In vitro degradability and gas production of corn silage ensiled with fibrolytic enzymes

E. C. Lara[1], F. C. Basso[2], C. H. S. Rabelo[2], F. A. Souza[2], L. G. O. Jorge[2], M. L. T. M. Polizeli[3], M. Cereia[3], R. A. Reis[2]

[1,2]Scientific Researcher, Animal Science Department, Faculty of Agricultural Sciences and Veterinary, Access Prof. Paulo Donato Castellane s/n 14.884-900. Jaboticabal - São Paulo, Brazil, [3]Faculty of Philosophy, Sciences and Letters – FFCLRP/USP, Ribeirão Preto/SP, Brazil. erikalarac@gmail.com.br.

Introduction Fibrolytic enzymes have been added to forage at ensiling with the goal to improve fermentation. Furthermore, enzymes can improve cell wall degradation, decreasing fiber content and enhancing the animal performance. An additional advantage could be that, by degrading the cell walls of the forage, the rate and extent of digestion of silage in the rumen may be increased (Bolsen et al., 1995). The objectives of this study were to evaluate *in vitro* degradability and gas production of corn silage ensiled with fibrolytic enzymes.

Materials and Methods Fibrolytic enzymes were produced by submersed fermentation (FSbm) of *Aspergillus niger* in a liquid medium (SR) containing 1% of wheat bran as substrate in stove 30ºC per 168 hours. Enzymatic extract contained 20.59 U mL^{-1} and 1.04 U mL^{-1} of xylanases and cellulases, respectively. Enzymatic extract was sprayed on the corn plant to manufacture the following treatments: Control - without enzymes; E1 – ensiling with 5.0 mL of enzymatic extract kg of fresh $forage^{-1}$; E2 - ensiling with 10.0 mL of enzymatic extract kg of fresh $forage^{-1}$; E3 - ensiling with 15.0 mL of enzymatic extract kg of fresh $forage^{-1}$. The forage was treated and ensiled into plastic buckets (5 L). The experimental silos were opened after 60 days of ensiling and all samples were dried, ground to pass through a 1-mm screen and weighed (0.5g) into filter bags (F57, ANKON). Then the bags were heat-sealed, placed into 115 ml vials and incubated with 60 ml of anaerobic buffer medium and ruminal fluid mixture (Goering and Van Soest, 1970). The diet of animals was composed of 60% of silage and 40% of concentrate (ingredients: corn meal, soybean meal, urea and mineral supplement) and ruminal fluid was colected 3 hours after feeding. The vials were sealed and incubated at 39°C in a water bath for 48 h. Head space gas production (GP) resultant of substrate fermentation was measured at 3, 6, 9, 12, 24 and 48 h post inoculation. At 24 and 48 h, vials were removed from the water bath, placed on ice and the bags were washed under cold tap water until excess water ran clear. Bags were dried at 55 °C for 48 h, and dry matter degradability (DMD) was determined following the procedures outlined by Van Soest et al. (1991) using thermostable α-amylase but without sodium sulfite in the Ankom 2000 Fiber Analyzer (Ankom Tecnologies, Macedon, NY, USA. After each analysis, bags were dried as described for DMD determination. All the statistical analyses were conducted using the MIXED procedure of SAS (SAS System, version 9.1, 2002). Orthogonal polynomial contrasts were performed to determine linear and quadratic effects of enzymatic level.

Results and Discussion Gas production was not significantly increased by enzymatic treatment (Table 1, $p>0.05$). According Nadeau et al. (2000), the most consistent effect of cell wall degrading enzymes such as cellulases and hemicellulases, is the reduction of structural carbohydrate concentration of silages. This decrease in fiber content could increase the degradability of feed, however, when the rapidly fermentable NDF is degraded, this extensive fermentation of forage before feeding can decrease the digestibility of remaining NDF (Van

Vuuren, 1995). In this research, the addition of different levels of enzymes in ensiling of corn plant not improved the degradability of DM and NDF after 24 or 48 hours. Colombatto et al. (2003) found which silages treated with commercial enzymes produced less gas and were degraded more slowly than control silage. Several factors contribute to the effectiveness of fiber degrading enzymes such as, for example, the activity of the enzymes used, the pH of the medium where the enzyme must act, as well as temperature and moisture content.

Table 1 Cumulative gas production (mL gDM^{-1}) of corn silage ensiled with different level of fibrolytics enzymes.

	Incubation time (hour)					
Treatment[1]	3	6	9	12	24	48
Control	62.41	99.11	130.10	153.36	183.54	235.17
E1	60.18	97.58	124.76	148.03	181.37	232.18
E2	61.71	100.38	129.39	151.85	182.17	226.83
E3	62.80	100.26	130.09	153.34	182.16	227.28
Mean	61.78	99.33	128.59	151.65	182.31	230.36
SEM	0.58	0.65	1.29	1.50	0.45	2.01
Significance	Ns	ns	ns	ns	ns	ns

[1]Control: without enzymes; E1: ensiling with 5.0 mL of enzymatic extract kg of fresh $forage^{-1}$; E2: ensiling with 10.0 mL of enzymatic extract kg of fresh $forage^{-1}$; E3: ensiling with 15.0 mL of enzymatic extract kg of fresh $forage^{-1}$.

Table 2 Apparent degradability (g kg^{-1}) of DM and NDF, from corn silage ensiled with fibrolytic enzymes after 12 and 48 h of incubation with ruminal fluid.

Treatment	Degradability			
	24 hours		48 hours	
	DM	NDF	DM	NDF
Control	35.43	17.08	43.92	40.08
E1	35.11	17.61	43.06	39.95
E2	36.94	17.37	42.64	38.58
E3	37.11	18.40	44.49	40.46
Mean	36.15	17.61	43.53	39.77
SEM	0.36	0.33	0.38	0.34
Significance	ns	ns	ns	ns

[1]Control: without enzymes; E1: ensiling with 5.0 mL of enzymatic extract kg of fresh $forage^{-1}$; E2: ensiling with 10.0 mL of enzymatic extract kg of fresh $forage^{-1}$; E3: ensiling with 15.0 mL of enzymatic extract kg of fresh $forage^{-1}$.

Conclusions Fibrolytic enzymes added in the ensiling of corn plant had no effect on gas production until 48 hours of incubation and dry matter and neutral detergent fiber degradation.