In-vitro Fermentation, Microbial Profile and Methanogenesis of Two Fibrous Feeds as Influenced by Exogenous Fibrolytic Enzymes Xylanase and Combination of Xylanase and Cellulase.

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Abstract

A series of experiments was conducted to determine effects of exogenous Xylanase and combination of Cellulase and Xylanase enzymes on *in-vitro* fermentation of *Panicum maximum* (PM) and *Oryza sativa* [Rice straw (RS)] under batch culture system. Xylanase (X) and a mixture of Xylanase and Cellulase (XC) 1:1 ratio were used as enzyme preparations. Four enzyme dosages (50µl /500mg DM (T1), 100µl/500mg DM (T2), 150µl/500 mg DM (T3) and 200µl/500 mg DM (T4) were used for two substrates. As the experiment design Complete Randomized Design with three replicates for each treatment, and the incubation period of 24h. was used. Addition of X and XC improved total *In vitro* gas production (IVGP) significantly ($P<0.05$) as compared to control for both PM and RS. The highest dose (200µ/500mg) of X and XC were associated with the highest IVGP for both PM and RS. Both X and XC improved *in vitro* dry matter digestibility (IVDMD) of PM and RS significantly ($P<0.05$) as compared to control. T4 of X had the highest IVDMD in both substrates. T1 of XC had the highest IVDMD for PM but for RS it was T3. The total Ammonia-N percentage (NH_3-N %) in fermentation liquid was highest for T4 with X for both PM and RS. Maximum NH_3-N production was obtained with T3 and T4 for XC for both substrates. Finally it can be concluded, that supplementation of X and XC improved IVDMD of both PM and RS. T4 of X and T1 of XC were most effective in digestibility of PM when T4 of X and T3 of XC were effective for digestibility of RS. The results reveal that use of fibrolytic enzymes shown to be an effective way to improve the ruminal fermentation characteristics of fibrous feeds as well as a significant way to reduce methanogenesis. However, further investigations are necessary to identify the correct doses.

Key words: Cellulase, in vitro gas production, *Panicum maximum*, Rice straw, Xylanase

Introduction

Guinea grass *(Panicum maximum* var.Jacq*)* and rice straw are generally use as main ruminant feeds in Sri Lanka especially during dry periods. But these type of tropical roughage composed of high percentage of fiber, and contribute to poor digestion, low nutritional value and greater enteric CH_4 production (Seresinhe *et al.,* 2012). Consequently improving fiber digestion is very much important in ruminant animal feeding practices.

Lack of rumen feed utilization efficiency also responsible for enteric methane production in ruminants. Domestic ruminants are accountable for 25% of total anthropogenic methane emission. Methane is the second most effective greenhouse gas emitted from anthropogenic sources and it loss 7-15% of gross energy intake of ruminants (Leng, 1991). Therefore, by enhancing the feed utilization through diet modification is important to reduce methane production.

Increasing the utilization efficiency of available feed resources using supplementation of exogenous fibrolytic enzymes has a great potential to improve fiber digestion and to reduce enteric methane production. Therefore, supplementing exogenous Cellulase contribute to catalyze hydrolysis of 1,4-β-D-

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glucosidic linkages in cellulose and Xylanases catalyze hydrolysis of 1,4-β-D-xylosidic linkages in xylans, the major component of hemicellulose. This will contribute to improve feeding value of roughage by enhancing cell wall digestion.

This study evaluated the effects of Xylanase alone or in combination of Cellulase : Xylanase enzyme mixtures on ruminal fermentation, rumen Ammonia Nitrogen (NH_3-N) synthesis and the effect on ruminal protozoa count on Guinea grass and rice straw. Further an attempt was taken to identify the best enzyme doses for both X and X:C.

Methodology

This procedure is based on the batch culture *in vitro* method described by Goering, H.K. and Van Soest, P.J. (1970).

***In vitro* gas production technique**

Rumen fluid was collected after morning feeding using a mouth tube donor ruminant animal (cattle). Rumen fluid was strained though three layers of cheese cloth in to pre-warmed vacuum flask.

Samples (500 mg) of Panicum maximum and rice straw were oven dried and subsequently weighed into 100 ml glass bottles and allowed for pre-incubation with relevant enzyme concentrations for 24 hours. Enzyme concentrations of 50 µl, 100 µl, 150 µl and 200 µl were for 500 mg of each substrate. Glass bottles filled with 42 ml of medium consisting with micro mineral solution, macro mineral solution, buffer solution, resazurin, Na_2S and tryptone. Medium was prepared incessant flow of CO_2 to ensure anaerobic conditions. Thereafter, glass bottles were sealed using aluminium crimp sealer and placed in a shaking water bath at 39 °C under dark environment to provide conditions same as rumen.

