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Development of Appropriate Fibrolytic Enzyme Combination for Maize Stover and Its Effect on Rumen Fermentation in Sheep

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ABSTRACT: *In vitro* studies were undertaken to develop an appropriate fibrolytic enzymes cocktail comprising of cellulase, xylanase and β-D-glucanase for maize stover with an aim to increase its nutrient utilization in sheep. Cellulase and xylanase added individually to ground maize stover at an increasing dose rates (0, 100, 200, 400, 800, 1,600, 3,200, 6,400, 12,800, 25,600, 32,000, 38,400, and 44,800 IU/g DM), increased ($p<0.01$) the *in vitro* dry matter digestibility and *in vitro* sugar release. The doses selected for studying the combination effect of enzymes were 6,400 to 32,000 IU/g of cellulase and 12,800 to 44,800 IU/g of xylanase. At cellulase concentration of 6,400 IU/g, IVDMD % was higher ($p<0.01$) at higher xylanase doses (25,600 to 44,800 IU/g). While at cellulase doses (12,800 to 32,000 IU/g), IVDMD % was higher at lower xylanase doses (12,800 and 25,600 IU/g) compared to higher xylanase doses (32,000 to 44,800 IU/g). At cellulase concentration of the 6,400 to 32,000 IU/g, the amount of sugar released increased ($p<0.01$) with increasing levels of xylanase concentrations except for the concentration of 44,800 IU/g. No effect of β-D-glucanase (100 to 300 IU/g) was observed at lower cellulase-xylanase dose (cellulase-xylanase 12,800 to 12,800 IU/g). Based on the IVDMD, the enzyme combination cellulase-xylanase 12,800 to 12,800 IU/g was selected to study its effect on feed intake and rumen fermentation pattern, conducted on 12 rams (6 to 8 months; 20.34±2.369 kg body weight) fed 50% maize stover based TMR. The total volatile fatty acids ($p<0.01$) and ammonia-N concentration was higher in enzyme supplemented group, while no effect was observed on dry matter intake, ruminal pH and total nitrogen concentration. (**Key Words:** Exogenous Fibrolytic Enzymes, *In vitro*, Maize Stover, Sheep, Rumen Fermentation Pattern)

INTRODUCTION

In many tropical countries including India, ruminants subsist on low quality grasses, crop residues, and agro-industrial by-products due to the depletion of grazing lands in day to day life. These crop residues and poor quality roughages need to be processed to increase the nutrient utilization and performance of animals. Recently, supplementation of exogenous fibrolytic enzymes (EFE) as feed additives for ruminants has attracted the interests of researchers. It has been demonstrated that exogenous fibrolytic enzymes work in synergy with the endogenous rumen microbiological enzymes to enhance the digestibility and nutritive value of a high fibrous diet (Morgavi et al., 2000), thereby increasing the economic benefits for the farmer. Most of the researchers have reported a positive effect of supplementing exogenous fibrolytic enzymes by enhancing the *in vitro* dry matter or fiber degradability from alfalfa hay (Eun and Beauchemin, 2007), corn stover or corn silage (Gallardo et al., 2010) and from TMR (Giraldo et al., 2008; Pinos Rodriguez et al., 2008). But most of the research on the use of EFE was focused on silages, hay and grasses. Further response to the level of enzyme addition was non-linear *in vitro* (Colombatto et al., 2003), indicating the need to determine the optimum dose rate of enzymes for individual feeds (Yang et al., 1999). The type of enzyme preparations or their dose levels used for hay, silages and concentrate based diets may not be applicable for the rations used in India, as the enzymes are target specific (Pinos Rodriguez et al., 2002) and scant research has been done in India on this aspect. Maize crop is one of the most important principal crops grown throughout the year in India. Moreover, Andhra Pradesh stands second in total crop yield (4,354 kg/ Hectare) for the year 2011 to 2012, in India. The maize stover, after harvesting of maize cobs, is

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used as staple feed for animals in most of the tropical countries like India. Any improvement in the nutrient utilization of such roughages would benefit farmers. Hence the maize stover was selected as substrate for present investigations.

In the view of the above, the proposed study was aimed to develop a suitable fibrolytic enzymes cocktail comprising of cellulase, xylanase, β-D-glucanase for maize stover by *in vitro* studies and later study the effect of supplementing the enzyme cocktail developed from *in vitro* studies on rumen fermentation in sheep.

