Lactobacillus buchneri and storage periods affect the fermentation profile and aerobic stability of corn silage

Adir de Sá Neto, Maity Zopollatto, Álvaro Wosniask Bispo, Daniel Junges, João Luiz Pratti Daniel, Mateus Castilho Santos and Luiz Gustavo Nussio

University of São Paulo, ESALQ, Department of Animal Science Avenida Pádua Dias, 11, Piracicaba - SP, Brazil
Contact email: adirneto@hotmail.com

Keywords: Yeasts, microbial additive, corn silage.

Introduction

Previous studies have shown that the utilization of *Lactobacillus buchneri* reduces the yeast population and improves the aerobic stability of corn silage (Driehuis *et al.* 1996) mainly because of the conversion of lactic acid to acetic acid and 1,2-propanediol (Oude Elferink *et al.* 1998; Driehuis *et al.* 1999). Recent studies have also shown that some microbial processes occur during prolonged storage and this may improve the quality of corn silage. For instance, Hoffman *et al.* (2011) suggested that proteolytic activity was responsible for improvements in starch digestion of high moisture corn over extended periods of ensiling and Kleinschmit and Kung (2006) reported that *L. buchneri* remained fairly active for prolonged periods of time (up to a year) in silage, even under anaerobic conditions at a low pH.

Thus, the objective of this trial was to evaluate the effects of increasing levels of *L. buchneri* and different storage periods on the fermentation profile and aerobic stability of corn silages.

Methods

Pioneer (Pioneer Hi-Bred International, DesMoines, IA) hybrid 30F90 *Bt* was sown in the spring of 2008 at the experimental farm of the University of São Paulo in Piracicaba, Brazil. Whole plant corn was harvested using a Menta Suprema (Menta Mit, Cajuru, Brazil) pull-type harvester when the dry matter content was at approximately 33%. The experiment was carried out in a completely randomized design, with five dosages, four storage periods and four replications, totalizing 80 20-L laboratory-scale silos. The following dosages were applied onto the fresh forage prior to ensiling: (1) control (no additive); (2) *L. buchneri* at a theoretical level of 1×10^5 cfu/g of fresh forage; (3) *L. buchneri* at a theoretical level of 3×10^5 cfu/g of fresh forage; (4) *L. buchneri* at a theoretical level of 5×10^5 cfu/g of fresh forage; and 5) *L. buchneri* at a theoretical level of 1×10^6 cfu/g of fresh forage. The microbial inoculants were mixed in deionized water and applied with a hand sprayer at a rate of 4 mL/kg of fresh forage. Silos were prepared with approximately 14 kg of fresh corn and kept in a closed barn at ambient temperature for 15, 60, 90 and 150 days.

After opening the silo,representative samples were collected and a water extract was prepared for each sample. The concentration of acetic acid was determined by gas chromatograph (Hewlett-Packard 5890 GC, Hewlett-Packard, Avondale, PA). Lactic acid bacteria (LAB) and yeast counts were determined by plating 1-mL aliquots using a 3M Petrifilm aerobic count plate and Petrifilm Yeast and Mold Count plates (The 3M Products, St. Paul, MN), respectively. Aerobic stability (defined as the time (hours) that silage remained stable before a 2°C rise in temperature above the ambient temperature threshold) was determined by loosely adding 3 kg of a representative sample of silage into a 20-L bucket and exposing it to air at 23 ± 1°C. The Mixed procedure of SAS (Version 9.2, SAS Institute Inc., Cary, NC) was used to analyse the data. Mean separation was performed using Tukey's test at the 0.05 level of significance.

Results

The population of LAB and yeasts, acetic acid concentration and aerobic stability were affect by both dosage and storage period (Table 1). Longer storage periods decreased LAB counts, especially for the control silage (approximately 3 log reduction from 15 to 150 days of storage), whereas for treated silages and at the same interval the average reduction in the LAB population was 1.1 log. When silos were opened at 15 days of storage, the inoculation with increasing levels of *L. buchneri* did not affect the yeast counts when compared with control silages. The lower concentration of acetic acid detected at this time point, may explain the lack of response of the microbial additive. On the other hand, at prolonged storage periods (90 and 150 days), the population of yeasts were reduced, especially for silages treated with *L. buchneri* at rates $>5 \times 10^5$ cfu/g of fresh forage. After 90 days of storage, the heterofermentative activity of *L. buchneri* may have resulted in an accumulation of acetic acid and, consequently, these silages were more stable after silo opening.

