



Effect of inoculants combining *Lactobacillus buchneri* strain LN40177 in different strata of the silo

Tânia Mara Becher Ribas¹, Mikael Neumann¹, André Martins de Souza¹, André Dochwat¹, Eduardo Rodrigues de Almeida¹ and Egon Henrique Horst^{2*} 

¹Universidade Estadual do Centro-Oeste, Guarapuava, Paraná, Brazil. ²Universidade Estadual de Londrina, Rodovia Celso Garcia Cid, 445, Km 380, 86057-970, Londrina, Paraná, Brazil. *Author for correspondence. E-mail: egonhh@yahoo.com.br

ABSTRACT. The objective of this work was to evaluate the chemical composition and rumen disappearance rate of dry matter of corn silages with inoculants combining *L. buchneri* strain LN40177 in different strata of the silo. The experimental design was a 3x2 randomized complete block design, with three treatments: Control: corn silage without inoculant; 11CFT: corn silage with inoculant which combines *L. buchneri* strain LN40177 (1.1×10^{11} CFU g⁻¹) with *L. casei* (1.1×10^{11} CFU g⁻¹); and 11C33: corn silage with inoculant which combines *L. buchneri* strain LN40177 (1.1×10^{11} CFU g⁻¹) with *L. plantarum* (1.1×10^{11} CFU g⁻¹) and *Enterococcus faecium* (1×10^{10} CFU g⁻¹), associated with two strata of the silo (lower and upper). The silage inoculated with 11C33 presented higher contents of crude protein and NDF and lower hemicellulose content in relation to the control treatment and 11CFT. The use of both inoculants resulted in silages with higher concentrations of soluble nutrients. Lower stratum silage had a higher rumen disappearance rate of dry matter compared to the upper stratum. In general, the combinations of *L. buchneri* promoted nutritional improvements in corn silage, but in presence of *L. casei*, there were more outstanding improvements.

Keywords: bromatology; corn silage; *Enterococcus faecium*; ruminal digestibility.

Received on October 3, 2018.
Accepted on November 1, 2018.

Introduction

The preservation of silage nutrients depends on the lack of oxygen for adequate development of lactic acid bacteria, which will use, as a matter of priority, soluble carbohydrates to synthesize organic acids. Depending on species of lactic acid bacteria present in the medium, the type of fermentation may differ and vary mainly by the different concentrations of acetic and lactic acid (Tabacco, Piano, Revello-Chion, & Borreani, 2011).

Commercial bacterial inoculants have mostly lactic acid bacteria species, and *Lactobacillus plantarum* and *L. buchneri* are the most frequently used, besides *L. casei* and other species such as *Pediococcus faecium* and *Enterococcus faecium* (Muck, 2010). The main function of these inoculants is to promote higher concentrations of organic acids during fermentation, inhibiting the growth and/or presence of spoilage microorganisms. However, studies have demonstrated the potential of certain strains of *L. buchneri* to produce enzymes that degrade fiber and improve its utilization (Lynch, Baah, & Beauchemin, 2015; Tabacco et al., 2011).

LN40177 strain has recently been registered and needs to be investigated for its use in combination with other lactic acid bacteria. Oliveira et al. (2017) point out that different combination of lactic acid bacteria in inoculants may increase the benefits to silage, but these combinations require constant evaluation.

Silva, Jobim, Poppi, Tres, and Osmari (2015) mentioned that about 65% of silage producers in southern Brazil use bacterial inoculants during the manufacturing, however, the success of this technology is linked to other points, such as mass (Zopollatto, Daniel, & Nussio, 2009), which normally differs between silo strata and is accompanied by differences in the nutritional quality of silage. The concentration of fiber may also interfere with the mentioned factors, so it is expected that changes from the bacterial action will restrict such distinctions.

In this context, the objective was to evaluate the rumen disappearance and rumen disappearance rate of dry matter of silages with inoculants combining *L. buchneri* strain LN40177 in the upper and lower strata of the silo.

