In vitro gas production of corn silage with different levels of enzymatic extract

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Introduction The use of filamentous fungi to produce enzymes of commercial interest is advantageous due to the fact that these microorganisms excrete these enzymes to the culture medium, which facilitates the process of obtaining and purifying these molecules (Reis, 2007). The addition of these enzymes in the diets of ruminants aims to increase the digestibility of fibrous feed. However, few studies that have tested their addition to diets are available, and found a great variability of results, making comparison between studies and validation of the efficacy of the product. A significant factor is great variability and level of enzyme to be applied. Nsereko et al. (2002) speculated that the application of a moderate level of enzymes in ruminant diets caused a beneficial break on the surface structure of the food before or after eating. Thus, it is noted that the optimal level of enzyme used depends on the diet, and high amounts reduce microbial attachment and limited digestion of food.

Materials and methods The enzymatic extract of *Aspergillus niger* was obtained by submerged fermentation (FSbm) SR in a liquid medium containing 1% wheat bran as substrate. The vials were kept for 72 hours at 30°C under 100 rpm agitation. The xylanolytic and cellulolytic activities were assayed using 3´,5´-dinitrosalicylic acid (DNS) (Miller, 1959), using 1% (v/w) birchwood xylan and CMcellulose as substrates, at 39°C, respectively. One unit (U) was defined as the amount of enzyme that releases 1 µmol of reducing sugar per min. Gas production was determined by the technique of gas production, according to the methodology described by Mauricio et al. (1999). The rumen fluid was taken from crossbred sheep Dorper x Santa Inês fitted with rumen cannula and maintained with diet based on corn silage. The enzyme doses were 0.0 (control), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 mL, added at the time of incubation. The experimental design was completely randomized design with nine treatments and four replications. Means were analyzed by regression test at 5% probability for the effect of time.

Results and discussion The enzymatic extract added to the silage at the time of incubation contained 25.78 U / mL xylanase and 1.54 U / mL cellulase at 39 ° C and pH 6.0. Table 1 shows the adjusted equations of cumulative gas production along with the optimal time of fermentation and the maximum gas production for each dose. Curves estimated cumulative gas production are shown in Figure 1. The curves of gas production showed quadratic trend, in which it was observed little difference between the equations. It was observed that the addition of fibrolytic enzymes increased the gas production when compared with treatment without enzymes. In general, it was found that the maximum gas production occurred between 42 and 43 hours of fermentation, noting that the dose was 4.0 mL with the highest gas production (331.34 mL / g DM). In another experiment of gas production, Jalilvand et al. (2008) found that the addition of enzymes (commercial) affected ruminal fermentation of forages differently, according to the fiber content and composition of the polysaccharides present and also that the concentration of enzyme addition is an important factor in the effectiveness them.

Conclusion The addition of enzyme extract containing fibrolytic enzymes increased the gas production of corn silage.

References

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Table 1. Equations and coefficient of determination adjusted for cumulative gas production.

Enzyme doses (mL)	Regression Equations	Time[1]	Gas production[2]	R^2
0.0	$y = -0.1103x^2 + 9.4574x + 104.88$	42.87	307.61	0.93
0.5	$y = -0.1128x^2 + 9.6318x + 109.95$	42.69	315.56	0.92
1.0	$y = -0.1150x^2 + 9.7826x + 104.24$	42.53	312.28	0.92
1.5	$y = -0.1203x^2 + 10.234x + 111.46$	42.54	329.11	0.92
2.0	$y = -0.1120x^2 + 9.7182x + 108.91$	43.38	319.72	0.91
2.5	$y = -0.1173x^2 + 9.9904x + 106.16$	42.58	318.88	0.92
3.0	$y = -0.1249x^2 + 10.527x + 109.36$	42.14	331.17	0.92
3.5	$y = -0.1226x^2 + 10.405x + 106.47$	42.43	327.24	0.92
4.0	$y = -0.1238x^2 + 10.473x + 109.85$	42.30	331.34	0.92

[1] Optimal time of fermentation. [2] Maximum cumulative gas production.

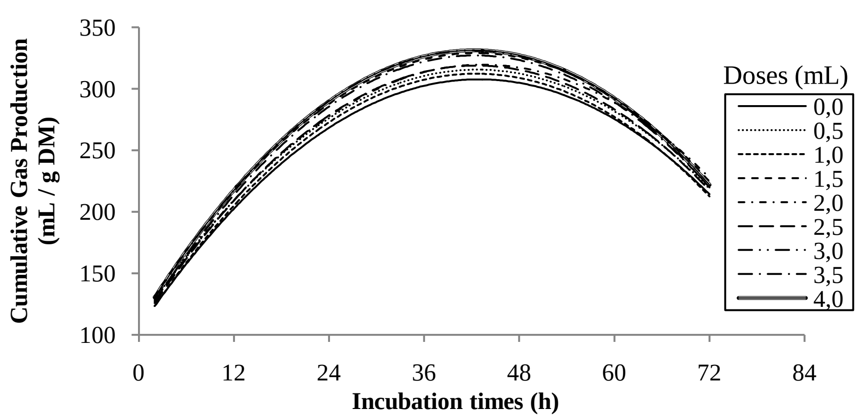

Figure 1. Cumulative gas production of corn silage with different enzyme levels.