Journal of Animal and Feed Sciences, 13, Suppl. 1, 2004, 67–70

Effect of exogenous fibrolytic enzymes on *in vitro* rumen fermentation of tropical forages*

L.A. Giraldo[1], M.J. Ranilla[2,3], M.L. Tejido[2] and M.D. Carro[2]

[1]Facultad de Ciencias Agropecuarias, Universidad Nacional de Colombia
Calle 64xCarrera 65, Autopista Norte, A.A. 1027 Medellín, Colombia
[2]Departamento de Producción Animal I, Universidad de León
24071 León, Spain

ABSTRACT

The aim of the study was to evaluate the effects of exogenous fibrolytic enzymes on the *in vitro* ruminal fermentation of three tropical forages (Kikuyo grass 1 and 2 and Angleton grass). Three different enzyme preparations were tested: cellulase (CEL), xylanase (XYL) and a 1:1 mixture cellulase:xylanase (MIX). Dry matter disappearance was increased ($P<0.05$) by CEL and MIX for Kikuyo grass 1. The treatment of forages with CEL and MIX increased ($P<0.05$) NDF degradability, except for Kikuyo grass 2. CEL treatment also increased ($P<0.05$) total VFA production of Kikuyo grass 2 and Angleton grass. The results indicate that the treatment of tropical forages with cellulase stimulate their *in vitro* ruminal fermentation, but the xylanase enzyme used did not produce any positive effect.

KEY WORDS: rumen, cellulase, xylanase, tropical forages, batch cultures

INTRODUCTION

Dietary fibre is an important energy source for ruminants. However, its digestion in the rumen is slow and incomplete. Preparations of exogenous cell wall-degrading enzymes, such as cellulases and xylanases, have the potential to hydrolyse forage fibre, thus improving the digestion of some ruminant feedstuffs (Beauchemin et al., 2003). Most of studies have been conducted with enzyme-treated good-quality forages, but data involving application of exogenous enzymes to fibrous feeds are much limited. The objective of this study was to evaluate the effects of exogenous fibrolytic enzymes on the *in vitro* ruminal fermentation of three tropical forages.

* Supported by M.C.Y.T. of Spain, Project AGL2001-0130 and the Excma. Diputación Provincial de León
[3] Corresponding author: e-mail: DP1MRG@UNILEON.ES

MATERIAL AND METHODS

Samples of three tropical forages (Kikuyo grass 1 and 2 and Angleton grass) were ground through a 1-mm screen and fermented *in vitro* with buffered rumen fluid. The chemical composition of forages is given in Table 1. Samples of 500 mg of each forage were accurately weighed into 120-ml serum bottles. Three different enzyme preparations were tested: cellulase from *Trichoderma longibrachiatum* (CEL; Fluka Chemie GmbH), xylanase from rumen microorganisms (XYL; Megazyme International Ireland Ltd.), and a 1:1 mixture cellulase:xylanase (MIX). Solutions of each enzyme containing 5 IU per ml were prepared in 0.1 M sodium phosphate buffer, pH 6.5. Two ml of the corresponding solution were added directly to each bottle 24 h before starting the incubation, and bottles were kept at room temperature (21-23ºC) until incubation. Two ml of 0.1 M sodium phosphate buffer, pH 6.5 were added to bottles corresponding to control treatment. Rumen fluid was obtained before the morning feeding from four rumen-cannulated Merino sheep fed medium-quality lucerne hay *ad libitum*, and mixed with a buffer solution in a proportion 1:4 (v:v) at 39ºC under continuous flushing with CO_2. Bottles were prewarmed (39ºC) prior to the addition of 50 ml of buffered rumen fluid into each bottle under CO_2 flushing. Bottles were sealed with rubber stoppers and aluminium caps and incubated at 39ºC for 24 h. Four incubation runs were performed on different days, so that each treatment was conducted in quadruplicate. In each incubation run, two blanks were included to correct the gas production values for gas release from endogenous substrates and enzyme treatment.

Table 1. Chemical composition of forages incubated *in vitro*, g/kg dry matter

Forage	Organic matter	Crude protein	Neutral-detergent fibre	Acid-detergent fibre
Kikuyo grass 1	901	186	630	264
Kikuyo grass 2	906	133	696	310
Angleton grass	889	39	736	400

Bottles were withdrawn from the incubator 24 h after inoculation and total gas production was measured with a calibrated syringe. Bottles were uncapped and the pH was measured immediately with a pH meter. One ml of the bottle content was added to 1 ml of deproteinizing solution (10% of metaphosphoric acid and 0.06% crotonic acid; w/v) for VFA analysis. The content of the bottle was then transferred to previously weighed filter crucibles and the residue of incubation was washed with 50 ml of hot distilled water and dried at 50ºC for 48 h to calculate DM apparent disappearance. Residues were analysed for NDF to estimate fibre degradability.

