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Limits of Exogenous Fibrolytic Enzymes to Improve Digestion and Intake of a Tropical Grass

C. Assoumaya, M. Boval, J. L. Weisbecker[1], G. Saminadin and H. Archimède*
INRA UR143, Unité de Recherches Zootechniques
Prise d'eau, Petit-bourg, 97170 Guadeloupe, French West Indies

ABSTRACT : The effect of the addition of exogenous fibrolytic enzymes (mainly xylanase and cellulase activities, 15 ml/15 kg of fresh forage), on intake, total tract digestibility and nylon bag degradability of a chopped fresh *Digitaria decumbens* grass was studied at 2 stages of regrowth (21 and 56-day old grasses). Moreover, comparisons between ground and chopped grass were done using the nylon bag degradability method. DM intake (g/kg $BW^{0.75}$) and organic matter total tract digestibility for control and enzyme treatments respectively were 69.1 vs. 65.9 ($p>0.05$) and 0.723 vs. 0.727 ($p>0.05$) with the 21-day old regrowth. Based on the same parameters, values for the 56-day old grass were 58.1 vs. 52.7 ($p>0.05$) and 0.621 vs. 0.591 ($p>0.05$). Nylon bag degradation at 24 h of the dry matter for control *versus* enzyme treatments were 0.653 vs. 0.70 ($p<0.05$) and 0.644 vs. 0.733 ($p<0.0001$) for the 21-day old chopped and ground forage respectively, whereas with the 56-day old grass, corresponding values were 0.321 vs. 0.392 ($p<0.0001$) and 0.463 vs. 0.481 ($p>0.05$). The positive impact of exogenous fibrolytic enzymes (EFE) on degradability of the young and ground pangola grass may suggest that in some cases, enzyme accessibility to potentially digestible cell wall is a limiting factor in their digestion. (**Key Words :** *Digitaria decumbens*, Sheep, Exogenous Enzyme, Intake, Digestion)

INTRODUCTION

Tropical grasses are often characterised as low quality forages when compared to temperate ones because of their low intake and digestibility (Minson, 1990). Research into technology aiming at increasing digestibility and intake of tropical grass is a challenge.

Some researchers have shown that the use of exogenous fibrolytic enzymes (EFE) can enhance fibre degradation, *in vitro* (Forwood et al., 1990; Varel et al., 1993; Feng et al., 1996; Hristov et al., 1996) and *in situ* (Lewis et al., 1996; Baah et al., 2005). While some authors (Beauchemin et al., 1999; Yang et al., 1999) have confirmed these results *in vivo*, others authors (Firkins et al., 1990; Varel and Kreikemeier, 1994; Kung et al., 2000) have found the opposite result. In a review, Wang and McAllister (2002) indicated that there is ample evidence that EFE exert dissimilar effects on different feed types and that each preparation reacts differently according to substrate. All published experiments have been done on temperate forage or straw. No results have been published on tropical grass which represents a specific and original biological model. Cell wall content is lower in temperate than tropical grasses. The latter are characterised by cell types with thickened secondary wall, such as in the vascular bundles, sclerenchyma strands, epidermis and parenchyma bundles (Wilson, 1994). Wang and McAllister (2002) indicated that commercially available enzyme preparations still lack the model activities that can overcome the factors limiting ruminal digestion of plant cell walls, especially for low quality forage. The objective of this study was to test this hypothesis on tropical forage.

* Corresponding Author: H. Archimède. Tel: +590-25-59-43, Fax: +590-25-59-36, E-mail: Harry.Archimede@antilles.inra.fr
[1] INRA UE 503, Unité Expérimentale en Production et Santé Animale, Petit-bourg, 97170 Guadeloupe, France.
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MATERIALS AND METHODS

Location

Research was carried out in 2004 at the experimental animal station at the National Agronomic Research Institute (INRA) in the French West Indies (Guadeloupe, latitude 16.16N, longitude 61.30W). Temperatures ranged on average from 21°C to 31°C. Mean rainfall on the experimental site was 3,000 mm/year. Rainfall was regular during the experiment.

