Effect of natamycin and *Lactobacillus buchneri* on yeast counting of maize silages during aerobic degradation

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Introduction According to Adesogan and Queiroz (2009) yeasts are the main microorganism to start aerobic degradation of silages, due to its affinity to lactate and glucose. Reich and Kung Jr. (2010) found high acetic acid concentrations and lower yeast counts in silages added with *Lactobacillus buchneri* when compared to control treatments. Natamycin is an antifungal compound used to avoid yeasts and molds in foods, such as cheeses and salami. Recently, Schmidt et al. (2012) verified positive effect of natamycin added to maize silages decreasing gases losses. This trial aimed to evaluate pH and yeast counting of maize silages added with natamycin and *Lactobacillus buchneri*.

Materials and Methods The trial was carried out at the Centro de Pesquisas em Forragicultura (CPFOR) of Federal University of Parana. Maize was harvested by self propelled chopper at a theoretical particle length of 12 mm. Four treatments were applied: Control – no additives (C); *Lactobacillus buchneri* at $5x10^4$ cfu g^{-1} fresh matter (LB); Natamycin at 8 g t^{-1} fresh matter (NA); and *Lactobacillus buchneri* plus natamycin at the same dosages (NLB). Forage was ensiled in 20-liter experimental silos provided with Bunsen valves for gases outflow. Each silo was filled with 12.5 kg of fresh forage, assuring 600 kg m^{-3}. Silos were sealed and stored for 90 days. After opening, samples were collected for pH evaluation and silages were exposed to air at 23±1ºC. For yeast counting, silages were sampled at days 0, 3 and 5 of aerobic exposure (D0, D3 and D5, respectively). Preparation for counting procedure was made by adding 25 g of silage to 225 mL of NaCl solution at 8.5%. The mixture was stirred and filtered, and 2 mL samples were taken for serial dilutions ranging from 10^{-1} to 10^{-7}. Diluted samples were seeded in triplicate on Sabouraud agar added with tartaric acid at 10% (resulting pH of 4.5) by Pour Plate method. The plates were BOD incubated at 26 ºC. From 72 to 144 hours after incubation yeast colonies were counted. Mean values of triplicates were transformed by base 10 logarithm and expressed in colonies forming units per gram of silage (cfu g^{-1}). Experimental design was completely randomized with four replicates. Data were analyzed by ANOVA and means of treatments were compared by Tukey test at a probability of 0.05 using STATISTIX (9.0).

Results and Discussion Means of pH and yeast counts are shown in Table 1. The NLB treatment showed the lowest pH (3.76), which was different from NA and control treatments ($P<0.05$). On day zero, NLB showed lower yeast counting than NA and control treatments ($P<0.05$). Natamycin decreased yeast population and might have enhanced *L. buchneri* fermentation, thus reducing pH and yeast counting of exposed silages. This effect was also verified by Hondrodimou et al. (2011) during lactic fermentation of olives added with natamycin.

On day 3, NLB yeast count was only different from control silage (P<0.05). Schmidt et al. (2012) observed decreased greenhouse gases emission from maize silages added with natamycin, when compared with untreated silages, which was explained by natamycin effect of controlling yeasts growth. In this trial, NA treatment was not effective for avoiding yeast growth, but promoted synergistic effect when added to LB. After five days of aerobic exposure NA showed higher yeast count than control and NLB treatments.

Table 1 pH and yeast population of maize silage aerobically exposed (log cfu g^{-1} of fresh matter).

	Treatments[1]					
Variable[2]	C	NA	LB	NLB	Mean	SEM
D0	2.95^{a}	2.23^{a}	1.92^{ab}	0.26^{b}	1.84	0.31
D3	7.67^{a}	6.63^{ab}	6.47^{ab}	5.99^{b}	6.69	0.23
D5	6.85^{b}	8.74^{a}	7.78^{ab}	6.32^{b}	7.42	0.29
pH	3.85^{a}	3.85^{a}	3.83^{ab}	3.76^{b}	3.8	0.01

[1]C, Control; NA, Natamycin; LB, *Lactobacillus buchneri*; NLB, Natamycin + *L. buchneri*. Different lower case letters within a line differ by Tukey test (P<0.05). [2]Log_{10} (yeast count) on days 0, 3 and 5 after silo opening.

Conclusion The combination of natamycin and *Lactobacillus buchneri* promoted decrease of maize silages pH and inhibit yeast growth after the silo opening until the third day of air exposure.

References

Adesogan, A.T. and Queiroz, O.C.M. 2009. Silage pathogenicity and implications for the ruminant production chain. Proc., International symposium on forage quality and conservation, São Paulo, Brazil, 7-22.

Hondrodimou, O., Kourkoutas, Y. and Panagou, E.Z. 2011. Efficacy of natamycin to control fungal growth in natural black olive fermentation, Food Microbiology, 28, 621-627.

Reich, L.J., Kung Jr. L. 2010. Effects of combining Lactobacillus buchneri 40788 with various lactic acid bacteria on the fermentation and aerobic stability of corn silage, Animal Feed Science and Technology, 159:105-109.

Schmidt, P.; Novinski, C.O.; Carneiro, E.W.; Bayer, C. 2012.Greenhouse gas emissions from fermentation of corn silage. Page 428 in Proc. XVI International Silage Conference. Hämeenlinna, Finland.