In vitro gas production of corn silages inoculated with *Lactobacillus buchneri* CNCM I-4323 associated with three ruminal fluids

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Introduction Silages resultant from homofermentation generally presents low aerobic stability. Therefore, inoculants containing heterofermentative lactic acid bacteria (LAB), such as *Lactobacillus buchneri* are used to improve the aerobic stability of the silage by producing high levels of acetic acid (inhibits action of yeasts and molds). This strategy should result in an improvement of silage quality. According to Weinberg et al. (2003), LAB can survive in ruminal fluid and improve animal performance. In this sense, the technique of *in vitro* gas production can be used to evaluate the feed quality and if really these LAB are surviving in ruminal conditions. Thus, our aim was to evaluate the influence of *L. buchneri* CNCM I-4323 inoculation associated with three ruminal fluids on *in vitro* gas production of corn silages.

Material and Methods A corn hybrid Impacto Víptera (Syngenta) was sown on 2011, harvested at 279 g/kg of dry matter (DM) on 2012 using a Premium Flex forage harvester. Forages were chopped to achieve a theoretical length averaging 10 mm and ensiled without (control) or with $1x10^5$ cfu of *Lactobacillus buchneri* CNCM I-4323 per gram of fresh forage. Inoculant was dissolved in water (0.7 L/t) and then applied with spray mounted on the fresh forage under constant mixing. The similar amount of water was applied in control silage. Eight silos were filled with 350 kg of corn forage each (remained closed for 229 days). After opening the silos, an *in vitro* assay was performed incubating wet samples (1 g) in a water bath at 39°C in serum bottles (115 mL) with 60 mL buffered rumen fluid, according to Maurício et al. (1999). Rumen fluid was collected from 6 rumen-cannulated sheep in the morning, before feeding. The rumen fluid was filtered through four layers of cheesecloth into pre-warmed thermal-flasks, homogenized and mixed with solution media. The sheep were fed with 70% of corn silage and 30% concentrate, on DM basis. Three different ruminal fluids were used. Two animals fed control silage (Control); two animals fed inoculated silage ($1x10^5$ cfu of *L. buchneri*) (*L. buchneri*); and two animals fed control silage and a daily-dose of *L. buchneri* was applied directly into the rumen ($1x10^7$ cfu of *L. buchneri*/g of silage provided) (rumen applied). Accumulated headspace gas pressure measurements were made using a needle attached to a pressure transducer connected to a visual display (readings after 3, 6, 9, 12, 24 and 48 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 48 h. Experiment was conducted in a completely randomized design with a 2 x 3 factorial arrangement with eight 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. Significance was declared at 5%.

Results and Discussion Gas production in the corn silage inoculated with *L. buchneri* was greatest mainly when incubated with control rumen fluid (Table 1). 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, these results can be explaining by higher non-structural carbohydrates content in the inoculated silage (58.6 *vs.* 55.2 g/kg of

DM in control silage). According to Nsereko et al. (2008), some strains of *L. buchneri* can produce the acid ferulic enzymes that act on the fiber content (reduce or alter the structure). The LAB can survive in ruminal conditions (Weinberg et al., 2003), thus, perhaps the *L. buchneri* can change the profile ruminal microorganisms profiles and improve the utilization efficiency of the silage. In relation to relative gas production, we found values among 75 to 82% of the total gas production after 24 h of incubation.

Table 1 Gas production of corn silages inoculated with *Lactobacillus buchneri* associated with three ruminal fluids in various times of fermentation (hours).

Treatments	3	6	9	12	24	48
Gas production, mL/g of organic matter						
Control silage						
Control	30.02	44.96	64.72	85.20	161.45	211.15
L. buchneri	38.36	48.18	66.76	87.21	151.98	192.11
Rumen applied	31.40	50.56	69.45	84.82	168.10	213.99
Inoculated silage						
Control	30.58	44.82	66.92	90.66	176.71	225.56
L. buchneri	33.08	53.77	74.91	94.08	178.09	220.18
Rumen applied	34.68	53.89	79.36	91.75	175.69	213.01
SEM[1]	0.526	0.593	0.640	0.740	0.908	0.803
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Relative gas production						
Control silage						
Control	0.13	0.20	0.29	0.39	0.75	-
L. buchneri	0.18	0.23	0.33	0.43	0.76	-
Rumen applied	0.15	0.24	0.34	0.41	0.80	-
Inoculated silage						
Control	0.13	0.19	0.29	0.40	0.78	-
L. buchneri	0.15	0.24	0.34	0.43	0.82	-
Rumen applied	0.16	0.25	0.36	0.42	0.81	-
SEM[1]	0.003	0.002	0.003	0.004	0.005	-
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-

SEM = standard error of the mean.

Conclusions Corn silage inoculated with *L. buchneri* present higher gas production. *L. buchneri* applied directly in ruminal fluid increases the gas production in the control silage.

References

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