = 0.001$). All strains increased the concentration of ethanol in comparison with the untreated control silage ($p = 0.024$; Table 3).

Table 3. The chemical composition (% of dry matter) of maize silages treated with *Lactobacillus buchneri* strains after 45 d of ensiling

Tabela 3. Composição química (% da matéria seca) das silagens de milho tratadas com cepas de *Lactobacillus buchneri* após 45 dias de ensilagem

Item	Control	*L. buchneri* strains				SEM[1]	*P*-value
		56.22	56.27	56.28	56.29		
WSC[2]	3.01	3.22	3.47	2.98	2.29	0.15	0.156
Lactic acid	3.58	3.95	4.11	3.64	3.55	0.072	0.392
Acetic acid	0.901[a]	0.805[b]	0.810[b]	0.753[c]	0.722[c]	0.018	0.002
Propionic acid	1.22[a]	0.986[ab]	1.02[ab]	0.900[b]	0.921[b]	0.032	0.001
Butyric acid	0.022	0.026	0.022	0.036	0.025	0.003	0.344
Ethanol	0.383[b]	0.844[ab]	1.03[a]	0.936[ab]	0.772[ab]	0.093	0.024

[1]Standard error of mean.

[2]Water-soluble carbohydrates.

[a–c]Means with different letters within a row differ ($p < 0.050$).

4 Discussion

In our study, the DM and WSC contents of fresh chopped maize (35.5 and 9.41 g/kg, respectively; Table 1) at ensiling were considered adequate for ensiling process (McDonald et al., 1991). All silages showed typical fermentation pattern and low DM loss, which is expected in maize silage due to its concentrations of DM and WSC in the fresh crop. In short, the maize silages inoculated with the *L. buchneri* strains had lower pH, lower acetic acid and greater ethanol concentrations in comparison with the untreated control silage.

The average values of pH observed in the present study for all silages (3.67 to 3.72) range in the pH interval considered adequate for maize silage (Kung et al., 2018). However, the inoculated silages with the strains 56.22, 56.27 and 56.28 showed a slight decrease in the pH when compared to the untreated control silage. It was not expected because some *L. buchneri* strains can degrade lactic acid into acetic acid raising the pH, but this only occurs when glucose is depleted from growing medium (Driehuis et al., 1999; Oude Elferink et al., 2001). In our study, the strains probably produced lactic acid and ethanol instead of acetic acid.

Although the inoculant has not increased the acetic acid concentration, which would be desirable, there is evidence that the strains 56.22 and 56.27 survived due to a slight increase in LAB count observed in these inoculated silages. However, the low LAB population in the inoculated silage with the strain 56.29 probably was due to the fact that in acid conditions some LAB may decrease viability, and just specialized LAB can remain active (Oude Elferink et al., 2000); it is related to resistance of LAB to acidic conditions (Assis et al., 2014; Li & Nishino, 2011).

Regarding the storage time, according to Muck et al. (2018) greater concentrations of acetic acid in silages treated with *L. buchneri* are observed from 56 d of ensiling onward. In high moisture corn, Taylor and Kung (2002), with inoculation of *L. buchneri,* report an increase in the acetic acid concentration from the storage length of 281 d. However, Driehuis et al. (1999) applying *L. buchneri* on maize silage, at 14 d, did not observed any difference in concentration acetic acid between treated and untreated control, but at 28 d acetic acid was increasing and lactic acid was beginning to decrease in the *L. buchneri* treatment.

In our study, an important factor is that the population of yeast and mould in the fresh forage were greater than other studies with maize silage (Assis et al., 2014; Filya, 2003; Zhou et al., 2016). This difference in population could be attributed to factors such as crop maturity stage and environmental conditions. A high population of yeast and mould before ensiling may be a reason why the treatments had no effect in this variable on silage. An unexpected fact was the increased concentration of ethanol in inoculated silages, but some strains of heterofermentative LAB, such as *L. buchneri*, can convert sugars into ethanol (Liu et al., 2008). However, the ethanol contents of silages in this study (0.38 to 1.03% of DM) are in the interval acceptable (Kung et al., 2018; Li & Nishino, 2011).