Table 1. Composition and activities of the experimental fibrolytic enzyme

Ingredients	
Trichoderma reesei fermentation extracts	80-86%
Sorbitol	10-12%
Salts	4-5%
Preservatives	0.25-0.3%
Cellulase activity (DNS-CMC)	5,500 IU/g
Xylanase activity (DNS-ABX)	>10,000 IU/g

Experimental design, animals, diets and feeding

Eight Black-belly rams (mean liveweight: 51.28±3.62 kg) were used in this experiment. They were fed during 2 successive 30-days periods with 21- and 56-day old regrowth fertilised fresh *Digitaria decumbens* (pangola) grasses respectively. During the first period, all the animals received the 21-day regrowth (or immature forage), four animals without enzyme (E-) and four with enzyme (E+). During the second period, all the animals received the 56-day regrowth (or mature forage), four animals without enzyme (E-) and four with enzyme (E+). Throughout the experiment, animals with enzyme during the first period was maintained with enzyme during the second period. The harvest has been planned to have daily a 21-day grass during the first period and a 56-day grass during the second period respectively. All the animals were fitted with ruminal cannulae and maintained in metabolism cages. The 30-day experimental period consisted of 14 days of adaptation to the diet, 5 days of intake and total tract digestibility measurements and 7 days of nylon bag incubation in the rumen. The pangola grass was cut early every morning and chopped (5 cm-length) using a forage chopper before being offered. The amount of forage provided was 1.15 times greater than the voluntary intake estimated during periods of adaptation. Water and mineral blocks were offered for *ad libitum*.

Trial 1. Intake and total tract digestibility : Four rams consumed chopped grass mixed with tap water without enzymes (one litre to 15 kg of fresh forage). Four other rams were fed with chopped forage mixed with a commercial fibrolytic enzyme solution (Nutreco Rumizyme-alpha) (Table 1). 15 ml of this enzyme were diluted with 100 times its volume in tap water. One litre of this diluted enzyme was mixed with 15 kg of fresh grass. The enzyme contained mainly xylanase and cellulase activities.

Trial 2. In sacco degradability : Three rams were fed with chopped forage mixed with tap water (1 litre to 15 kg of fresh forage). Nylon bags filled with 15 g of fresh chopped or 3 g of dry (freeze dried) ground forage mixed for 10 minutes with tap water were incubated in their rumen for 24 or 48 h. At the same time, three others rams were fed with chopped forage mixed with the diluted fibrolytic enzyme solution (1 litre to 15 kg of fresh forage). Nylon bags filled with 15 g of fresh chopped or 3 g of dry (freeze dried) ground forage mixed for 10 minutes with the diluted fibrolytic enzyme solution were incubated in their rumen for 24 or 48 h.

Nylon bags mixed with tap water were incubated in rumen of animals fed with forage mixed with tap water. Nylon bags mixed with enzyme were incubated in rumen of animals fed with forage mixed with enzyme.

Measurements

Intake and total tract digestibility were measured by weighing the daily amounts of food offered, refusals and faeces. Degradability of forage offered was measured using the nylon bag method. The nylon bags were 10 cm×5 cm, with a pore size of 50×50 μm and filled with 15 g of fresh chopped forage or 3 g of freeze dried then ground forage in the basal diet. Fresh grass was manually cut into particles of 2 mm mean length. Frozen dry forage was ground (1 mm). Incubation times in the rumen were 24 and 48 h.

Chemical analyses

DM content of fresh forage and refusals was determined daily by drying at constant weight at 60°C in a forced-draught oven. DM content of faeces was determined in similar conditions using a representative sub-sample. The latter came from a sample obtained by pooling 10% of the daily amount of faeces excreted by each animal. It was then stored (-20°C). Samples were ground (1 mm) prior to chemical analysis. Organic matter (OM) content was measured after a 10 h pyrolysis at 550°C. Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL) were estimated following the methods of Van Soest et al. (1991). Nitrogen concentration of forage was determined using the Dumas method (AFNOR, 1988).

