Fibrolytic enzymes may improve *in vitro* degradability of tropical forages

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Background

Tropical forages typically may have poor nutritional value due to its high amount of fiber in their chemical composition compromising the sustainability of livestock production and resulting in high enteric methane (CH_4) emissions. In order to increase fiber degradability and energy utilization efficiency from low quality forages, exogenous fibrolytic enzymes have been studied. The aim of this study was to evaluated the effects of increasing dose levels of fibrolytic enzymes extract (FEE) produced by *Trichoderma reesei* using sugar-cane coproduct as substrate, on *in vitro* organic matter degradability, fermentation parameters, total gas and CH_4 production of tropical forages.

Material and Methods

The procedures of this experiment was approved by the Internal Commission for Ethics in Experimentation with Animals (CEUA – CENA/USP 014/2016). Forages samples were analyzed for chemical compostion. The FEE were submited to xylanase (Bailey et al. 1992) and endoglucanase (Wood and Bhat 1988) activities determined at 39°C and pH 6.6. The xylanase activity was 102.5 IU and endoglucanase was 1.45 IU. To determine the total gas and CH_4 production, substrate degradability, and fermentation parameters the *in vitro* gas production technique was used according to Bueno et al. (2005) in a 4 x 3 + 1 factorial arrangement with four dose levels of FEE (5, 50, 500, and 5000 L), three substrates (*Cynodon* spp., *Panicum maximium*, and *Cenchrus ciliares* L.) plus one control (without enzymes addition). Means were compared by Tukey's test ($p<0.05$) using the SAS software.

Figure 1 – Enzyme preparation and *in vitro* gas production technique: inoculation using inoculum from Santa Ines sheep, incubation at 39°C for 24 hours, pressure reading, organic matter degradability and methane quantification.

Results

Table 1 – *In vitro* total gas and CH_4 production and nutrient degradability of tropical forages with increasing dose levels of fibrolytic enzymes extract.

***Cynodon* spp.**		**Dose levels (µL)**					
	Control	**5**	**50**	**500**	**5000**	**S.E.M.**	**p-Value**
GP (mL/g TDOM)	29.97^{c}	36.66^{b}	35.89^{bc}	36.67^{b}	47.71^{a}	1.43	<.0001
TDOM (g/kg)	348.52^{c}	353.01^{bc}	350.65^{c}	365.62^{b}	380.47^{a}	3.55	<.0001
CH_4 (mL/g TDOM)	1.09^{b}	1.65^{b}	1.44^{b}	1.97^{b}	3.05^{a}	0.21	<.0001
Panicum maximum		**Dose levels (µL)**					
	Control	**5**	**50**	**500**	**5000**	**S.E.M.**	**p-Value**
GP (mL/g TDOM)	35.03^{c}	39.70^{bc}	41.95^{b}	39.87^{bc}	55.83^{a}	1.58	<.0001
TDOM (g/kg)	419.49^{b}	403.74^{b}	403.03^{b}	408.21^{b}	450.24^{a}	6.99	<.0001
CH_4 (mL/g TDOM)	1.08^{b}	1.25^{b}	1.37^{b}	1.66^{b}	2.91^{a}	0.16	<.0001
***Cenchrus ciliares* L.**		**Dose levels (µL)**					
	Control	**5**	**50**	**500**	**5000**	**S.E.M.**	**p-Value**
GP (mL/g TDOM)	28.20^{c}	32.64^{bc}	32.94^{bc}	39.19^{b}	47.42^{a}	1.81	<.0001
TDOM (g/kg)	337.81^{b}	317.51^{b}	333.28^{b}	343.37^{b}	375.46^{a}	6.79	<.0001
CH_4 (mL/g TDOM)	1.11^{c}	1.61^{bc}	1.76^{bc}	2.21^{b}	3.11^{a}	0.22	<.0001

GP = gas production; OM = organic matter; TDOM = truly degraded organic matter; CH_4 = methane production; S.E.M. = standard error of the mean. Means with different superscript letters within a same row differ at $p<0.05$ (Tukey's test).

Table 2 – *In vitro* fermentation parameters of tropical forages with increasing dose levels of fibrolytic enzymes extract.

***Cynodon* spp.**		**Dose levels (µL)**					
	Control	**5**	**50**	**500**	**5000**	**S.E.M.**	**p-Value**
C4 (mmol L^{-1})	6.36^{b}	7.77^{a}	7.59^{ab}	8.18^{a}	8.32^{a}	0.31	0.0012
C2:C3	4.53^{a}	3.87^{b}	4.00^{b}	3.96^{b}	4.01^{a}	0.10	<.0001
pH	6.82^{b}	6.83^{ab}	6.87^{a}	6.87^{a}	6.72^{c}	0.01	<.0001
Panicum maximum		**Dose levels (µL)**					
	Control	**5**	**50**	**500**	**5000**	**S.E.M.**	**p-Value**
C4 (mmol L^{-1})	5.39^{b}	7.40^{a}	7.74^{a}	8.09^{a}	7.39^{a}	0.33	<.0001
C2:C3	4.95^{a}	4.10^{b}	4.16^{b}	4.05^{b}	4.25^{b}	0.16	0.0004
pH	6.90^{a}	6.88^{a}	6.85^{ab}	6.87^{ab}	6.82^{b}	0.02	0.0046
***Cenchrus ciliares* L.**		**Dose levels (µL)**					
	Control	**5**	**50**	**500**	**5000**	**S.E.M.**	**p-Value**
C4 (mmol L^{-1})	5.68^{b}	7.45^{a}	7.70^{a}	9.01^{a}	7.51^{a}	0.38	<.0001
C2:C3	4.63^{a}	4.14^{ab}	4.15^{ab}	3.88^{b}	4.16^{ab}	0.16	0.0251
pH	6.81^{b}	6.85^{b}	6.95^{a}	6.82^{b}	6.83^{b}	0.01	<.0001

C4 = butyrate; C2:C3 = acetate-to-propionate ratio; S.E.M. = standard error of the mean. Means with different superscript letters within a same row differ at $p<0.05$ (Tukey's test).

Conclusion

These results indicated that the use of fibrolytic enzymes produced through agricultural resources can be a reliable way to improve degradability of low quality forages, contributing to the sustainability and intensification of livestock production in tropical countries.

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Acknowledgments

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