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Effects of enzyme application method on *in vitro* rumen fermentation of tropical forages*

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ABSTRACT

The objective of this study was to investigate the effect of method of delivery of a solution contaning cellulase on the *in vitro* rumen fermentation (24 h) of three tropical forages. Enzyme was applied to forages either at the time of incubation or 24 h before. Both cellulase treatments increased (P<0.05) acetate, propionate and total VFA production, as well as neutral-detergent fibre degradability (NDFD) with all forages. NDFD increased significantly (P<0.05) with all forages when 24 h pre-treatment of forage with the enzyme was allowed, and this pre-treatment also tended to increase (P<0.05) gas production with two of the three forages. The results indicate that 24 h pre-treatment of forages with cellulase could increase the efficacy of this enzyme, but the incubated forage affects the results.

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

INTRODUCTION

In the last years many studies have explored the possibility of improving the nutritive value of forage for ruminants by using exogenous enzymes. However, results have been highly variable, and these inconsistencies could be attributed to differences in crude enzyme preparations, type of diets fed to the animals and enzyme application methods (Beauchemin et al., 2003). Some studies (Lewis et al., 1997; Hong et al., 2003) have compared methods of enzyme application, but results were variable. The aim of this study was to evaluate the effects of two application methods on the *in vitro* rumen fermentation of three tropical forages.

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MATERIAL AND METHODS

One sample of Kikuyo grass and two samples of Angleton grass were fermented *in vitro* with buffered rumen fluid. Samples of 500 mg of each forage were accurately weighed into 120 ml serum bottles. One solution of cellulase from *Trichoderma longibrachiatum* (Fluka Chemie GmbH) containing 5 international units (IU) per ml was prepared in 0.1 M sodium phosphate buffer (pH=6.5). Two ml of the solution were added directly to each bottle either 24 h before incubation (E24) or just at time of incubation (E0). Bottles dosed 24 h before incubation were kept at room temperature (21-23ºC), and 2 ml of buffer were added to bottles corresponding to control treatment. Rumen fluid was obtained from four rumen-cannulated Merino sheep fed medium-quality lucerne hay *ad libitum*. Rumen fluid was 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. 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 for each treatment were included to correct the gas production values for gas release from endogenous substrates and enzyme treatment. After 24 h of incubation total gas production was measured with a calibrated syringe using a pressure transducer; bottles were then uncapped, the pH was immediately measured and one ml of the bottle content was taken for volatile fatty acid (VFA) analyses. The content of the bottles was transferred to previously weighed filter crucibles and the residues of incubation were analysed for neutral-detergent fibre (NDF) to estimate fibre degradability (NDFD).

Data relative to fermentation parameters were analysed for each forage by ANOVA with enzyme treatment (control, E24 and E0) and rumen inocula as main factors. The sums of squares were further partitioned by orthogonal polynomial contrasts to analyse differences among treatments, and the contrasts were distributed as follows: 1. control vs both enzyme treatments, and 2. E24 vs E0. The GLM procedures of SAS (SAS Inst., Inc., Cary, NC) were used for all statistical analyses.

RESULTS

The 24 h pre-treatment of forages with cellulase decreased ($P<0.05$) the NDF content of all forages (Table 1). Acid-detergent fibre content of forages was decreased ($P<0.05$) by this enzyme pre-treatment with Kikuyo grass and a trend ($P<0.10$) was observed with Angleton grass-1.

The treatment with cellulase enzyme increased ($P<0.05$) acetate, propionate and total VFA production, as well as NDFD, with all forages. Gas production was increased ($P<0.05$) by cellulase treatment with Kikuyo grass and Angleton grass-1 and a trend ($P<0.10$) to a higher gas production was observed with Angleton grass-2. Enzyme application method did not affect ($P>0.05$) VFA production with Kikuyo grass

Table 1. Influence of 24 h pre-treatment of tropical forages with a cellulase preparation (E24) on their neutral-detergent fibre (NDF) and acid-detergent fibre (ADF) content, g/kg DM

Forage	NDF			ADF		
	control	E24	SED	control	E24	SED
Kikuyo grass	630[b]	613[a]	0.20	264[b]	258[a]	0.11
Angleton grass-1	736[b]	711[a]	0.41	400	394	0.15
Angleton grass-2	733[b]	709[a]	0.42	332	328	0.27

[a,b] - for each parameter means within the same row with different superscript differ (P<0.05)

and Angleton grass-1, but pre-treatment of Angleton grass-2 with cellulase increased (P<0.05) acetate production. NDFD increased significantly (P<0.05) with all forages when a pre-treatment of forage with the enzyme was allowed, and this pre-treatment also tended to increase (P<0.05) gas production with both Angleton grass forages.

