In vitro ruminal fermentation, gas production and true digestibility of the corn silages inoculated with microbial additives

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Introduction Homofermentative lactic acid bacteria (^{ho}LAB) are used to rapidly decrease the pH of forage during ensilage, thereby avoiding undesired fermentation. Inoculants containing heterofermentative LAB, such as *Lactobacillus buchneri*, or *Bacillus* species are used to improve the aerobic stability of the silage. Moreover, some strains of LAB and *Bacillus* ssp. can produce ferulate esterase enzyme, which may increase the susceptibility of plant cell walls to enzymatic hydrolysis (Nsereko et al., 2008; Donaghy et al., 1998). Furthermore, *in vitro* cumulative gas production techniques were developed to predict fermentations of ruminant feedstuffs (Rymer et al., 2005). Thus, the purpose of this study was to evaluate the effects of microbial additives on the *in vitro* ruminal fermentation, gas production and true digestibility of the corn silages.

Material and Methods During three consecutive years (2010, 2011 and 2012) microbial additives were evaluated in corn silage in farm silos, which remained closed for 165, 70 and 88 d, respectively. The corn hybrid studied were Maximus (Syngenta), 2B688Hx (Dow AgroSciences) and Impacto Víptera (Syngenta). The whole corn plants were harvested, chopped and the following treatments were applied: *2010* - untreated (control 1), *L. buchneri* "NCIMB 40788" (LB 1), and the combinations *L. buchneri* and *L. plantarum* "MA18/5U" (LBLP 1); *2011* - untreated (control 2) and *L. buchneri* "NCIMB 40788" (LB 2) and; *2012* - untreated (control 3), the combinations *L. buchneri* "CNCM I-4323" and *L. plantarum* "MA18/5U" (LBLP 2) and *B. subtilis* "AT553098" and *L. plantarum* "MA18/5U" (BSLP). All inoculants were applied at a rate of 1 x 10^5 cfu/ g. Inoculants were diluted in water and applied by spraying with a constant mixing. Samples were collected during feedout, taken to oven (55°C for 72h), processed in order to pass through 1 mm screen sieves and weighed (0.5g) into filter bags (F57, ANKON). The bags were heat-sealed, placed into 115 ml vials (duplicate for sample) and incubated with 60 ml of anaerobic buffered rumen fluid (Menke et al., 1979). The vials were sealed and incubated at 39°C in a water bath for 24 h. Rumen fluid was collected from 2 rumen-cannulated wethers fed 60% corn silage without inoculant and 40% concentrate (on DM basis), in the morning before feeding. Head space gas production (GP) was measured at 3, 6, 12 and 24 h post inoculation. Flasks containing buffered rumen fluid without samples were used as blanks. At 24 h, vials were removed from the water bath, placed on ice. The serum bottles were opened, samples were collected to measure pH values and volatile fatty acids (VFA). The bags were washed until excess water ran clear. Neutral detergent fiber (NDF) was determined according to Van Soest et al. (1991) using thermostable α-amylase in the Ankom 2000 Fiber Analyzer (Ankom Tecnologies) to determine the *in vitro* true dry matter digestibility (IVDMD). Data were analyzed using a completely randomized design with 6 replicates and as mixed model using the MIXED procedure of SAS (v. 9.0). Differences between the means were determined using DIFF (level of significance 5%).

Results and Discussion During the years the parameters response to microbial inoculants was different. On 2010, LB silage had the highest GP and, the highest total VFA was produced from LB and LBLP silages incubation**.** On the other hand, on 2011

a greater total VFA was produced from control silage. Ever on 2012, the control silage presented the highest IVDMD. Aside years, hybrids and strains studied, factors that may have contributed to the different effects include differences in epiphytic bacterial population, water activity, water soluble carbohydrate content and cell wall component concentrations (McDonald et al., 1991).

Table 1. *In vitro* gas production (GP), true dry matter digestibility (IVDMD) and ruminal fermentation of the corn silages inoculated with microbial additives in different years.

Treatments	Variables[1]								
	GP	IVDMD	pH	AA	PA	AAPA	BA	Others VFA	Total VFA
					2010				
Control 1	153.70[b]	47.90	6.67	35.80	13.60[b]	2.66[a]	2.60[b]	10.61[b]	73.18[b]
LB 1	164.00[a]	49.14	6.64	40.83	15.50[a]	2.65[a]	3.66[a]	12.63[a]	85.21[a]
LBLP 1	155.90[b]	48.40	6.66	40.50	15.83[a]	2.56[b]	4.00[a]	11.71[a]	83.48[a]
SEM[2]	2.19	0.89	0.01	1.47	0.57	0.02	0.22	0.49	3.04
P value	0.01	0.35	0.26	0.05	0.04	0.01	0.01	0.03	0.03
					2011				
Control 2	196.80	60.02	6.57	41.33	17.83	2.35	3.83	12.45	77.77
LB 2	194.20	61.87	6.58	36.66	15.11	2.42	3.16	10.42	67.77
SEM	4.15	0.70	0.01	0.71	0.29	0.02	0.31	0.54	1.34
P value	0.67	0.09	0.75	0.01	0.01	0.04	0.16	0.23	0.01
					2012				
Control 3	210.95	67.43[a]	6.53	37.16	17.00	2.20	3.83	11.51[ab]	69.51[a]
LBLP 2	199.00	60.88[b]	6.58	37.00	15.50	2.40	3.40	13.26[a]	69.16[a]
BSLP	211.38	62.62[b]	6.55	34.00	15.20	2.25	3.83	10.05[b]	63.05[b]
SEM	4.90	0.64	0.02	1.02	0.62	0.06	0.18	0.59	1.89
P value	0.16	0.01	0.28	0.08	0.12	0.05	0.17	0.01	0.04

[*]Means follows of the same letter did not differ to 5% of significance.[1]GP - mL/g; IVDMD - %DM; AA: acetate – mmol/g; PA: propionate – mmol/g; AA:PA: acetate to propionate ratio; BA: butyrate – mmol/g; Others VFA: sum of valerate, isovalerate and isobutyrate – mmol/g; Total VFA: Total of volatile fatty acids – mmol/g. [2]SEM: Standard error of the mean.

Conclusion The microbial inoculation changed the *in vitro* ruminal fermentation, gas production and true digestibility of the corn silages, depending on year, hybrid and strain studied.

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