***In vitro* gas production (IVGP)**

Gas production was recorded after 4,8,12,16,20,24 hours of incubation.

Methane production

Gas samples in first 4 hour time interval were collected to pre-evacuated exetainer and analyzed by using gas chromatograph.

***In vitro* dry matter digestibility (IVDMD)**

At the end of fermentation period, fermented residue was filter into pre-weighed filter bag and oven dried for 48 hours at 55°C.

Ammonia production

Ammonium concentration in fermentation liquid was determined using kjeldhal method.

Protozoa counts

Protozoa counts were taken using a Burker type counting chamber.

Statistical analysis

Analysis of variance (ANOVA) was performed on IVGP, IVDMD, ammonia nitrogen % with SPSS 17.0 statistical package and the mean differences were tested using the Least Significant Difference (LSD). Descriptive analysis was done using Microsoft Excel 2010 package.

Results and discussion

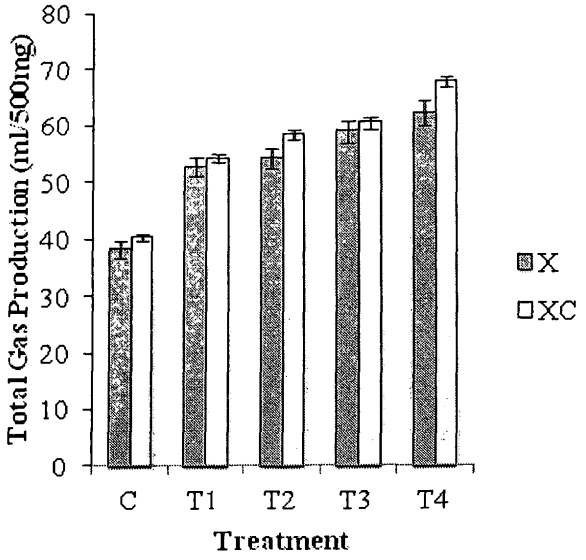

Figure 1 (a). *In-vitro* total gas production in different enzyme treatments for *Panicum maximum*

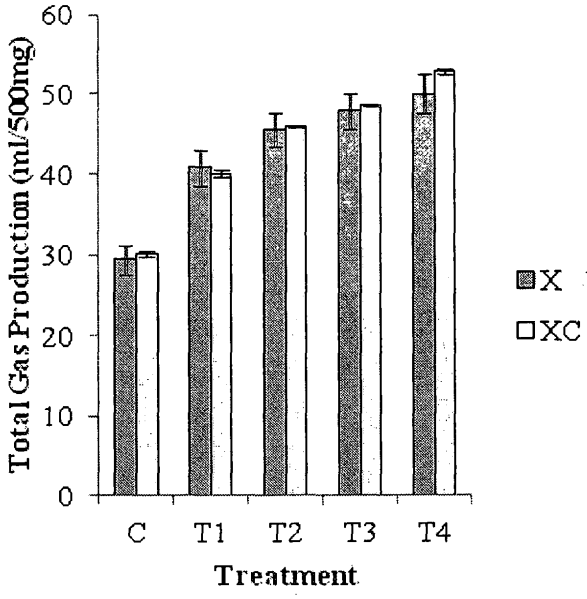

Figure 1 (b). *In-vitro* total gas production in different enzyme treatmentsfor Rice straw

Xylanase and XC resulted a significantly higher total gas production ($p<0.05$) in rice straw and *Panicum maximum* as compared to control. Although not significant ($p>0.05$), T4 had the highest gas production as compared to other treatments. Higher total gas production in enzyme treatments is an indication of improved digestibility particularly carbohydrates (Menke and Steingass,1988). Methane is a result of incomplete digestion and in this study there was no significant difference between the control and treatments for methane production. Percent Methane production in *Panicum maximum* with X was 7.14±0.83 and with XC, 4.11±0.48 and for rice straw with X was 10.95±1.37 and with XC, was 6.37±0.79. The results suggests that, X:C mixture significantly reduced methane production when compared with the X.

Table 01. Influence of different enzymatic treatments (X and XC) on IVDMD, Ammonia Nitrogen % and protozoa count of Panicum maximum and Oryza sativa (Rice straw) after 24h in-vitro rumen fermentation.