Material and methods

Two bacterial inoculants were tested at the Animal Production Center (NUPRAN) of the State University of Central-West (UNICENTRO) in Guarapuava, State of Paraná, Brazil, to evaluate the efficiency of *Lactobacillus buchneri* strain LN40177 combined with distinct heterofermentative bacteria. The inoculants were diluted in water and applied by means of a spray nozzle located in the forage trough of silage to harvest 20×10^{10} CFU g^{-1} of product per ton of fresh biomass. Harvesting of hybrid maize (P2866H; Pioneer®) was performed in a hard grain stage, with 42% dry matter and grains presenting around $\frac{2}{3}$ of the milk line.

The silages were accommodated in three trench silos, one for each treatment, with dimensions of 15 m in length, 4 m in width and 1.2 m in height. During the filling, samples contained inside bags of known weight were deposited in the upper stratum and in the lower stratum of the silos. The designation bags refers to a 100% polyamine malleable nylon bag with 85 micrometer pores, 12 cm x 50 cm in diameter and length, respectively. Four bags were placed in the lower layer (0.4 m high) and four bags in the upper layer (0.8 m high) of the silo, remaining centralized in relation to the side walls of each silo. The opening of the silos occurred simultaneously, at 160 days after ensiling. Samples similar to those packaged in the bags were analyzed immediately after harvest.

The experimental design was a randomized block design, with the input weight of the animals being the criterion used for blocking, in a 3x2 factorial scheme, with three treatments: Control: corn silage without inoculant; 11CFT: corn silage with Pioneer® brand 11CFT inoculant, which combines *L. buchneri* strain LN40177 (1.1×10^{11} CFU g^{-1}) with *L. casei* (1.1×10^{11} CFU g^{-1}); and 11C33: corn silage with Pioneer® brand 11C33 inoculant, which combines *L. buchneri* strain LN40177 (1.1×10^{11} CFU g^{-1}) with *L. plantarum* (1.1×10^{11} CFU g^{-1}) and *Enterococcus faecium* (1×10^{10} CFU g^{-1}), associated with two strata of the silo (lower and upper). After opening silo for supply to animals, samples of the recovered material in each bag were pre-dried in forced air oven at 55° C for 72 hours for determination of the partially dried matter and milled in a Wiley mill with a 1 mm mesh sieve. The total dry matter (DM) in the oven at 105° C and crude protein (CP) by the micro Kjeldahl method, according to Association Official Analytical Chemist (AOAC, 2005) were determined in the pre-dried samples. Neutral detergent fiber contents (aNDF) were determined according to Van Soest, Robertson, and Lewis (1991) using thermostable α -amylase and acid detergent fiber (ADF) and lignin contents according to Goering and Van Soest (1970). The hemicellulose contents were estimated by the difference between aNDF and ADF, as well as the cellulose contents were estimated by the difference between ADF and lignin.

The rumen disappearance rate of the dry matter was estimated by *in situ* technique using nylon bags measuring 12x8 cm and with 40 – 60 μ m pores, containing approximately 5 g of each dry material and ground to 1 mm, for later incubation in the rumen (Nocek, 1988). The incubation times used were 0, 6, 12, 18, 24, 36 and 48 hours. For this purpose, two 36-month-old steers, average body weight of 650 kg, with ruminal cannula were used. Eight replications were performed per treatment, four of them referring to the upper stratum and four to the lower stratum.

Data were subjected to the Shapiro-Wilk and Bartlett tests to check the assumptions of normality and homogeneity of variance, respectively. Once these assumptions were met, the F-test was applied through analysis of variance (ANOVA) and then the Tukey's test at 5% and 1% of significance. Data referring to the rumen disappearance rate were subjected to polynomial regression analysis, considering the variable hours of evaluation, using the "procreg" procedure of the Statistical Analysis System (SAS, 2004) software (v. 9.1.2, SAS Institute Inc., Cary, NC).

Results and discussion

Regardless of using inoculant, the dry matter content of the silage differed between the silo strata, with higher moisture present in the silage of the lower stratum (38.7% vs. 40.5%; Table 1) due to the gravitational effect of part of the water released by cellular disruption and organic acids produced during the fermentation process. The use of inoculants did not affect the dry matter content of the silage, as well as no variations were detected in the contents of ash, ADF, cellulose, lignin and *in situ* dry matter digestibility (ISDDM) in 24 hours.

