End products of *in vitro* fermentation 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 improve of silage quality. According to Weinberg et al. (2003), LAB can survive in ruminal fluid (*in vitro* conditions) and improve animal performance. Thus, the objective of this trial was to evaluate the influence of *L. buchneri* CNCM I-4323 inoculation in corn silage associated with three ruminal fluids on the end products of *in vitro* fermentation.

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). In this assay, 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 daily-a dose of *L. buchneri* was applied directly into the rumen ($1x10^7$ cfu of *L. buchneri*/g of silage provided) (Rumen applied). At 9 and 48 h, the serum bottles were opened and pH values and volatile fatty acids (VFA) were analyzed. The VFA were determined by gas chromatograph. Experiment was conducted in a completely randomized design with a 2 x 3 factorial arrangement with eight replicates. All data were 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 We observed higher production of total VFA in the corn silage inoculated with *L. buchneri* mainly when incubated with control and *L. buchneri* rumen fluid after 48 h of incubation. As a result, pH values decreased in these treatments as well as when it was compared to the 9 h of incubation. The inoculation in the ensilage resulted in higher production of propionic acid after 48 h of incubation when compared to control silage, and consequently, lower acetate: propionate ratio was observed (Table 1). Even with these

challenges, the commercial inoculant produced significant shifts in pH values and *in vitro* end fermentation products, indicating that it had affected the final outcome of silage fermentation. Incubation of control silage with ruminal fluid from animals fed with inoculated corn silage promotes higher acetic acid production.

Table 1 End products of *in vitro* incubation (9 and 48 h) of corn silages inoculated with *Lactobacillus buchneri* associated with three ruminal fluids.

Treatments	pH	Acetate	Propionate	Butyrate	AC:P ratio	Total VFA
9 h						
Control silage						
Control	6.64	35.30	13.29	4.16	3.52	53.97
L. buchneri	6.62	44.57	15.09	7.08	3.10	69.99
Rumen applied	6.62	39.59	13.38	5.68	3.28	62.28
Inoculated silage						
Control	6.60	40.74	13.19	6.06	3.51	63.33
L. buchneri	6.59	40.78	14.00	6.92	3.15	64.07
Rumen applied	6.59	41.10	13.80	5.20	3.57	61.83
48 h						
Control silage						
Control	6.59	48.94	16.62	8.76	3.06	79.45
L. buchneri	6.59	60.10	18.74	11.40	3.00	92.29
Rumen applied	6.56	56.21	18.63	10.35	3.08	102.25
Inoculated silage						
Control	6.58	67.50	19.66	11.94	3.10	115.44
L. buchneri	6.52	64.17	21.43	13.39	3.00	122.03
Rumen applied	6.55	62.94	18.70	9.78	3.06	105.28
SEM	0.008	3.382	0.708	0.664	0.052	6.612
Silage (S)	<0.0001	0.0006	0.0133	0.0047	0.0467	<0.0001
Ruminal fluid (RF)	<0.0001	0.0713	0.0005	<0.0001	<0.0001	0.0306
S x RF	0.0099	0.0065	0.3082	0.0014	0.1618	0.0036
Time	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

SEM = Standard error of the mean; AC:P = acetate: propionate ratio.

Conclusions *Lactobacillus buchneri* inoculated in corn silage increased the volatile fatty acids production in an *in vitro* condition.

References

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