The effect of rumen isolated bacteria on degradation of sugarcane pith processed with steam and or exogenous enzyme in *in vitro* culture condition

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Introduction Rumen cellulolytic bacteria, such as Ruminococcus albus have a key role in ruminal digestion of plant cell walls, due to their numerical predominance and metabolic diversity (Cheng *et al.,* 1991). Many methods have proved successful in disrupting cell wall material e.g. using enzyme (Eun and Beauchemin, 2007), and or steam (Castro and Machado, 1990). Steam explosion resulted in to disrupt lignocellulosics and partial or complete hydrolysis of hemicellulose fraction in a way which allows improved utilization of cell wall polysaccharides by rumen microbes and improving enzymatic accessibility and digestibility (Castro and Machado, 1990). Researchers reported increasing of *in vitro* DM digestibility for sugarcane pith treated with steam by about 14% (Chaji and Naserian, 2006). The objective of this experiment was to estimate the *in vitro* disappearance of dry matter (DM) and neutral detergent fiber (NDF) of untreated sugarcane pith and treated with steam and or enzyme (4 g/kg DM) by rumen isolated bacteria.

Material and methods Four fistulated sheep was used to collect rumen fluid which fed 250 g concentrate, 550 g lucerne hay and 200 g wheat straw, then centrifuged (1000 rpm, 10 min). Supernatant was used to grow bacteria in medium containing fungicides (benomyle: 500 ppm/ml medium and metalaxyle: 10 mg/ml medium) under anaerobic conditions at 39 ºC for 24 h. These isolates were then used as a source of inoculum for culturing bacteria in a serum bottle containing 45 ml of culture medium of rumen bacteria (Galdwell and Bryant, 1966) and 1g of sugarcane pith as untreated (USP), treated with steam (SSP, at 19 bar for 3 min 70% moisture) and or with exogenous enzyme (ESP, 4 g/kg DM, the enzyme mixture composition was Cellulase, Xylanase, Betaglucanase, Alpha amylase, Pectinase, Phytase, Protease and Lipase as 0.03, 6.6, 10, 0.7, 0.7, 0.07, 0.5 and 3 MU/kg, respectively; Bioproton Pty. Ltd. Co.) under anaerobic conditions (using three times subculture), at 39 ºC for 12, 24, 48, 72 and 96 h (3 replicates per time). The residual substrates of each bottle were then filtered and used to determine the DM and NDF concentrations. Data of DM and NDF disappearance in different times were analyzed as a completely randomized design using the General Linear Model (GLM) procedure of SAS (1990). Duncan's multiple range test was used to compare the means at $P < 0.05$.

Results Disappearance of DM and NDF of samples using rumen isolated bacteria culture are given in Table 1. Sugarcane pith treated with steam had the highest disappearance rate of DM and NDF of in each incubation time ($P < 0.05$) in compared with the other samples, using *in vitro* bacterial culture.

Table 1 Disappearance of DM and neutral detergent fibre of sugarcane pith treated with steam and or enzyme by rumen bacteria

Incubation time (h)	DM disappearance (g /100g)					NDF disappearance (mg/ g)				
	USP	SSP	ESP	s.e.d	P	USP	SSP	ESP	s.e.d	P
12	34.2^{c}	45.0^{a}	39.3^{b}	0.65	<.0001	87.2^{c}	148.0^{a}	105.3^{b}	0.62	<.0001
24	36.4^{c}	51.1^{a}	45.6^{b}	0.51	<.0001	164.4^{c}	217.1^{a}	199.6^{b}	0.71	<.0001
48	47.2^{c}	63.2^{a}	55.1^{b}	0.72	<.0001	236.2^{c}	302.2^{a}	271.1^{b}	0.80	<.0001
72	56.1^{c}	71.1^{a}	60.2^{b}	0.70	<.0001	244.1^{c}	314.1^{a}	285.2^{b}	0.75	<.0001
96	58.3^{c}	73.3^{a}	63.3^{b}	0.42	<.0001	247.3^{c}	317.3^{a}	287.3^{b}	0.62	<.0001

Means with different letters within each row, differed significantly ($P< 0.05$)

Conclusions The present experiment showed *in vitro* bacterial digestion of DM and NDF of sugarcane pith was increased by steam or enzyme, and the effect of steam was more than enzyme. Results of the present study confirmed results Nsereko *et al.* (2000) that reported enzyme products containing xylanases and esterases had stimulatory effects on fibre degradation of alfalfa hay. Also Toussaint *et al.* (1991) reported the increase in enzymic hydrolysis after steam treatment. Results of the present study indicated DM and NDF digestion of sugarcane pith by bacteria could be improved by steam and or exogenous enzyme.

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