Table 1. Chemical composition of corn silage inoculated with *Lactobacillus buchneri* in different combinations, according to the silo stratum.

Variable	Upper stratum			Lower stratum			CV	Probability		
	Control	11CFT	11C33	Control	11CFT	11C33		I	E	I x E
Dry matter	41.21	41.13	39.12	41.49	35.69	38.96	5.26	ns	*	ns
Ash	2.47	2.27	2.42	2.52	2.19	2.58	9.55	ns	ns	ns
Crude protein	6.06	5.16	6.77	5.58	5.73	5.64	7.02	*	ns	**
aNDF	45.44	46.84	47.11	38.04	39.49	46.89	8.05	**	**	ns
Hemicellulose	15.58	19.63	22.57	13.18	14.97	22.45	17.28	**	ns	ns
ADF	29.86	27.21	26.47	24.86	24.52	26.24	7.31	ns	**	ns
Cellulose	24.09	22.09	21.21	20.97	20.67	21.99	8.39	ns	ns	ns
Lignin	5.77	5.13	5.33	3.89	3.85	4.25	10.65	ns	**	ns
ISDDM24	54.06	53.15	49.36	56.50	59.08	54.75	10.74	ns	*	ns
ISDDM48	62.73	70.93	65.03	63.25	68.81	66.24	5.49	**	ns	ns

* p < 0.05; ** p < 0.01; ns: non-significant; I: inoculant; E: silo stratum. Control: corn silage without inoculant; 11CFT: corn silage with inoculant which combines *L. buchneri* strain LN40177 (1.1×10^{11} CFU g⁻¹) with *L. casei* (1.1×10^{11} CFU g⁻¹); and 11C33: corn silage with inoculant which combines *L. buchneri* strain LN40177 (1.1×10^{11} CFU g⁻¹) with *L. plantarum* (1.1×10^{11} CFU g⁻¹) and *Enterococcus faecium* (1×10^{10} CFU g⁻¹).

The silage of the upper stratum showed higher contents of aNDF, FDF and lignin (48.92, 27.02 and 5.41%, respectively) in relation to the lower stratum silage (43.7, 25.21 and 4.00%, respectively). These results may be due to a dilution effect, in which soluble carbohydrates and soluble nitrogen are displaced to the lower part of the silo together with the water mobilization, concentrating the fiber fractions in the upper stratum. These results are similar to those described by Zanette et al. (2012) and Lynch et al. (2015).

The use of 11C33 inoculant provided higher contents of aNDF and hemicellulose (48.9 and 22.5%) when compared to 11CFT inoculant (43.2 and 17.3%) and control silage (41.7, 14.4%). However, this higher value of aNDF found in the silage with 11C33 inoculant cannot be taken into account in isolation, since this fraction had a high concentration of hemicellulose in both silages with inoculant, being the most digestible part of the fiber and easily hydrolyzed. Tabacco et al. (2011) also reported improvements in the NDF quality of silages inoculated with new strains of *L. buchneri*, highlighting the capacity of producing fibrolytic enzymes of these microorganisms.

There was significant interaction between inoculant and silo strata for the crude protein content of the different silages, in which the silage of the upper stratum with 11C33 inoculant showed the highest value, and the silage of the same stratum with 11CFT inoculant had the lowest value (6.8 and 5.2%, respectively). It is possible to infer that the crude protein content of the silage suffers greater interference from the use of bacterial inoculants according to the stratum of the silo in which the silage is found. The similarity in the protein content between the strata of the silo is fundamental (Marquardt, Jobim, Bueno, & Ribeiro, 2017), mainly in properties that use small amounts of silage daily and do not have mixers.