Table 2. Influence of application method of a cellulase preparation on final pH, volatile fatty acid production (VFA; μmol), gas production (ml/500 mg sample) and neutral-detergent fibre degradability (NDFD; %) after incubating three forages with buffered rumen fluid for 24 h

Indices	Treatment			SED	Statistical significance of the treatment effect (P =)	
	control	E-24	E-0		C1	C2
Kikuyo grass						
pH	6.70	6.67	6.65	0.007	0.011	NS
total VFA	1911	2120	2189	28.9	0.003	NS
acetate	1342	1468	1525	22.0	0.007	NS
propionate	395	444	456	5.89	0.002	NS
butyrate	111	135	134	3.26	0.005	NS
gas	59.2	66.0	64.4	0.75	0.004	NS
NDFD	41.3	46.0	43.6	0.39	0.002	0.019
Angleton grass-1						
pH	6.65	6.61	6.63	0.008	NS[†]	NS
total VFA	1781	2216	2112	61.9	0.012	NS
acetate	1284	1578	1496	43.1	0.015	NS
propionate	336	436	422	13.0	0.006	NS
butyrate	125	149	148	5.49	0.045	NS
gas	59.8	65.6	61.9	0.88	0.041	NS[†]
NDFD	35.4	40.0	37.6	0.49	0.007	0.047
Angleton grass-2						
pH	6.63	6.60	6.63	0.010	NS	NS
total VFA	1989	2234	2089	31.2	0.019	NS[†]
acetate	1407	1570	1439	20.0	0.030	0.017
propionate	377	441	441	7.53	0.003	NS
butyrate	165	171	157	7.50	NS	NS
gas	68.4	74.7	68.8	1.06	NS[†]	0.031
NDFD	40.8	45.6	43.0	0.66	0.023	NS[†]

[1] treatments, C - control, E24: cellulase applied 24 h before *in vitro* incubation, E0 - cellulase applied immediately before *in vitro* incubation

[2] orthogonal contrasts; C1 - C vs enzyme treatments; C2 - E24 vs E0; NS - P>0.05; NS[†] - P<0.10

DISCUSSION

The observed effects of 24 h pre-treatment with cellulase on the forages NDF content suggest that an hydrolytic action prior to rumen incubation took place. These results agree with those reported by Nsereko et al. (2000), who studied the effects of 2 h pre-treatment with several fibrolytic enzymes on fibre concentration of lucerne hay and observed that some of the enzymatic preparations produced a decrease on the NDF content. In agreement with our results, these authors reported that 2 h pre-treatment with several fibrolytic enzymes increased ($P<0.05$) NDF degradation of lucerne hay at 12 h when compared to 0 h pre-treatment.

Compared to E0 treatment, E24 increased total VFA production by 4.7 and 6.5% for Angleton grass-1 and Angleton grass-2, respectively, but a 3.3% decrease was observed for Kikuyo grass. Hong et al. (2003) obtained no differences in VFA concentrations in the rumen of goats when comparing the effects of 24 h enzyme pre-treatment of a mixed diet with the addition of enzyme to the diet just before feeding the animals, but observed an increase in NDF digestibility when the diet was pre-treated with the enzyme. A lack of effect on rumen VFA concentrations has also been found by Lewis et al. (1996) in steers fed a 70:30 grass hay:barley diet.

CONCLUSIONS

The results seem to indicate that 24 h pre-treatment of forages with a cellulase solution could be more effective in enhancing forage *in vitro* degradability than the addition of the enzyme to forage just prior to incubation, but the results could be affected by the incubated forage.

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