Since the inoculants were isolated from maize crop, probably they exist quite frequently also in the untreated control maize silage. Thus, this also can be a reason for the absence of improvements in maize silage. Besides the ensiling process has been done properly and the fermentation is appropriate naturally. However, future research with these wild strains should consider higher application rates, in addition to longer silage storage time to verify its real effects.

5 Conclusion

These wild strains of *L. buchneri* are not indicated to increase the acetic acid concentration and possibly the aerobic stability of maize silage with 45 d of storage. However, they promoted a pH reduction.

References

ASSIS, F. G. V.; ÁVILA, C. L. S.; PINTO, J. C.; SCHWAN, R. F. New inoculants on maize silage fermentation. *Revista Brasileira de Zootecnia*, Viçosa, v. 43, n. 8, p. 395-403, 2014. http://dx.doi.org/10.1590/S1516-35982014000800001

CHIBA, S.; CHIBA, H.; YAGI, M. *A guide for silage making and utilization in the tropical regions*. Tokyo: Japanese Livestock Technology Association, 2005. 29 p.

DOONAN, B. M.; KAISER, A. G.; STANLEY, D. F.; BLACKWOOD, I. F.; PILTZ, J. W.; WHITE, A. K. Silage in the farming system. In: KAISER, A. G.; PILTZ, J. W.; BURNS, H. M.; GRIFFITHS, N. W. (Ed.). *Successful silage*. Ottawa: NSW Agriculture, 2004. p. 1-24.

DRIEHUIS, F.; OUDE ELFERINK, S. J.; SPOELSTRA, S. F. 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*, Hoboken, v. 87, n. 4, p. 583-594, 1999. https://doi.org/10.1046/j.1365-2672.1999.00856.x

DRIEHUIS, F.; WILKINSON, J. M.; JIANG, Y.; OGUNADE, I.; ADESOGAN, A. T. Silage review: animal and human health risks from silage. *Journal Dairy Science*, Amsterdam, v. 101, n. 5, p. 4093-4110, 2018. https://doi.org/10.3168/jds.2017-13728

FILYA, I. 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*, Hoboken, v. 95, n. 5, p. 1080-1086, 2003. https://doi.org/10.1046/j.1365-2672.2003.02081.x

JOBIM, C. C.; NUSSIO, L. G.; REIS, R. A.; SCHMIDT, P. Methodological advances in evaluation of preserved forage quality. *Revista Brasileira de Zootecnia*, Viçosa, v. 36, suppl., p. 101-119, 2007. http://dx.doi.org/10.1590/S1516-35982007001000013

KUNG JR, L.; SHAVER, R. D.; GRANT, R. J.; SCHMIDT, R. J. Silage review: interpretation of chemical, microbial, and organoleptic components of silages. *Journal Dairy Science*, Amsterdam, v. 101, n. 5, p. 4020-4033, 2018. https://doi.org/10.3168/jds.2017-13909

LI, Y.; NISHINO, N. Monitoring the bacterial community of maize silage stored in a bunker silo inoculated with *Enterococcus faecium*, *Lactobacillus plantarum* and *Lactobacillus buchneri*. *Journal of Applied Microbiology*, Hoboken, v. 110, n. 6, p. 1561-1570, 2011. https://doi.org/10.1111/j.1365-2672.2011.05010.x

LIU, S.; SKINNER-NEMEC, K. A.; LEATHERS, T. D. *Lactobacillus buchneri* strain NRRL B-30929 converts a concentrated mixture of xylose and glucose into ethanol and other products. *Journal of Industrial*

Microbiology and Biotechnology, Berlin, v. 35, n. 2, p. 75-81, 2008. https://doi.org/10.1007/s10295-007-0267-8

MCDONALD, P.; HENDERSON, A. R.; HERON, S. J. E. *The biochemistry of silage*. Marlow: Chalcombe, 1991. 340 p.