Regardless of the use of inoculant, ISDDM in 24 hours was higher for lower stratum silage than for upper stratum silage (56.8 vs. 52.2%), but this behavior was not the same for the ISDDM in 48 hours (66.1 and 66.2%, respectively), suggesting that there was a higher concentration of soluble nutrients carried with water to the lower stratum, as already described, which are rapidly fermented. However, the efficacy of the non-soluble fractions allowed similar digestibility after longer incubation time.

The ISDDM in 24 hours showed no difference between inoculant use, but the ISDDM in 48 hours of the 11CFT inoculant silage had superior digestibility in relation to the control silage (69.9 vs. 62.99%), without statistical difference of both from the silage with 11C33 inoculant (65.6%). The *L. buchneri* strain LN40177 used in the present assay is a producer of ferulate-esterase enzymes, which according to Lynch et al. (2015) break the fiber structure during the silage fermentation process. Accordingly, Tabacco et al. (2011) related the use of *L. buchneri* with changes in fiber structure and the increase in digestibility provided by these alterations.

The use of the 11CFT inoculant provided a higher rumen disappearance rate of the silage dry matter of both strata of the silo, being 0.81% h⁻¹ for the upper stratum and 0.59% h⁻¹ for the lower stratum (Figure 1). As no difference was found between the strata for these variables, the data were not presented.

Control silage presented the highest rumen disappearance rate of dry matter, but these results are related to the lower concentration of soluble nutrients represented by the intercept of the curve on the y axis of the graph. Nsereko, Smiley, and Rutherford (2008) emphasize that enzymatic degradation of fiber compounds during silo fermentation increases soluble nutrients and fiber utilization.

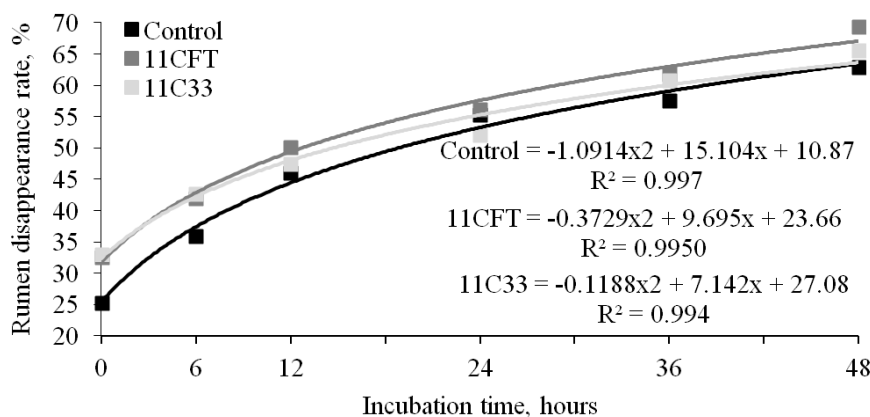


Figure 1. Rumen disappearance rate of dry matter of corn silage inoculated with *Lactobacillus buchneri* in different combinations. Control: corn silage without inoculant; 11CFT: corn silage with inoculant which combines *L. buchneri* strain LN40177 (1.1×10^{11} CFU g^{-1}) with *L. casei* (1.1×10^{11} CFU g^{-1}); and 11C33: corn silage with inoculant which combines *L. buchneri* strain LN40177 (1.1×10^{11} CFU g^{-1}) with *L. plantarum* (1.1×10^{11} CFU g^{-1}) and *Enterococcus faecium* (1×10^{10} CFU g^{-1}).

Regardless of the rumen disappearance rate, the silage inoculated with 11CFT showed ruminal digestibility at the end of the 48 hours of evaluation superior to the silage inoculated with 11C33, an effect promoted by the lower concentration of aNDF of the silage when *L. buchneri* is combined with *L. casei*, having a high lactic acid production potential that assists in fiber hydrolysis and lowers the pH of the ensiled mass to levels adequate for the performance of enzymes produced by the former.

Conclusion

The silage of the lower stratum of the silo presented lower concentrations of fiber and lignin in relation to the upper stratum, leading to improvements in dry matter digestibility in 24 hours.

