In vitro gas production of the corn silages inoculated with microbial additives

F.C. Basso[1], E.C. Lara[1], C.H.S. Rabelo[1], L.G.O. Jorge[1], G.R. Siqueira[2], T.T. Berchielli[1], R.A. Reis[1]

[1]*Sao Paulo State University (UNESP) – Jaboticabal 14883-900, SP, Brazil, Email: fcarvalhobasso@yahoo.com.br.* [2]*APTA – Alta Mogiana, Colina 14770-000, SP, Brazil.*

Introduction Microbial additives containing homofermentative lactic acid bacteria (^{ho}LAB) such as *Lactobacillus plantarum* are used to rapidly decrease the pH of forage during ensilage, thereby avoiding undesired fermentation. However, corn plants have a high content of water soluble carbohydrates (WSC), which are fermented by the natural LAB, resulting in lactic acid (LA). The WSC and LA are consumed by yeast and mold in the post-opening of silos, raising the silage pH and resulting in aerobic deterioration, which reduces the silage quality. Therefore, inoculants containing heterofermentative LAB (he LAB), such as *L. buchneri* are used to improve the aerobic stability of the silage by producing high levels of acetic acid. It has been suggested that other types of inoculants, such as propionic acid bacteria and *Bacillus* species, can be used as microbiological additives to overcome the problem of silage aerobic spoilage. Moreover, some strains of LAB and *Bacillus* ssp. can produce ferulate esterase enzyme (FAE), which may increase the susceptibility of plant cell walls to enzymatic hydrolysis because ferulic acid is released from cell wall arabinoxylans (Donaghy et al., 1998; Nsereko et al., 2008). Thus, the purpose of this study was to evaluate the effects of microbial additives on the *in vitro* gas production of corn silage.

Material and Methods An AG1051 corn hybrid was harvested, chopped and the following treatments were applied to the fresh forages: untreated (**control**), *L. buchneri* NCIMB 40788 (**LB**), *B. subtilis* AT553098 (**BS**), *Propionibacterium acidipropionici* MA26/4U (**PA**), *L. plantarum* MA18/5U (**LP** - 1 x 10^5 cfu/ g), and the combinations *L. buchneri* and *L. plantarum* (**LBLP**), *B. subtilis* and *L. plantarum* (**BSLP**) and *P. acidicipropionici* and *L. plantarum* (**PALP**). All inoculants were applied at a rate of 1 x 10^5 cfu/ g. Inoculants were diluted in water (5 mL/kg of fresh forage) and applied by spraying with a constant mixing. The control silage received a similar amount of water. An amount of chopped forage from each treatment was packed into 7L plastic bucket silos in quadruplicate; these silos were sealed with a lid and adhesive tape, stored at room temperature and remained closed for 96 d. Thus, the *in vitro* gas production was performed. Dry samples (200 mg) were incubated in a water bath at 39°C in serum bottles (115 mL) with 30 mL buffered rumen fluid (Menke et al., 1979). Rumen fluid was collected from 2 rumen-cannulated steers fed 60% corn silage without inoculant and 40% concentrate (on DM basis), in the morning before feeding. The accumulated headspace gas pressure measurements were made using a needle attached to a pressure transducer connected to a visual display. Readings were taken 2, 4, 6, 8, 10, 12, 24, 48 and 72 h post-inoculation. Flasks containing buffered rumen fluid without samples were used as blanks. However, the blank correction was omitted according to Cone et al. (1997). The relative gas production was calculated by dividing the gas production at a given time by the gas production at 72 h. Data were analyzed using a completely randomized design with 4 replicates and as a 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 Silages containing LP had higher gas production volumes and faster relative rates of gas production than the control silage until 24 h (Table 1). In silages containing LP, we observed that 50% of the total of gas production (72 h)

occurred between 10 and 12 h. The FAE breaks the ester linkage between ferulic acid and the attached carbohydrate, releasing ferulic acid from the cell walls of the plant, which leaves the remainder of the polysaccharide chain open for further hydrolysis by other cell wall degrading enzymes (Yu et al., 2005). This could increase the digestibility of silage or gas production volumes as we found in the present study.

Table 1 *In vitro* gas production and relative *in vitro* gas production of the corn silages inoculated with microbial additives for incubation times (IT).

