



SHORT COMMUNICATION

Effect of Feeding Total Mixed Rations Supplemented With or Without Exogenous Fibrolytic Enzymes on Rumen Degradation Kinetics in Buffalo Bulls

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ABSTRACT

The present study was carried out to determine the *in situ* degradation kinetics of total mixed rations supplemented with or without exogenous fibrolytic enzymes (EFE) using Nylon bag technique in bulls. Four dietary treatments *viz.*, TMR with R: C ratio of 60: 40 (T₁), T₁ supplemented with exogenous fibrolytic enzymes (T₂), TMR with R: C ratio of 70: 30 (T₃) and T₃ supplemented with exogenous fibrolytic enzymes (T₄) were evaluated for their rumen degradable DM, CP, NDF and ADF using three buffalo bulls fitted with a permanent rumen fistula. *In sacco* study revealed that the effective degradability (ED) of DM, CP, NDF and ADF were higher (P<0.01) in TMR with R: C ratio of 60: 40 (T₁) when compared to that with 70: 30 (T₃). Further, the % effective degradability of DM, CP, NDF and ADF increased (P<0.01) with the supplementation of EFE in TMRs irrespective of R: C ratio. It is therefore concluded that supplementation of EFE in TMRs has a positive effect irrespective of the R: C ratio as evidenced by their increased degradation rates in the rumen.

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INTRODUCTION

Cereal crop residues form the main source of feed for ruminants especially during the dry season and winter. However, these crop residues are poor in nutritive value owing to their low energy and high fibre content. Improving the degradability of fibrous and non fibrous carbohydrates in the rumen is important for feed utilization in ruminants. Supplementing ruminant diets with feed enzymes to improve forage utilization has attracted growing attention (Beauchemin *et al.*, 2003). The beneficial effects of fibrolytic enzymes in ruminant diets appear to be a result of improved feed intake (Krueger *et al.*, 2008), which could be attributed to increased ruminal fibre digestion (Singh and Das, 2009). The use of fibrolytic enzymes as feed additives to improve degradation of fibre has been studied under *in vitro* (Bhasker *et al.*, 2012), *in sacco* (Giraldo *et al.*, 2008; Bassiouni *et al.*, 2011) and *in vivo* (Gaafar *et al.*, 2010) conditions, but the results are not consistent. Several factors such as enzyme doses (Colombatto *et al.*, 2007) and type of diet (Pinos-Rodriguez *et al.*, 2008) could

affect the fibrolytic activity of exogenous enzymes. Indeed, the role of fibrolytic enzymes in increasing the degradation of substrates depends on the proportion of concentrates in the diet (Giraldo *et al.*, 2008). Therefore, the objective of this study was to evaluate the effect of supplementation of exogenous fibrolytic enzymes in TMRs containing different roughage concentrate ratio on *in sacco* degradation kinetics in buffalo bulls.

MATERIALS AND METHODS

Preparation and analysis of samples

Maize stover procured locally, was ground in a hammer mill and used as roughage source. The four dietary treatments comprises of TMR's containing two different roughage concentrate ratios supplemented with and without EFE *viz.*, TMR with R: C ratio of 60: 40 (T₁), T₁ supplemented with EFE (T₂), TMR containing R: C in 70: 30 ratio (T₃) and T₃ supplemented with EFE (T₄). The ingredient composition of total mixed rations was presented in table 1. The exogenous fibrolytic enzyme (Fibrozyme; fermentation extracts of *Aspergillus niger*

and *Trichoderma viridae* containing cellulases and hemicellulases; 100 IU as xylanase/g) used in the present study was procured from M/s Alltech Inc., Nicholasville, USA. Samples of total mixed rations were then ground to pass through a 1 mm screen for nylon bag incubation. The samples were analyzed for proximate constituents (AOAC, 2007) and fibre fractions (Van Soest *et al.*, 1991).

Feeding regimen

Three buffalo bulls fitted with a permanent plastic rumen fistula were used in the present study. The bulls were fed daily a basal diet comprising of 5 kg hybrid napier, 4 kg paddy straw and 1.5 kg concentrate mixture to meet the nutrient requirements for maintenance (ICAR, 1998). The bulls were housed in well ventilated and hygienic stalls with individual feeding and watering arrangements. All the animals were offered fresh, clean drinking water free of choice.