The combinations of *L. buchneri* resulted in nutritional improvements in corn silage, however, in the presence of *L. casei*, there was a greater reduction in aNDF content, increasing rumen disappearance of dry matter. Therefore, inoculants with these combinations may be indicated as corn silage quality enhancers.

References

- Association Official Analytical Chemist [AOAC]. (2005). *Official Methods of Analysis* (18th ed.). Gaithersburg, MD: AOAC International.
- Goering, H. K., & Van Soest, P. J. (1970). *Forage Fiber Analysis. USDA Agricultural Research Service. Handbook number 379* (16th ed.). Washington, DC: US Government Printing Office.
- Lynch, J. P., Baah, J., & Beauchemin, D. K. A. (2015). Conservation, fiber digestibility, and nutritive value of corn harvested at 2 cutting heights and ensiled with fibrolytic enzymes, either alone or with a ferulic acid esterase-producing inoculant. *Journal of Dairy Science*, *98*(1214-1224). doi: 10.3168/jds.2014-8768.
- Marquardt, F. I., Jobim, C. C., Bueno, A. V. I., & Ribeiro, M. G. (2017). Altura de corte e adiç o de inoculante enzimbacteriano na composiç o qu mico-bromatol gica e digestibilidade de silagens de milho avaliada em ovinos. *Ci ncia Animal Brasileira*, *18*, 1-9. doi: 10.1590/1089-6891v18e-42888.
- Muck, R. E. (2010). Silage microbiology and its control through additives. *Revista Brasileira de Zootecnia*, *39*, 183-191. doi: 10.1590/S1516-35982010001300021.
- Nocek, J. E. (1988). *In situ* and other methods to estimate ruminal protein and energy digestibility: a review. *Journal of Dairy Science*, *71*(8), 2051-2069. doi: 10.3168/jds.S0022-0302(88)79781-7.
- Nsereko, V. L., Smiley, B. K., & Rutherford, W. M. (2008). Influence of inoculating forage with lactic acid bacterial strains that produce ferulate esterase on ensilage and ruminal degradation of fiber. *Animal Feed Science and Technology*, *145*, 122-135. doi: 10.1016/j.anifeeds.2007.06.039
- Oliveira, A. S., Weinberg, Z. G., Ogunade, I. M., Cervantes, A. A., Arriola, K. G., Jiang, Y., & Adesogan, A. T. (2017). Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. *Journal of Dairy Science*, *100*(6), 4587-4603. doi: 10.3168/jds.2016-11815.

- Statistical Analysis System [SAS]. (2004). *SAS/STAT User guide, Version 9.1.2*. Cary, NC: SAS Institute Inc.
- Silva, M. S. J., Jobim, C. C., Poppi, E. C., Tres, T. T., & Osmari, M. P. (2015). Production technology and quality of corn silage for feeding dairy cattle in Southern Brazil. *Revista Brasileira de Zootecnia*, *44*(9), 303-313. doi: 10.1590/S1806-92902015000900001.
- Tabacco, E., Piano, S., Revello-Chion, A., & Borreani, G. (2011). Effect of *Lactobacillus buchneri* LN4637 and *Lactobacillus buchneri* LN40177 on the aerobic stability, fermentation products, and microbial populations of corn silage under farm conditions. *Journal of Dairy Science*, *94*(11), 5589-5598. doi: 10.3168/jds.2011-4286.
- Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, *74*(10), 3583-3597. doi: 10.3168/jds.S0022-0302(91)78551-2.
- Zanette, P. M., Neumann, M., Faria, M. V., Ueno, R. K., Marafon, F., & Durman, T. (2012). Valor nutricional e perdas durante a fermentação de silagens de milho (*Zea mays* L) com açúcar ou inoculante. *Revista Brasileira de Milho e Sorgo*, *11*(2), 178-189. doi: 10.18512/1980-6477/rbms.v11n2p178-189.
- Zopollatto, M., Daniel, J. L. P., & Nussio, L. G. (2009). Aditivos microbiológicos em silagens no Brasil: revisão dos aspectos da ensilagem e do desempenho de animais. *Revista Brasileira de Zootecnia*, *38*, 170-189.