MUCK, R. E.; NADEAU, E. M. G.; MCALLISTER, T. A.; CONTRERAS-GOVEA, F. E.; SANTOS, M. C.; KUNG JUNIOR, L. Silage review: recent advances and future uses of silage additives. *Journal Dairy Science*, Amsterdam, v. 101, n. 5, p. 3980-4000, 2018. https://doi.org/10.3168/jds.2017-13839

NELSON, N. A photometric adaptation of the Somogyi method for the determination of glucose. *Journal of Biological Chemistry*, v. 153, p. 375-380, 1944.

OUDE ELFERINK, S. J. W. H.; DRIEHUIS, F.; GOTTSCHAL, J. C.; SPOELSTRA, S. F. Silage fermentation processes and their manipulation. In: ELECTRONIC CONFERENCE ON TROPICAL SILAGE, 2000, Roma. *Proceedings*… Roma: FAO. p. 17-30.

OUDE ELFERINK, S. J. W. H.; KROONEMAN, J.; GOTTSCHAL, J. C.; SPOELSTRA, S. F.; FABER, F.; DRIEHUIS, F. Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by *Lactobacillus buchneri*. *Applied and Environmental Microbiology*, Washington, DC, v. 67, n. 1, p. 125-132, 2001. https://doi.org/10.1128/AEM.67.1.125-132.2001

RABELO, C. H. S.; BASSO, F. C.; LARA, E. C.; JORGE, L. G. O.; HÄRTER, C. J.; MARI, L. J.; REIS, R. A. Effects of *Lactobacillus buchneri* as a silage inoculant or probiotic on in vitro organic matter digestibility, gas production and volatile fatty acids of low dry-matter whole-crop maize silage. *Grass and Forage Science*, Hoboken, v. 72, n. 3, p. 534-544, 2017. https://doi.org/10.1111/gfs.12273

SCHMIDT, P.; NUSSIO, L. G.; QUEIROZ, O. C. M.; SANTOS, M. C.; ZOPOLLATTO, M.; TOLEDO FILHO, S. G.; DANIEL, J. L. P. Effects of *Lactobacillus buchneri* on the nutritive value of sugarcane silage for finishing beef bulls. *Revista Brasileira de Zootecnia*, Viçosa, v. 43, n. 1, p. 8-13, 2014. http://dx.doi.org/10.1590/S1516-35982014000100002

SIEGFRIED, V. R.; RUCKEMANN, H.; STUMPF, G. Method for the determination of organic acids in silage by high performance liquid chromatography. *Landwirtschaftliche Forsch*, Berlin, v. 37, p. 298-304, 1984.

SILVA, L. D.; PEREIRA, O. G.; SILVA, T. C. LEANDRO, E. S.; PAULA, R. A.; SANTOS, S. A.; RIBEIRO, K. G.; VALADARES FILHO, S. C. Effects of *Lactobacillus buchneri* isolated from tropical maize silage on fermentation and aerobic stability of maize and sugarcane silages. *Grass and Forage Science*, Hoboken, v. 73, n. 3, p. 660-670, 2018. https://doi.org/10.1111/gfs.12360

TAYLOR, C. C.; KUNG JUNIOR. L. The effect of *Lactobacillus buchneri* 40788 on the fermentation and aerobic stability of high moisture corn in laboratory silos. *Journal Dairy Science*, Amsterdam, v. 85, n. 6, p. 1526-1532, 2002. https://doi.org/10.3168/jds.S0022-0302(02)74222-7

WEIßBACH, F.; STRUBELT, C. Correcting the dry matter content of maize silages as a substrate for biogas production. *Landtechnik*, Darmstadt, v. 63, n. 4, p. 82-83, 2008. http://dx.doi.org/10.15150/lt.2008.818

ZHOU, Y.; DROUIN, P.; LAFRENIÈRE, C. Effect of temperature (5°C-25°C) on epiphytic lactic acid bacteria populations and fermentation of whole-plant corn silage. *Journal of Applied Microbiology*, Hoboken, v. 121, n. 3, p. 657-671, 2016. https://doi.org/10.1111/jam.13198

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