Statistical analyses

Data were analysed using the General Linear Model procedure of SAS (1987). Concerning data on nylon bag degradation, the model included age of forage (df 1), enzyme treatment (df 1), forage granulometry (df 1), and enzyme×granulometry interaction. With regard to intake and digestibility, the model took into account the effects of forage (df 1), and enzyme treatment (df 1).

RESULTS

Dietary composition

The mean composition of *Digitaria decumbens* was reported in Table 2. The 56-day old grasses were characterised by lower nitrogen and higher fiber contents as compared with the 21-day old grasses.

Table 2. Chemical composition (g/kg DM) of a 21- and 56-day old fertilized *Digitaria decumbens* grass

Constituents (g/kg)	Regrowth age (days)	
	21	56
Dry matter content (g/kg)	180	192
Constituents (g/kg Dry matter)		
Organic matter	909	900
Crude protein	151	64
Neutral detergent fibre	727	751
Acid detergent fibre	362	406
Acid detergent lignin	54	72

The values are means of 7 samples.

Intake

DM intake and other components were reported in Table 4. No difference was observed between control and enzyme treatments irrespective of the forage age and the dry matter component. Moreover, intake was higher with the younger forage.

Total tract digestibility

DM, OM, NDF and ADF total tract digestibility of diets were reported in Table 4. No difference was observed between control and enzyme treatments irrespective of the forage age and some dry matter component (OM, NDF, ADF). Total tract digestibility was lower ($p<0.05$) with the older forage irrespective of the dry matter component.

In sacco degradability

Values for *in sacco* degradability were reported in Table 3. Dry matter degradability of 21-day forage increased significantly ($p<0.05$) with the enzyme supplement irrespective of the incubation time and the physical

Table 3. Degradability of a 21- and 56-day old fresh chopped (G-) and frozen-dry ground (G+) *Digitaria decumbens* grass, sprayed with tap water (E-) and a 1/100 diluted enzymatic solution (E+), in nylon bags incubated for 24 and 48 h in the rumen of six rams feeding similar grasses

Age of regrowth (days)	21			56		
Treatment	E-	E+	SE	E-	E+	SE
Degradability of dry matter (%)						
G- 24 h	65.30^{a}	70.04^{b}	1.30	32.14^{f}	39.25^{g}	1.50
G+ 24 h	64.42^{a}	73.30^{b}	1.30	46.27^{h}	48.10^{h}	1.26
G- 48 h	78.68^{c}	83.32^{de}	1.30	49.26^{h}	49.43^{h}	1.42
G+ 48 h	81.47^{cd}	85.80^{e}	1.26	59.19^{i}	57.02^{i}	1.26
Degradability of neutral detergent fiber (%)						
G- 24 h	58.01^{a}	63.13^{b}	1.65	19.24^{e}	26.20^{f}	1.90
G+ 24 h	54.83^{a}	66.52^{b}	1.65	32.44^{g}	35.26^{g}	1.60
G- 48 h	75.24^{c}	79.01^{cd}	1.65	40.24^{h}	35.57^{gh}	1.80
G+ 48 h	77.57^{c}	81.92^{d}	1.60	49.80^{i}	46.51^{i}	1.60
Degradability of acid detergent fiber (%)						
G- 24 h	43.89^{ac}	52.24^{e}	2.00	15.13^{h}	22.71^{i}	2.28
G+ 24 h	39.97^{ab}	58.39^{d}	2.00	29.28^{j}	34.63^{bk}	1.93
G- 48 h	66.19^{f}	70.07^{f}	2.00	36.99^{bk}	31.52^{jk}	2.17
G+ 48 h	70.78^{f}	76.65^{g}	1.93	48.81^{ce}	45.42^{c}	1.93

$^{a, b, c, d, e, \cdots}$ Means within each category of degradability of components lacking a common superscript letter differ ($p<0.05$).

