Clostridium control during the fermentation of sugarcane silages added with lime

L. Custódio[1], J.L.P. Daniel[1], G. Morais[1], C. Kleinshmitt[1], B.C. Bach[1], M.C. Santos[1] and L.G. Nussio[1]

[1]*University of São Paulo, Piracicaba, São Paulo 13418-900, Brazil, Email: leticiacustodio@usp.br*

Introduction Sugarcane silage has a predominantly alcoholic fermentation, which is associated with high dry matter (DM) and net energy losses. Therefore, chemical and microbial additives have been recommended to mitigate nutrient losses. Lime is a chemical additive recognized to inhibit yeast growing and improve DM recovery of sugarcane silages (Schmidt, 2009). However, several studies have reported high contents of butyric acid in sugarcane silages added with lime. Furthermore, farmers and nutritionists have observed a poorer performance than that expected for feedlot cattle fed diets based on sugarcane silage added with lime. It suggests that despite the benefits on nutrient preservation (throughout yeast inhibition), lime would favor clostridium growth and disimprove the hygienic quality of silages. Thus, the aim of this study was to compare strategies to prevent clostridium grow in sugarcane silages added with lime.

Materials and Methods Sugarcane variety IAC 93-3046 (12 mo. regrowth, 18°Brix) was harvested and ensiled in 20 L plastic buckets. Treatments were Control: no additives; L: 1.5% lime; L + LP: 1.5% lime + *Lactobacillus plantarum* Ma 18/5U (5×10^5 cfu/g fresh forage); L + LB: 1.5% lime + *Lactobacillus buchneri* 40788 (5×10^5 cfu/g fresh forage); L + N: 1.5% lime + 0.07% sodium nitrite; L + B: 1.5% lime + 0.15% sodium benzoate. At ensiling and opening, samples were collected to determine pH, microbial counts (yeasts, lactic acid bacteria and clostridia), DM, soluble carbohydrates (SC) and buffering capacity (BC). Fermentability coefficient was calculated as: FC = DM(g/kg) + [80 × SC(g/kg) / BC(g/kg)]. Acetic acid and butyric acid were determined by gas liquid chromatograph and lactic acid by a colorimetric method. Data were analyzed as a completely randomized design and means compared by Tukey test ($\alpha = 0.05$), using the mixed procedure of SAS.

Results and Discussion The composition of fresh and ensiled forages are shown in Table 1. As expected, untreated fresh forage had adequate DM, SC and BC, thus a suitable FC. However, lime addition led to increased BC, which in turn increased the risk of clostridium growth (Weissbach, 2011). Therefore, L, L+N, L+LB and L+LP treatments had higher counts of clostridia than the control, whereas L+B silage showed intermediate counts. The alkaline nature of lime raised the sugarcane pH, enabling clostridium growth. Consequently, treated silages had two- to three-fold more butyric acid than the untreated silage. Even the high content of lactic acid accumulated in lime treated silages was not enough to prevent clostridium grow, which was not expected based on traditional data. None of additives combined with lime were able to afford silages with low clostridium counts. Even the anti-clostridia effect of nitrite (Woods et al., 1981) did not offset the loss of hygienic quality of silages caused by lime. A higher dose of sodium nitrite should be further tested. Although clostridium count was fairly for L+B, butyric acid concentrations still high and there is no reason for combining those additives, because several studies has showed that sodium benzoate by self is good enough to preserve sugarcane as silage (Schmidt, 2009). Finally, the threshold developed by Weissbach to anticipate the run of silage

fermentation (FC ≥ 450 denotes anaerobic stable silages) cannot be used for sugarcane, because all fresh forages presented FC greater than 450 whereas still having butyric fermentation.

Table 1 Composition of fresh sugarcane and sugarcane silages

Item[1]	Treatments[2]							
Fresh forage	C	L	L+B	L+N	L+LB	L+LP		
DM, g/kg	266	291	287	285	281	280		
pH	5.95	10.05	10.38	9.62	10.38	9.61		
BC, g lactic acid/kg DM	21	161	155	162	151	179		
SC, g/kg DM	387	401	392	378	393	407		
FC	1830	480	480	470	490	460		
LAB, log cfu/g	4.94	3.85	4.52	4.94	5.4	3.48		
Yeasts, log cfu /g	5.00	3.00	3.60	3.90	4.32	4.18		
Clostridia, log cfu/g	3.81	3.40	3.00	3.60	3.78	3.70		
Silages	C	L	L+B	L+N	L+LB	L+LP	SEM	*P*
DM, g/kg	224^{c}	249^{b}	266^{a}	257^{ab}	253^{b}	252^{b}	2.7	<0.01
pH	3.62^{c}	4.78^{ab}	4.54^{ab}	4.83^{a}	4.41^{b}	4.51^{ab}	0.9	<0.01
SC, g/kg DM	28.0	30.8	30.5	53.6	34.1	37.1	6.6	0.13
LAB, log cfu/g	7.52	7.82	8.13	7.9	8.62	7.44	2.1	0.06
Yeasts, log cfu/g	2.70^{ab}	2.60^{ab}	$<2.0^{b}$	3.16^{a}	3.56^{a}	3.84^{a}	3.2	<0.01
Clostridia, log cfu/g	3.26^{c}	6.74^{a}	4.41^{bc}	5.24^{b}	5.96^{a}	4.63^{b}	2.9	<0.01
Lactic acid, g/kg DM	35.3^{c}	71.2^{b}	91.5^{b}	56.2^{b}	115^{a}	86.7^{b}	3.6	<0.01
Acetic acid, g/kg DM	75.4	72.3	76.7	62.8	82.0	68.6	4.2	0.07
Butyric acid, g/kg DM	6.70^{b}	19.4^{ab}	13.8^{ab}	22.3^{a}	14.3^{ab}	21.8^{ab}	3.5	<0.01

[1]DM: dry matter, BC: buffering capacity, SC: soluble carbohydrates, FC: fermentability coefficient, LAB: lactic acid bacteria.

[2]C: control, L: 1.5% lime, L+LP: 1.5% lime + *L. plantarum* (5×10^5 cfu/g), L+LB: 1.5% lime + *L. buchneri* (5×10^5 cfu/g), L+N: 1.5% lime + 0.07% sodium nitrite, L+B: 1.5% lime + 0.15% sodium benzoate.

Conclusions None of additives combined with lime were able to provide butyric acid free silages.

References

Schmidt, P. 2009. Improved efficiency of sugarcane ensiling for ruminant supplementation. In: Proc. International symposium on forage quality and conservation. São Pedro, Brazil, p. 47-72.

Weissbach, F. 2011. The future of forage conservation. In: Proc. II International symposium on forage quality and conservation. São Pedro, Brazil, p. 319-363

Woods, L.F., J.M. Wood, P.A. Gibbs. 1981. The involvement of Nitric Oxide in the inhibition of the phosphoroclastic system in *Clostridium sporogenes* by sodium nitrite. J. Gen. Microbiol. 125: 399-406.