

Effects of fibrolytic enzymes on *in vitro* ruminal fermentation and methane production from *Panicum maximum* (Wild Guinea grass- Ecotype A) and rice straw (*Oryza sativa*)

Thakshala Seresinhe^A, R Mayes^B and NE Odongo^C

^A Department of Animal Science, Faculty of Agriculture, University of Ruhuna, Sri Lanka

^B James Hutton Institute, Craigiebuckler, Aberdeen, Scotland

^C International Atomic Energy Agency, Vienna, Austria

Contact email: thakshas@ansci.ruh.ac.lk

Keywords: Methane production, animal productivity, feed utilisation.

Introduction

The wide gap between an animal's energy requirement and the nutrients available from feeds is a major constraint in animal productivity and should urgently be addressed with novel methods to improve feed utilization and efficiency (Murad and Azzaz 2010). Forages such as Guinea grass (*Panicum maximum*) and agricultural by products such as rice (*Oryza sativa*) straw play a vital role as animal feeds in the tropics. However, the total energy requirement of animal cannot be met due to the high fiber content and low digestibility of these feeds. The low digestibility of these feeds not only limits the available energy to the animal, it also accelerates enteric methane (CH₄) production a potent greenhouse gas. Supplementation of ruminant diets with exogenous enzymes has recently gained considerable attention as a promising area with potential to improve animal productivity through enhanced digestibility and environment impact. As a starting point in the screening and selection of suitable enzymes and/or enzyme additives to use as feed additives, this study aimed to determine the effects of fibrolytic enzymes on *in vitro* ruminal fermentation and methane production of two fibrous feeds in artificial ruminal conditions.

Methods

Experimental design

Samples of Wild Guinea grass (*Panicum maximum*) and rice straw (*Oryza sativa*) were collected and used as two substrates after drying at 55°C for 48 hours and grinding through a 1 mm screen. Six enzyme treatments including a control were evaluated in a randomized complete block design using the two substrates. The treatments tested were: No enzyme (CON), cellulase from microorganisms (Dyadic Cellulase Plus) at 80 µl (T1) and 120 µl (T2), xylanase from microorganisms (Dyadic Xylanase Plus) at 80 µl (T3) and 120 µl (T4), a mixture of Cellulase/Xylanase at 40:40 µl (T5) and 60:60 µl (T6). Enzymes (20 IU/g forage dry matter (DM)) were applied directly onto forages 24 h before incubation with buffered rumen fluid at 39°C.

In vitro gas production (IVGP)

In vitro gas production was determined as described by Menke and Steingass (1988). Rumen fluid was collected in a pre-warmed flask from two rumen-fistulated donor bulls before morning feeding at the experimental farm of the James Hutton Institute in Aberdeen, UK and strained through four layers of gauze. All laboratory handling of rumen fluid was carried out under a continuous flow of CO₂. Incubation of 200 mg of substrates with the six enzyme treatments and the control in 100 ml glass syringes fitted with plungers was done in triplicate. Artificial rumen conditions were provided by transferring 30 ml of buffered anaerobic rumen fluid (a mixture of 10 ml of rumen fluid and 20 ml of buffer solution) into each glass syringe. Two blank samples each containing 30 ml of medium were also included. The syringes were placed in an incubator set at 39°C and rotated during the first 4 h. Gas production was recorded after 4, 8, 12, 24 and 48 h of incubation and gas samples were collected in pre-evacuated exetainers for analysis of CH₄.

Determination of IVDMD and CH₄

The remaining solid component at the end of incubation was used to determine *in vitro* dry matter digestibility (IVDMD) using the oven dry method by drying the samples at 55°C for 48 hours. Methane was analyzed using a Hewlett Packard Gas Chromatograph (Model 5890, Series II, Avondale, PA, USA).

Statistical analysis

Analysis of variance (ANOVA) of all parameter was performed using SAS (2010) statistical package and the mean differences were tested using the Least Significant Difference (LSD) test.

Results and Discussion

IVGP of Guinea grass steadily increased ($P < 0.05$) with all enzyme treatments compared with the control. Similarly, IVGP was increased ($P < 0.05$) in rice straw over the CON. However, the highest activity was observed ($P < 0.05$) during 6 to 24 hour period. In both

Table 1. Cumulative gas production, methane production and dry matter degradability of wild guinea grass (*Panicum maximum*) and rice straw (*Oryza sativa*) incubated with enzymes

Substrate	Treatment	Cumulative gas production (ml /200mgDM)	Methane production (ml/200 mg DM)	Dry matter degradability (%)	Methane to Total gas ratio
Guinea grass	T0	31.0c ±4.2	4.0b ±1.0	0.45c ±0.1	0.13b
	T1	35.0bc ±3.8	4.4ba ±0.9	0.53ab ±0.1	0.13b
	T2	43.2ba ±3.5	4.4ba ±0.9	0.62a ±0.1	0.10a
	T3	50.0a ±3.5	5.1a ±0.9	0.68a ±0.1	0.10a
	T4	47.3a ±3.5	5.0a ±0.9	0.68a ±0.1	0.10a
	T5	42.0ba ±3.5	4.8ba ±0.9	0.63a ±0.1	0.11a
	T6	44.7ba ±3.5	4.3ba ±0.9	0.65a ±0.1	0.10a
	<i>P</i> -value	0.001	0.05	0.05	0.05
Rice straw	T0	23.1b±2.30	3.4b±0.7	0.36.b±0.1	0.15b
	T1	48.2a±3.20	5.6a±0.8	0.54a±0.1	0.12a
	T2	43.3a±2.40	5.6a±0.8	0.53a±0.1	0.13a
	T3	49.2a±3.21	5.7a±0.9	0.55a±0.1	0.12a
	T4	51.4a±3.45	6.4a±0.8	0.56a±0.1	0.12a
	T5	48.6a±3.11	6.2a±0.9	0.55a±0.1	0.13a
	T6	49.2a±3.14	5.8a±0.9	0.55a±0.1	0.12a
	<i>P</i> -value	0.001	0.05	0.05	0.05

Values are means of three replicates ± SE. C, Control ; T1, 80 µl Cellulase ; T2, 120 µl Cellulase ; T3, 80 µl Xylanase ; T4, 120 µl Xylanase ; T5, 40 µl : 40 µl Cell : Xyl ; T6, 60 µl : 60 µl Cell : Xyl.

substrates, the treatments with xylanase increased ($P<0.05$) cumulative IVGP followed by treatments with cellulase:xylanase mixtures. The DM degradation was not affected ($P>0.05$) but was higher ($P<0.05$) than for CON for both substrates. Methane production (ml/200 mg DM) was correlated with total IVGP for both substrates, as also reported by Soliva *et al.* (2008).

However, methane: total gas ratio was lower ($P<0.05$) with all enzyme treatments compared with CON. Xylanase (80µl and 120µl) was the most effective for both substrates followed by cellulase:xylanase mixtures (40:40 µl and 60:60 µl).

Conclusion

These results suggest that fibrolytic enzymes are able to

significantly reduce ruminal CH₄ production relative to total gas production however, further investigations are necessary to clarify these effects *in vivo*.

References

- Murad HA, Azzaz HH (2010) Cellulase and Dairy Animal Feeding. *Biotechnology* **9**, 238-256.
- Menke KH, Steingass H (1988) Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research and Development* **28**, 7-55.
- Soliva CR, Zeleke AB, Clement C, Hess HD, Fievez V, Kreuzer M (2008) *In vitro* screening of various tropical foliage, seeds, fruits and medicinal plants for low methane and high ammonia generating potentials in the rumen. *Animal Feed Science and Technology* **147**, 53-71.