Conclusion

Longer storage period of corn silages resulted in a microbial profile favourable to the fermentation and aerobic stability after silo opening. Despite a less pronounced increment in the concentration of acetic acid,

Table 1. The influence of levels of *Lactobacillus buchneri* on the nutritive value of corn silage ensiled at different storage periods.

Storage period (days)	Dosage[1]					SEM
	Control	LB 1 × 10^5	LB 3 × 10^5	LB 5 × 10^5	LB 1 × 10^6	
			Lactic acid bacteria (log_{10} CFU/g of fresh forage)			
15	8.28 Ac	8.63 Ab	8.74 Aab	8.92 Aab	8.99 Aa	0.026
60	7.25 A	7.84 A	8.53 AB	8.73 AB	8.44 AB	0.140
90	5.18 Bb	7.82 ABa	8.33 ABa	8.92 ABa	8.51 ABa	0.121
150	5.35 Bb	7.54 Ba	7.83 Ba	7.94 Ba	7.88 Ba	0.043
			Yeast (log_{10} CFU/g of fresh forage)			
15	5.63 Aa	5.48 Aa	5.57 Aa	5.64 Aa	5.52 Aa	0.03
60	4.96 Ba	4.14 Bb	< 3.0	< 3.0	< 3.0	0.40
90	4.83 ABa	4.23 Ba	2.78 Bb	1.86 Bb	1.98 Bb	0.11
150	4.43 Ba	3.84 Ba	2.77 Bb	2.27 Bb	2.33 Bb	0.08
			Acetic acid (% of DM)			
15	0.69 b	0.91 Bab	1.15 Ba	1.32 Ca	1.29 Ca	0.036
60	0.72 d	1.33 ABc	1.62 Ab	2.27 Aa	1.88 ABb	0.029
90	0.98 a	1.05 Ba	1.29 ABa	1.44 BCa	1.59 BCa	0.048
150	1.18 b	1.82 Aab	1.86 Aab	2.10 ABab	2.43 Aa	0.076
			Aerobic stability (hours)			
15	38.1 a	20.6 Bb	21.3 Cb	32.2 Cab	39.3 Ca	1.30
60	44.8 c	52.2 Abc	61.9 Bab	68.0 Ba	64.5 Bab	1.29
90	41.5 b	51.1 ABb	83.0 ABb	173.2 Aa	169.7 Aa	6.48
150	45.9 c	55.0 Ac	96.2 Ab	160.7 Aa	153.6 Aa	2.03

[1] LB 1 × 10^5 = *Lactobacillus buchneri* at a theoretical level of 1 × 10^5 cfu/g of fresh forage; LB 3 × 10^5 = *Lactobacillus buchneri* at a theoretical level of 3 × 10^5 cfu/g of fresh forage; LB 5 × 10^5 = *Lactobacillus buchneri* at a theoretical level of 5 × 10^5 cfu/g of fresh forage; LB 1 × 10^6 = *Lactobacillus buchneri* at a theoretical level of 1 × 10^6 cfu/g of fresh forage. Means within a row without a common lower-case letter following differ (P<0.05). Means within a column without a common upper-case letter differ (P<0.05).

doses of the inoculant higher than 5 × 10^5 cfu/g fresh forage reduced yeast population and maintained the aerobic stability in silages stored for more than 90 days. Higher doses of the inoculant may partly compensate for the shorter storage periods, however further studies should be conducted to confirm this hypothesis.

References

Driehuis F, Spoelstra SF, Cole SCJ, Morgan R (1996) Improving aerobic stability by inoculation with *Lactobacillus buchneri*. In ´Proceeding of the 11-th International Silage Conference´ (Eds DIH Jone, RR Dewhurt, R Merry. PM Haigh) pp. 106-107. (IGER. Aberystwyth).

Driehuis F, Elferink S, Spoelstra S (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.

Hoffman P, Esser N, Shaver R, Coblentz W, Scott M, Bodnar A, Schmidt R, Charley R (2011) Influence of ensiling time and inoculation on alteration of the starch-protein matrix in high-moisture corn. *Journal of Dairy Science* **94**, 2465-2474.

Kleinschmit D, Kung Jr, L (2006) The Effects of *Lactobacillus buchneri* 40788 and *Pediococcus pentosaceus* R1094 on the fermentation of corn Silage. *Journal of Dairy Science* **89**, 3999-4004.