In situ degradation kinetics

The *in sacco* degradability of DM, CP, NDF and ADF of TMR's containing two different roughage concentrate ratios supplemented with and without exogenous fibrolytic enzyme were determined as per the procedure of Orskov and Mc Donald (1979). The bags with feed samples were incubated in the ventral sac of rumen of each bull for 0, 3, 6, 12, 24, 36, 48 and 72 h and were removed in a sequential method (Osuji *et al.*, 1993). The bags containing samples of 0 h were washed without incubation in the rumen. After removal of bags from rumen they are placed in a bucket of cold water to stop ongoing microbial activity. Then bags were washed under slow running tap water by rubbing between fingers and the thumb finger for ten minutes, oven dried at 70°C for 48 h to a constant weight and DM loss during the incubation was calculated. The CP, NDF and ADF content in the residue was analyzed to determine their respective degradability's. From the degradability data obtained at different intervals, the constant a, b and c were obtained from the expression.

$$P = a + b(1 - e^{-ct})$$

Where, 'P' is percentage of degradability for response variables at t; 't' is time relative to incubation (h); 'a' is highly soluble or readily degradable fraction (%); 'b' is insoluble and slowly degradable fraction (%); 'c' is rate constant for degradation (h); 'e' is 2.7182 (Natural logarithm base). The effective nutrient degradability (%) of total mixed rations were calculated by time measurements and fitted values in NEWAY programme (Model based on Mc Donald, 1981) using a computer, assuming an outflow rate (K) of 0.05%/hr.

Statistical analysis

Data were analyzed statistically as per the procedures suggested by Snedecor and Cochran (1994) using SPSS version 17.0 (SPSS software products, USA) package. Group differences were compared using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The chemical composition of dietary treatments was presented in table 2 while the *in situ* degradation kinetics

of DM, CP, NDF and ADF of total mixed rations containing different roughage concentrate ratios supplemented with and without EFE were shown in Table 3.

The *in sacco* DM degradability values revealed that the instantly soluble fraction 'a', slowly degradable fraction 'b', potential degradable fraction 'a+b' and rate constant 'c' were similar among the treatments. These results corroborated with the findings of earlier workers (Gallardo *et al.*, 2010; Phakachod *et al.*, 2012). However, these results are contradictory to those of Pinos-Rodriguez *et al.* (2008) who reported improvement in potential disappearing fraction (b) and disappearance rate (c)/h by feeding EFE. Further, the present study indicated that supplementation of EFE increased (P<0.05) the EDDM (%) irrespective of the roughage: concentrate ratio. The increased EDDM (%) observed in enzyme treated total mixed rations might be due to increased enzyme activity in the rumen fluid. These results are very consistent with those reported earlier (Chopra *et al.*, 2007; Giraldo *et al.*, 2008; Bassiouni *et al.*, 2011).

The *in sacco* CP degradability values revealed that the instantly soluble fraction 'a' was higher (P<0.01) in TMR containing R: C ratio of 60: 40 than TMR containing R: C ratio of 70: 30. The present study revealed that, supplementation of EFE increased (P<0.01) fraction 'a' in TMR containing R: C ratio of 70: 30 while there was no effect in TMR containing R: C ratio of 60: 40. Further, the slowly degradable fraction 'b', potential degradable fraction 'a+b' and rate constant 'c' were similar among the treatments. The present study also indicated that supplementation of EFE increased (P<0.01) the EDCP (%) irrespective of the roughage concentrate ratio. This may be attributed to the direct effect of the EFE that can randomly release reducing sugars and possibly make more nutrients available for utilization by the microorganisms (McAllister *et al.*, 2001). Similar findings were also reported earlier (Gaafar *et al.*, 2010; Jalilvand *et al.*, 2008; Bassiouni *et al.*, 2011).

In sacco NDF degradability values revealed that the R: C ration had no effect on the instantly degradable fraction 'a'. Further, supplementation of EFE had no effect on instantly degradable fraction 'a' irrespective of the R: C ration in the TMR. This is in line with the findings of earlier workers (Giraldo *et al.*, 2008; Phakachod *et al.*, 2012). In the present study, the slowly degradable fraction 'b' was not affected by EFE supplementation. However, this is contradictory to the findings of previous reports (Giraldo *et al.*, 2008; Pinos-Rodriguez *et al.*, 2008; Phakachod *et al.*, 2012) who reported improvement in potential disappearing fraction (b) by feeding EFE. Moreover, it is observed that supplementation of EFE in TMR containing R: C ratio of 70: 30 had no effect on PD while it increased (P<0.05) the PD in TMR containing R: C ratio of 60: 40. However, supplementation of EFE increased (P<0.01) the EDNDF (%) irrespective of the roughage: concentrate ratio in the TMR. This may be the effect of EFE added to the TMRs which might have stimulated the proliferation of hemi-cellulose degrading bacteria in the rumen thereby leading to improved rate of NDF degradation in the rumen (Feng *et al.*, 1996; Nsereko *et al.*, 2002). These results corroborated with those reported earlier (Chopra *et al.*, 2007; Giraldo *et al.*, 2008).