Data for each forage were analyzed by ANOVA with four treatments (control (C), CEL, MIX and XYL) and rumen inoculum as main factors. The GLM procedures of SAS (SAS Inst., Inc., Cary, NC) were used for all statistical analyses.

RESULTS AND DISCUSSION

The effects of exogenous fibrolytic ezymes on *in vitro* rumen fermentation of the three forages are shown in Table 2. Final pH was not affected (P>0.05) by added enzymes for any substrate. For Kikuyo grass 1, CEL and MIX treatments increased (P<0.05) gas production, compared to the control and to XYL treatment, but there were no differences (P>0.05) for the other two substrates.

Table 2. Influence of enzyme treatment on final pH, gas production (ml/500 mg substrate), volatile fatty acid production (VFA; µmol), DM disappearance (DMD; %) and NDF degradability (NDFD; %) of tropical forages incubated in batch cultures of mixed rumen microorganisms for 24 h

Substrate and parameter	C	CEL	MIX	XYL	SED
Kikuyo grass1					
pH	6.70	6.67	6.66	6.67	0.045
gas	61.1^{b}	68.1^{a}	67.2^{a}	61.9^{b}	1.77
DMD	48.6^{b}	51.5^{a}	51.7^{a}	48.7^{b}	1.13
NDFD	41.3^{b}	46.0^{a}	44.7^{a}	42.2^{b}	0.79
total VFA	1911^{ab}	2120^{a}	2018^{ab}	1785^{b}	103.5
Kikuyo grass2					
pH	6.68	6.66	6.67	6.68	0.007
gas	56.5	59.9	58.7	55.6	2.38
DMD	42.8	42.1	42.0	42.4	1.42
NDFD	36.5	35.4	36.2	36.9	1.85
total VFA	1718^{b}	1948^{a}	1686^{b}	1784^{ab}	82.3
Angleton grass					
pH	6.65	6.61	6.62	6.62	0.038
gas	61.9	67.8	62.3	62.2	2.50
DMD	37.6	40.8	39.6	38.4	1.14
NDFD	35.4^{b}	40.0^{a}	38.6^{a}	35.2^{b}	1.23
total VFA	1781^{b}	2216^{a}	1961^{ab}	1675^{b}	144.8

[1] treatments, C - control, CEL - cellulase, MIX - cellulose:xylanase mixture (1:1), XYL - xylanase; [a,b] mean values within a row not sharing a common superscript letter were significantly different, P<0.05

DM disappearance was increased (P<0.05) by CEL and MIX just for Kikuyo grass 1. Similarly, the treatment of forages with CEL and MIX increased (P<0.05) NDFD, compared to the control, except for Kikuyo grass 2. Similar results were obtained by Feng et al. (1992), who reported that treatment of dry

grass with fibrolytic enzymes improved *in vitro* ruminal fibre digestion. Zinn and Salinas (1999) also showed that a fibrolytic enzyme supplement increased ruminal digestion of diet NDF. However, XYL had no effect ($P>0.05$) on cell wall degradability of any substrate.

It would be expected that, as degradation of a feedstuff increases, as indicated by DMD and NDFD, there would be a concomitant increase in the end-products of that fermentation (i.e. VFA). Compared to control, the treatment of Kikuyo grass 2 and Angleton grass with CEL increased ($P<0.05$) total VFA production. In line with our results, Lewis et al. (1996) reported that enzymes sprayed onto a grass hay:barley diet increased VFA production and NDF digestion. However, in the present experiment, XYL and MIX treatments had no effect ($P>0.05$) on VFA production for any substrate. These results, and those obtained for DMD and NDFD suggest that effectiveness of enzymes varies with the substrate and that the xylanase used in the present experiment did not contribute to ruminal fibrolytic activity.

CONCLUSIONS

Under the conditions of the present experiment, the treatment of three tropical forages with cellulase seemed to stimulate their in vitro ruminal fermentation, but the xylanase used did not produce any positive effect. Although these and other results demonstrate that exogenous fibrolytic enzymes may enhance ruminal utilization of fibrous diets, further study is warranted to investigate specific, optimal enzyme-substrate combinations.

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