Table 4. Dry matter, organic matter, neutral detergent fiber and acid detergent fiber Intake and total tract digestibility of a 21- and 56-day old fresh chopped and frozen-dry ground *Digitaria decumbens* grass, sprayed with tap water (E-) and a 1/100 diluted enzymatic solution (E+) given *ad libitum* to eight Black-belly rams

Age of regrowth (days)	21			56		
Enzyme treatment	E-	E+	SE	E-	E+	SE
Intake (g/day/$LW^{0.75}$)						
Dry matter	69.09^{a}	65.96^{a}	4.56	58.10^{b}	52.72^{b}	4.56
Intake (g/day)						
Dry matter	1,258.82^{a}	1,284.54^{a}	76.49	1,108.04^{ab}	1,017.39^{b}	76.49
Organic matter	1,155.52^{a}	1,179.42^{a}	67.24	949.81^{ab}	850.75^{b}	86.37
Neutral detergent fiber	914.26^{a}	928.96^{a}	57.07	835.86^{a}	763.84^{a}	57.07
Acid detergent fiber	449.85^{a}	455.23^{a}	29.37	450.34^{a}	410.15^{a}	29.37
Total tract digestibility						
Dry matter	0.663^{a}	0.689^{a}	0.019	0.560^{b}	0.532^{b}	0.019
Organic matter	0.723^{a}	0.727^{a}	0.015	0.621^{b}	0.591^{b}	0.015
Neutral detergent fiber	0.688^{a}	0.702^{a}	0.023	0.557^{b}	0.529^{b}	0.023
Acid detergent fiber	0.652^{a}	0.656^{a}	0.022	0.549^{b}	0.525^{b}	0.022

$^{a, b}$ Intake and total tract digestibility means within a row lacking a common superscript letter differ ($p<0.05$).

treatment. The same tendency was observed with NDF and ADF although differences were not always significant. Concerning the mature forage, significant positive effect of enzyme was registered only for the chopped forage that was incubated for 24 h. Irrespective of the forage regrowth, the interaction between incubation time and enzymatic treatment was always significant ($p<0.001$).

The interaction between physical treatment and enzyme supplement was not significant irrespective of the grass regrowth. Physical treatment improved ($p<0.05$) the degradability of old forage whereas no difference was observed with young forage.

DISCUSSION

The main objective of this work was to test the efficacy of EFE in increasing cell wall digestion in tropical grass. Due to large variation of feed value of these last grasses with their maturity, two extreme regrowth ages (21 vs. 56-days) have been studied. Several studies have shown that EFE can improve the rate of feed digestion but their ability to increase the extent of digestion may be limited by a lack of the enzyme that breaks down the core structure of lignin-cellulosic complex (Wang and McAllister, 2002). In our experiment, we used grinding as a means of increasing the total diet particle surface area and the accessibility of potential digestible cell wall to enzymatic activity in the rumen (Journet and Demarquilly, 1967).

In the present study where EFE were added to tropical grass both the degradation and the extent of cell wall digestion (DM, NDF, ADF) increased according to the level of maturity of the forage. This adverse result has to be analysed taking into account of the chemical composition of the plant cell wall. The chemical analysis of forage indicated an increase in the level of lignin with maturation and consequently an increase in the linkage between polysaccharide (cellulose and hemicelluloses) and lignin. This linkage limits the ability of the EFE which did not contain the enzymes (e.g., esterase) to break down the esterified bonds between lignin and carbohydrates. Our results for nylon bags (positive action of grinding on the degradation of the grass) illustrate limited enzyme accessibility to the potentially digestible cell wall. In reality, the positive effect of grinding on dry matter (or NDF and ADF) degradation of the old pangola grass in the control treatment could indicate that enzyme accessibility to potentially digestible cell wall is a limiting factor in their digestion. This hypothesis has been evocated by Archimede et al. (2000) working on similar forages. Consequently, like Eugene et al. (2002), we can hypotheses that with mature fertilized forage the cellulolytic capacity in the rumen (amount of microbial enzymes) is not the first limiting factor in the digestion of old forage but rather the range of colonisation of feed particle by microbes. On the contrary, the lack of effect of grinding on dry matter degradation in the control treatment of the young forage could illustrate that maximal degradability reached.