IT (h)	*In vitro* gas production (mL/ g DM)									
	Control	LB	BS	PA	LP	LBLP	BSLP	PALP	*P value*	SEM[1]
2	67.9^{c}	75.5^{bc}	73.6^{cb}	76.5^{ab}	86.2^{a}	78.7^{ab}	80.1^{ab}	78.8^{ab}	0.006	2.702
4	97.7^{c}	106.6^{bc}	106.6^{bc}	107.7^{b}	117.6^{a}	109.3^{ab}	115.1^{ab}	111.5^{ab}	0.008	3.183
6	125.9^{c}	140.4^{b}	140.4^{b}	138.4^{b}	154.2^{a}	151.4^{a}	152.3^{a}	145.5^{b}	0.003	3.753
8	146.7^{d}	158.0^{bcd}	160.3^{bc}	156.8^{cd}	170.5^{a}	166.6^{ab}	169.9^{ab}	163.1^{abc}	0.008	4.162
10	162.3^{c}	177.2^{ab}	179.8^{ab}	173.7^{cb}	188.9^{a}	182.9^{ab}	189.5^{a}	180.8^{ab}	0.005	4.414
12	178.9^{c}	194.7^{ab}	195.4^{ab}	190.0^{bc}	205.2^{a}	192.3^{b}	204.9^{a}	197.4^{ab}	0.008	4.401
24	254.9^{c}	277.8^{ab}	277.9^{ab}	272.3^{b}	284.6^{ab}	274.6^{ab}	290.1^{a}	277.1^{ab}	0.013	5.648
48	336.4	352.5	359.6	349.2	347.1	346.3	351.1	329.3	0.846	3.456
72	380.7	396.0	411.5	396.5	409.7	388.5	398.3	377.9	0.852	7.540
	Relative *in vitro* gas production									
2	0.178^{c}	0.191^{bc}	0.180^{c}	0.193^{bc}	0.211^{a}	0.203^{ab}	0.201^{ab}	0.209^{a}	0.001	0.005
4	0.257^{c}	0.269^{bc}	0.260^{c}	0.272^{bc}	0.287^{ab}	0.282^{ab}	0.289^{ab}	0.295^{a}	0.006	0.007
6	0.331^{c}	0.354^{bc}	0.342^{c}	0.349^{c}	0.377^{ab}	0.390^{a}	0.382^{a}	0.384^{a}	0.001	0.008
8	0.385^{c}	0.399^{bc}	0.391^{bc}	0.396^{bc}	0.416^{ab}	0.428^{a}	0.426^{a}	0.432^{a}	0.004	0.009
10	0.426^{d}	0.447^{bcd}	0.439^{cd}	0.439^{cd}	0.461^{abc}	0.471^{ab}	0.476^{ab}	0.478^{a}	0.005	0.010
12	0.470^{d}	0.492^{bcd}	0.477^{cd}	0.480^{bcd}	0.501^{abc}	0.495^{bcd}	0.514^{ab}	0.522^{a}	0.014	0.010
24	0.700^{b}	0.702^{ab}	0.679^{b}	0.687^{b}	0.695^{ab}	0.707^{ab}	0.728^{a}	0.734^{a}	0.034	0.014
48	0.883	0.890	0.879	0.881	0.848	0.892	0.883	0.872^{a}	0.707	0.017

[*]Means follows of the same letter did not differ to 5% of significance. Silages - Control: without inoculant; LB: *L. buchneri*; BS: *B.subtilis*; PA: *P. acidipropionici*; LP: *L. plantarum*; LBLP: *L. buchneri* and *L. plantarum*; BSLP: *B. subtilis* and *L. plantarum*, PALP: *P. acidipropionici* and *L. plantarum*. [1]SEM: Standard error of the mean for the incubation time.

Conclusion The microbial inoculation changed the *in vitro* gas production of the corn silages.

References

Cone, J.W., van Gelder, A.H., Driehuis, F. Description of gas production profiles with a three-phasic model. Anim. Feed Sci. Technol. 66, 31-45.

Donaghy, J., Kelly, P.F., McKay, A.M., 1998. Detection of ferulic acid esterase production by *Bacillus* spp. and lactobacilli. Appl. Microbiol. Biotechnol. 50, 257-260.

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D., Schneider, W., 1979. The estimation of the digestibility and metabolisable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor *in vitro*. J. Agric. Sci. 93, 217-222.

Nsereko, V.L., Smiley, B.K., Rutherford, W.M., Spielbauer, A., Forrester, K.J., Hettinger, G.H., Harman, E.K., Harman, B.R., 2008. Influence of inoculating forage with lactic acid bacterial strains that produce ferulate esterase on ensilage and ruminal degradation of fiber. Anim. Feed Sci. Technol. 145, 122-135.

Yu, P., McKinnon, J.J, Christensen, D.A., 2005. Hydroxycinnamic acids and ferulic acid esterase in relation to biodegradation of complex plant cell walls. Can. J. Anim. Sci. 85, 255-267.