Substrate	Treatment	IVDMD%	NH_3-N%	Protozoa count (Number/ml)
	XC	47.44±1.4^{a}	100±0^{a}	2960±854^{a}
	XT1	51.53±1.02^{b}	111.35±7.41^{ab}	2220±427^{a}
	XT2	52.96±0.27^{b}	118.04±8.25^{b}	1973.33±26^{a}
	XT3	52.13±4.82^{b}	123.83±10.6^{b}	2713.33±246^{ac}
	XT4	53.29±0.56^{b}	125.75±9.65^{b}	3700±1130^{bc}
Guinea grass				
	XCC	46.96±0.01^{a}	100±0^{a}	244±244^{a}
	XCT1	52.64±0^{a}	111.6±3.14^{a}	733±423^{a}
	XCT2	50.62±0.02^{c}	109.28±24.64^{a}	978±244^{a}
	XCT3	50.04±0.01^{c}	134.39±16.64^{a}	2200±733^{b}
	XCT4	51.91±0.01^{b}	114.87±7.65^{a}	1467±423^{a}
	XC	32.27±0.47^{a}	100±0^{a}	2960±740^{a}
	XT1	36.31±0.02^{b}	108.88±6.78^{b}	2960±854^{acd}
	XT2	38.09±1.04^{c}	113.03±7.17^{b}	2651.67±222^{a}
	XT3	38.82±1.16^{c}	112.46±2.58^{b}	5180±1281^{bc}
	XT4	39.16±0.21^{c}	122.62±3.49^{c}	4933.33±889^{bd}
Rice Straw				
	XCC	34.84±0.01^{a}	100±0^{a}	1222±244^{a}
	XCT1	37.58±0.01^{b}	106.45±8.85^{a}	2200±0^{b}
	XCT2	37.76±0^{bc}	104.41±3.98^{a}	978±488^{a}
	XCT3	38.29±0^{b}	103.54±2.13^{a}	489±244^{b}
	XCT4	36.89±0^{c}	111.57±2.79^{b}	733±423^{a}

within a column with differing superscripts are significantly different ($P< 0.05$).

All enzyme treatments had significantly higher IVDMD than the control. T4 had highest IVDMD for X while, T1 for *Panicum maximum* and T3 for rice straw had highest IVDMD with XC. Colombatto and Beauchemin (2003) reported exogenous enzymes improve digestibility through removing physical barriers and enhancing bacterial colonization. The correlation (R^2) between IVGP and IVDMD for XC was 0.59 for *Panicum maximum* and 0.65 for rice straw. This suggests that nearly 60% or of IVDMD was explained by the gas production for *Panicum maximum* and rice straw respectively. Menke and Steingass (1988) reported that *in-vitro* gas production technique could be adopted to estimate the in-vivtro fermentation of forages.

Enzyme treatments had higher NH_3-N production than control and T4 had the highest NH_3-N production when X was added while, T3 for *Panicum maximum* and T4 ($p<0.05$) for rice straw with XC added. According to present results it could be suggested that enzyme addition with roughages has influence to increase NH_3-N production that provides evidence for synergetic relationship between enzyme and ruminal microbial growth (Seresinhe *et al.,* 2012). The correlation (R^2) between total mean NH_3-N production and cumulative gas production for X was 0.95 in Panicum maximum and in rice straw it was 0.92 which indicates a strong correlation between in-vitro gas production and NH_3-N production.

Ruminal protozoa assist in the plant cell wall digestion. However, according to reports of increased population protozoa in the rumen contributes to more enteric methane production (Leng, 1991). In this experiment, T1, T2 and T3 treatments of *Panicum maximum* had lower protozoa count with X, compare to control but not significantly different. T2, T3 and T4 treatments for rice straw added with XC had lower protozoa count than control and T3 had significantly higher count with control.

According to present results it can be concluded that, supplementation of X and XC with *Panicum maximum* and rice straw has ability to improve IVDMD and provide evidence to that through increase of total gas production when compare to control. In addition, enzyme supplementation has effect on reduction of rumen protozoa count and rumen microbial protein synthesis, the indicator of synergistic effect between enzyme and rumen microorganisms. Enzyme combination was more effective in reducing methane production. However, further experiments should be necessary for identify actual effect on rumen microorganisms. Considering all aspects T4 (200µl enzyme/500mg) was found to be the best enzyme treatment for X and T1 (50 µl enzyme/500 mg DM substrate) with Panicum maximum and T3 (150 µl enzyme/500 mg DM substrate) with rice straw was best for XC.

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