Table 1: Ingredient composition of total mixed rations fed to buffalo bulls

Ingredient	Total Mixed Rations	
	TMR-1	TMR-2
Maize stover	60.0	70.0
Maize grain	10.8	3.0
DORB	12.0	7.5
Cotton seed cake	14.0	10.5
Sunflower cake	2.0	8.1
Mineral mixture	0.8	0.6
Salt	0.4	0.3
Total	100	100

Table 2: Chemical composition (% DMB) of total mixed rations fed to buffalo bulls

Nutrient	Maize stover	TMR - 1	TMR - 2
Dry matter	92.13	94.38	94.87
Organic matter	92.60	92.64	92.57
Total ash	7.41	7.36	7.43
Crude protein	5.28	10.48	10.92
Ether extract	1.67	0.83	0.85
Crude fibre	37.13	25.01	26.50
Nitrogen free extract	48.52	56.33	54.30
Neutral Detergent Fibre	63.95	65.95	67.62
Acid Detergent Fibre	42.30	38.94	40.81
Acid Detergent Lignin	6.72	6.40	6.81
Hemi-cellulose	21.65	27.02	26.80
Cellulose	32.02	29.91	32.18
Silica	2.53	2.05	2.07
Calcium (%)	0.44	1.22	1.02
Phosphorus (%)	0.21	0.84	0.58

Table 3: Average *in sacco* degradation kinetics of total mixed rations supplemented with and without exogenous fibrolytic enzymes in buffalo bulls

Incubation period (h)	T-1	T-2	T-3	T-4
DM degradability				
a*	30.11 ^b	31.11 ^b	25.62 ^a	25.05 ^a
b	43.43	43.85	45.87	47.60
c	0.033	0.035	0.033	0.034
PD (a+b)	73.54	74.96	72.49	73.65
EDDM (0.05%)**	46.0 ^b	49.1 ^c	42.83 ^a	45.2 ^b
CP degradability				
a**	16.80 ^b	18.54 ^b	14.24 ^a	16.96 ^b
b	55.72	55.93	56.89	55.31
c	0.055	0.059	0.049	0.060
PD (a+b)*	72.52 ^{ab}	74.47 ^b	71.13 ^a	72.27 ^{ab}
EDCP (0.05%)*	46.5 ^b	48.5 ^c	43.5 ^a	46.6 ^b
NDF degradability				
a*	12.38 ^{ab}	15.60 ^b	10.33 ^a	12.34 ^{ab}
b	50.93	51.60	52.71	51.04
c*	0.051 ^b	0.049 ^b	0.039 ^a	0.053 ^b
PD (a+b)*	63.34 ^a	67.20 ^b	63.04 ^a	63.38 ^a
EDNDF (0.05%)*	36.4 ^b	38.6 ^c	32.3 ^a	36.7 ^b
ADF degradability				
a*	5.18 ^{ab}	7.02 ^b	3.75 ^a	5.45 ^{ab}
b	48.27	47.40	50.09	48.30
c*	0.042 ^b	0.047 ^b	0.033 ^a	0.041 ^{ab}
PD (a+b)	53.45	54.42	53.84	53.75
EDADF (0.05%)*	24.30 ^b	26.40 ^c	21.50 ^a	24.30 ^b

^{abc} Values in the rows bearing different superscripts differ significantly; *P<0.05 **P<0.01

The *in sacco* ADF degradability values revealed that the instantly soluble fraction 'a', slowly degradable fraction 'b', potential degradable fraction 'a+b' and rate constant 'c' were similar among the treatments. These results corroborated with the findings of earlier workers

(Gallardo *et al.*, 2010; Phakachoe *et al.*, 2012). Further, the present study indicated that supplementation of EFE increased (P<0.05) the EDADF (%) irrespective of the roughage: concentrate ratio. This improved fibre digestion and increased rate of digestion may be due to the action of EFE possibly by enhancing attachment and colonization to the plant cell matrix by rumen microorganisms (Nsereko *et al.*, 2000; Wang *et al.*, 2001) and/or by synergism with enzymes in rumen fluid (Morgavi *et al.*, 2000). These results are very consistent with those reported earlier (Chopra *et al.*, 2007).

Conclusion

The *in situ* degradation kinetics indicated that TMR containing R: C ratio of 60: 40 (T₁) is better utilized by bulls when compared to that containing R: C ratio of 70: 30 (T₃). It is further concluded that supplementation of EFE in TMRs has a positive effect irrespective of the R: C ratio as evidenced by their increased degradation rates in the rumen.

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