In vitro gas production of corn silages produced in different maturity stages and inoculated with lactic acid bacteria

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Introduction Silage is a common method of preserving forage and is based on the conversion of carbohydrates into organic acids by the action of lactic acid bacteria (LAB) under anaerobic conditions. As a result, the pH decreases and the forage are preserved against deterioration caused by microorganisms (McDonald et al., 1991). According to Filya (2004), the stage of maturity when the plant is harvested can be considered the main determinant of the nutritional value of silage. Moreover, the knowledge of the real importance of the LAB application in each point of development of plant is important to analyze the impact this strategy on the silage quality. Thus, the technique of *in vitro* gas production is used in the assessment of feed quality. The objective of this study was to evaluate the *in vitro* gas production in corn silages produced in different maturity stages and inoculated with LAB.

Material and Methods A corn hybrid BM3061 was harvested in five maturity stages: without milk line (WML), 1/3 of milk line (ML), 1/2 ML, 2/3 ML and black layer (BL), corresponding to 261.3; 290.9; 321.1; 340.2 and 385.8 g of dry matter (DM)/kg fresh matter (FM). When established the ideal harvest (although observing reduction of the milk line in the grain), all plants of the sub-plot corresponding to the stage of maturity for silage were harvested and cut in particles close to 2.0 cm. Each maturity stage was inoculated or not with LAB. The treatments evaluated were: uninoculated (control); forage inoculated with Silobac® (*Lactobacillus plantarum* and *Pediococcus pentosaceus*, 2.5 x 10^{10} cfu per gram of product); forage inoculated with Maize All® (*Enterococcus faecium* and *L. plantarum*, 1.0 x 10^{10} cfu per gram of product, *P. acidilactici*, 1.0 x 10^{9} cfu per gram of product, amylolitic and cellulolytic enzymes (1.5%), and proteolytic enzymes (2.0%)). As experimental silos were used PVC tubes (4 L; specific mass between 540-605 kg FM/m^3). After 55 days of ensiling, the silos were opened and samples were taken for characterization of silages and evaluation of gas production. The assay *in vitro* was conducted incubating dry samples (200 mg) in a water bath at 39°C in serum bottles (115 mL) with 30 mL buffered rumen fluid, according to Maurício et al. (1999). Accumulated headspace gas pressure measurements were made using a needle attached to a pressure transducer connected to a visual display (readings after 2, 4, 6, 8, 10, 12, 24, 48, 60 and 72 h post-inoculation). Relative gas production was calculated by dividing the gas production at a given time by the gas production for that bottle at 72 h. Experiment was conducted in a completely randomized design, with four replicates in a factorial arrangement 5 x 3. The test of *in vitro* gas production was conducted as split plot, where the factor of plots was the treatments, and the factor attributed to the sub-plots was the time, with four replicates All data was analyzed as mixed model with repeated measures in the time using MIXED procedure of SAS (v. 9.0). Differences among means were tested using the LSMEANS statement with the PDIFF option, and significance was declared at 5% and tendencies between 5 and 10%.

Results and Discussion Gas production and relative gas production were changed by use of inoculant, stage of maturity and interaction between factors ($P<0.0001$). Gas production

technique considers the conversion of all the main rich sources of metabolizable energy, such as pectins, starch, cellulose and hemicellulose into gases. Thus, we observed that silages produced with high moisture presented more non-structural carbohydrates (NSC) contents in relation to silages harvested with dry matter content ideal (WML = 250.32; 1/3 ML = 242.22; 1/2 ML = 226.57; 2/3 ML = 221.20 ; BL = 249.10 g/kg of DM). These results help us to explain the higher gas production in corn silages harvested in the stages WML and 1/3 ML. However, in the stages 2/3 ML and BL, the LAB inoculation resulted in higher gas production, and this can be explaining by presence of fibrolytic enzymes in the inoculant Maize All. The relative gas production ranged 7 to 10% of the total gas production (72 h) after 2 h of incubation, whereas after 48 h ranged 86 to 90%.

Table 1 Gas production (mL/g of organic matter) of corn silages inoculated with lactic acid bacteria in different stages of maturity.

Stage	Silage	Time of fermentation, h					
		2	6	10	24	48	72
WML	Control	27.40	47.20	82.20	191.02	238.08	269.12
	Silobac	26.45	50.12	74.75	175.19	250.75	282.87
	Maize All	25.10	47.75	71.14	166.96	232.66	270.08
1/3 ML	Control	25.90	53.11	82.97	201.51	265.61	299.01
	Silobac	24.33	54.81	82.03	163.59	200.07	231.92
	Maize All	25.40	55.68	77.36	197.15	250.56	279.88
1/2 ML	Control	27.62	53.33	84.02	185.42	251.08	275.92
	Silobac	21.69	41.32	68.03	156.18	210.81	241.42
	Maize All	23.96	47.34	69.77	179.65	236.22	265.77
2/3 ML	Control	21.91	46.51	71.36	171.13	229.65	257.27
	Silobac	23.87	56.93	86.82	207.09	271.59	300.07
	Maize All	22.01	47.68	73.77	203.07	269.30	298.75
BL	Control	23.84	47.00	70.64	171.40	229.27	262.63
	Silobac	24.06	48.97	75.18	163.91	206.68	234.41
	Maize All	26.06	59.03	90.74	189.18	247.10	286.95
SEM		0.486	0.834	0.957	1.385	1.624	2.305
P value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

*SEM = standard error of the mean.

Conclusions Corn silages produced higher *in vitro* gas when the plants are harvested with high moisture. Lactic acid bacteria increase the gas production when applied in silages produced in maturity stages more advanced.

References

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