The lack of positive effect of EFE on the *in sacco* degradability (DM, NDF, ADF) of the 56-day pangola grass at 48 h irrespective of the physical treatment and the incubation time coincides strongly with the hypothesis of Wang and McAllister (2002). The positive effect measured at 24 hours with the same forage probably illustrates a higher rate of degradation with addition of EFE as is typically observed in other studies (Wang and McAllister, 2002). Concerning the 21-day pangola grass, the efficacy of EFE has to be explained by the nature of the cell wall. Our results are similar to those of Wang et al. (2004) who have studied the effect of alkali pre-treatment of straw on the efficacy of EFE. They found that EFE had a significant effect on both the rate and extent of degradation of the pre-treated straw whereas no significant effect was registered with control. They suggested that removal of the phenolic barriers that impede the microbial digestion of the feed may be an additional factor in the alkali treatment. In our study phenolic barriers may have been low for the immature forage and high for the mature forage.

Our results concerning total tract digestibility in which no effect of EFE has been registered irrespective of the forage age, differed significantly from those recorded for the *in sacco* degradation. We hypothesis that nylon bag degradation of cell wall is correlated with rumen digestion, however physical breakdown of feed particle and rumen turnover is not taken into account. Nevertheless precedent results (Archimede et al., 2000) indicated that mean retention time in the rumen with the immature forage was around 24 h against 48 h for the mature. Consequently we hypothesize that the degradability of the ground forage incubated during 24 and 48 h were estimate of rumen digestibility of the immature and mature forages respectively. Generally, 90% of the total tract digestibility of cell wall occur in the rumen but significant variations can be observed (Archimede et al., 1995). Really, contribution of intestine in cell wall digestion can increase if large amount of potentially digestible carbohydrate arrive in the intestine as compensatory intestine cell wall digestion (Ulyatt and Macrae, 1974). The higher nylon bag degradability with EFE applied to immature forage is probably the consequence of higher rate of degradation (Yang et al., 1999) and this last is not compensated by the retention time in the rumen for the no treated forage. Consequently, we hypothesize that contrary with mature forage and immature forage treated for which contribution of rumen in digestion is maximized, with immature forage

no treated with EFE, larger amount of potentially digestible particle arrive in the intestine where a larger part is digested as compensatory intestine cell wall digestion.

Concerning the impact of EFE on intake, our results, like those reported by Feng et al. (1996), Mc Allister et al. (1999), Kung et al. (2000), Wang et al. (2004) on temperate forage or straw, revealed that they have no significant effect. These results seem logical. Really, there is a positive correlation between intake and digestibility of forage and driving force of intake is the rate of rumen turnover. This last one is controlled by the rate of reduction of size of particles mainly via rumination and chewing Kennedy (1995); Forbes (1994) and Poppi 1980. EFE improve rate of degradation (Yang et al., 1999) whereas no effect has been registered on rate of reduction of particle size.

CONCLUSION

In conclusion, in this study, applying EFE to tropical forage had no effect on intake and total tract digestibility irrespective of the age of the grass. Concerning rumen digestion, addition of EFE seems to have beneficial effects on degradation of immature tropical forage (<28 d) whereas no improvement was recorded for mature forage. In order to confirm these preliminary results, further investigations on tropical forage will have to be carried out.

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