Ruminal fermentation end-products in wethers fed diets containing inoculated corn silage and supplemented with amylolytic enzyme

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Introduction The search for increased starch utilization of whole plant silages has been the goal of many researches aming to improve ruminant performance and reduce feed costs, especially during periods in which grain prices are relatively high (Ferrareto and Shaver, 2012). The choice of corn hybrids, harvest period (% of DM), storage time and grain processing may be some management strategies aimed at improving the use of starch from grain and corn silage by ruminants. However, when these factors are uncontrollable, some technologies are adopted, such as the use of microorganisms and enzymes in ruminant diets. Silage inoculants are used to improve the fermentation process and reduce dry matter (DM) losses (McDonald et al., 1991), however some strains produce enzymes that help in the degradation of fiber and starch of the silage (i.e. *B. subtilis*). On the other hand, the direct-fed amylolytic enzymes addition may increase the availability of starch hydrolysis products in the rumen and alter the ruminal fermentation process providing greater intake of nutrients for metabolism and animal production (Tricarico et al., 2008). From this, we aim to evaluate the changes in ruminal fermentation of wethers fed diets containing inoculated corn silage and supplemented with amylolytic enzyme.

Material and Methods A flint corn hybrid (2B710, Dow AgroSciences Cravinhos, São Paulo, Brazil) was harvested with dry matter content between 33 to 35%. Forage was chopped and two stack silos were filled in for two consecutive days with approximately 20 tons each treatment (uninoculated and inoculated with *Lactobacillus plantarum* associated with *Bacillus subtilis*). Both inoculants were diluted in distilled water and sprayed on the forage during filling of silo. Four diets (treatments) were evaluated: (1) Corn silage uninoculated and no amylase added to total mixed ration (TMR); (2) Corn silage uninoculated and amylase added to TMR; (3) Corn silage inoculated with 1×10^5 CFU *L. plantarum* [MA 18/5U] and 1×10^5 CFU *B. subtilis* [AT553098] and no amylase added to TMR; (4) Corn silage inoculated with 1×10^5 CFU *L. plantarum* [MA 18/5U] and 1×10^5 CFU *B. subtilis* [AT553098] and amylase added to TMR. Diets were composed of 400 g/kg of DM corn silage and 600 g/kg of DM of concentrated. Alpha amylase from *Aspergillus oryzae* (Amaize, Alltech Inc.) was added at feeding applied at a rate of 2 g/kg of DM total diet. Eight Dorper × Santa Ines crossbred wethers, fitted with ruminal silicone-type cannulas (diameter 6.4 cm) were housed in individual metabolism crates. The animals were fed ad libtum (approximately 10% of orts) once a day (0700 h) with free access to drink water. The study consisted of four experimental periods of 16-d each consisting of 15 d for diet adaptation and one day for collection of ruminal fluid. Sample of ruminal fluid (50 mL) was collected from each wether before feeding (0 h), and at 3, 6 9 and 12 h post-feeding. The samples were squeezed through four layers of cheesecloth and its pH was immediately measured. The resulting solution was stored at -20°C until further analysis of volatile fatty acids (VFA) and NH3-N. Data were analyzed as a replicated 4 × 4 Latin square design and the treatments were arrangement in a 2 × 2 factorial (corn silage inoculated or not with *L. plantarum* and *B. subtilis*,

supplemented diet or not with amylase). All data were analyzed using the PROC MIXED procedure of SAS (version 9.0; SAS Inst. Inc., Cary, NC).

Results and Discussion The treatments did not alter the ruminal pH, NH3-N and total VFA. There were interactions (P<0.05) between silages and amylase supplementation in the acetic acid and propionic acid proportion, as well as in acetic acid:propionic acid ratio (Figure 1). Wethers fed uninoculated silage and supplemented with amylase had lower acetic acid and higher propionic acid proportion (65.66 and 24.14), respectively. Consequently, wethers fed uninoculated silage and supplemented with amylase had lower acetic:propionic acid ratio in ruminal fluid. Conversely, when amylase was associated with inoculated silage, wethers showed higher acetic acid and lower propionic acid proportion (24.14 and 19.75), respectively. The propionic acid is the main end product of starch fermentation and can be used to synthesize glucose and may offer an energetic benefit to the ruminant host (Ørskov, 1977). Nozière et al. (2014) also found similar response, with no effects on pH, NH3-N and total VFA production when amylase was added to the high starch diets, however, it was observed high propionic acid and lower acetic acid proportions. However, no change in the VFA profiles following amylase supplementation was also verified by DeFrain et al. (2005) and Hristov et al. (2008). The differences in the end products of rumen fermentation in the various studies evaluated may be due to the use of different enzymatic products, which contains enzymes from different microorganisms as well as variable enzymatic activity and due to the different ways of enzyme applying to the diet.

Conclusion The association of amylase in diets containing uninoculated silage altered the final products of ruminal fermentation, with a higher proportion of propionic acid.

Table 1 Ruminal parameters of wethers fed diets containing corn silage (Uninoculated or Inoculated) and supplemented with amylase at moment of feeding (Amylase or No Amylase).

	Uninoculated		Inoculated		SEM	*P*-value[1]			
	No Amylase	Amylase	No Amylase	Amylase		S	A	S × A	T
pH	5.98	5.94	5.91	5.96	0.04	0.462	0.895	0.234	<.0001
NH_3-N, mg/dL[2]	24.92	22.92	25.25	25.08	0.77	0.231	0.294	0.375	<.0001
Total VFA, mM/L	78.43	79.26	71.82	78.63	1.67	0.274	0.246	0.365	<.0001
Molar proportion, mM/100 Mm									
Acetic acid	68.34ab	65.66b	67.98ab	68.48a	0.32	0.124	0.173	0.047	<.0001
Propionic acid	19.64b	24.14a	21.20ab	19.75b	0.38	0.139	0.111	0.002	<.0001
Butyric acid	11.20	10.23	10.90	11.58	0.14	0.094	0.923	0.133	<.0001
Acetic:propionic	3.49a	2.79b	3.30ab	3.56a	0.08	0.083	0.183	0.005	<.0001

*Corn silage uninoculated or inoculated at ensiling with 1 × 10^5 cfu/g of fresh forage of *L. plantarum* [MA 18/5U] (Lallemand Animal Nutrition, Goiânia, GO, Brazil) combined with 1 × 10^5 cfu/g of fresh forage of *B. subtilis* [AT553098] (FATEC Nutrição e Saúde Animal, Arujá, Sao Paulo, Brazil).
[1]S, silage; A: amylase; S×A: interaction between silage and amylase; T, time; E×S×T, interaction among amylase, silage and time.
[2]NH_3-N: Ammonia nitrogen