MATERIALS AND METHODS

The EFE under investigation were Cellulase (EC 3.2.1.4), Xylanase (EC 3.2.1.8) and β-D-glucanase (EC 3.2.1.6) enzymes in powder form, had an activity of 1,000,000 and 1,600,000 IU/g, respectively, and active in alkaline pH, which were procured from Advanced Bio-Agrotech Limited, Pune, India. The source of these enzymes was *Trichoderma sps*. The maize stover used in the *in vitro* studies was of GK- 3017 variety, of 3 to 4 months maturity, procured after harvesting of maize cobs, sundried and chaffed to 1 to 2 cm with chaff cutter. The Organic matter (OM), Crude protein (CP), Ether extract (EE), Crude fibre (CF), Nitrogen free extract (NFE), Total ash (TA), Calcium (Ca) and Phosphorus (P) contents were 86.25, 3.84, 0.76, 48.31, 33.34, 9.62, 0.79 and 0.62 percent. The stover required for *in vitro* studies was ground to 1 to 2 mm particle size with a hammer mill. The calculated concentration of enzyme was mixed manually with the stover a day before the *in vitro* studies had to be carried out. The *in vitro* dry matter digestibility (IVDMD) was studied by modified two stage *in vitro* technique (Goering and Van Soest, 1970) using sheep rumen liquor. The *in vitro* total sugar release from maize stover supplemented with various doses and combination of fibrolytic enzymes was estimated as per the procedure described by Nsereko et al. (2000). The total sugar released due to supplementing fibrolytic enzymes was quantified by the phenol-sulphuric acid method as described by Dubois et al. (1956). The assay for each sample was carried out in triplicate.

During *in vivo* studies, a total mixed ration (TMR) was formulated with maize stover as the sole roughage source in a roughage concentrate ratio of 50:50 (TMR-MS). The maize stover was chopped to 0.5 to 1.0 cm with a chaff-cutter and mixed with a concentrate mixture consisting of 35% groundnut cake, 40% maize, 22% deoiled rice bran, 2% salt and 1% mineral mixture and vitamin AD_3 (20 g/qt). The experimental ration was the TMR-MS to which the fibrolytic enzyme cocktail (cellulase-xylanase 12,800-12,800 IU/g maize stover) was supplemented (TMR-MS +EFE). The calculated quantity of enzyme was accurately weighed and mixed in the required concentrate mixture and then mixed with chopped maize stover manually for about 10 minutes before feeding to sheep daily. The CP and CF of TMR-MS and TMR-MS+EFE were 10.43, 10.84 and 28.7, 28.25% respectively.

Twelve Deccani ram lambs (6 to 8 months) with an average body weight of (20.34±2.369 kg) were randomly distributed into 2 dietary groups, one group was fed with control TMR (TMR-MS) and the other group was fed on TMR supplemented with EFE (TMR-MS+EFE). The respective rations were offered twice daily at 9.00 AM and 3.00 PM to meet the nutrient requirements (ICAR, 1998). The sheep of both groups were fed the dry matter requirement as per ICAR, 1998 recommendations, during the experiment. The leftover was weighed on the next day morning before cleaning and feeding. Clean, fresh and wholesome water was made available to each group of animals at all times.

At the end of preliminary feeding period of 30 d, rumen liquor was collected for two consecutive days from 4 lambs of each group with the help of stomach tube fitted with vacuum pump, four times a day at 0 h (before feeding), 2 h, 4 h and 6 h post feeding.

Feed offered samples were analyzed for CP and CF (AOAC, 1997). The TVFA in rumen liquor samples was estimated by the method of Barnet and Reid (1957). The total nitrogen in SRL was estimated as per methods of Singh et al. (1968) and ammonia nitrogen was estimated by the method of Schwartz and Schoeman (1964). The data were subjected to statistical analysis in one-way classification under a completely randomized design using General Linear Model of SPSS 15.0. Comparison between means was done using Duncan multiple ranges test (Duncan, 1955) at 5% and 1% level.

RESULTS AND DISCUSSION

In vitro studies

The IVDMD and corresponding release of total sugars from maize stover for various concentrations of cellulase and xylanase enzymes is given in Table 1. The IVDMD % was comparable among cellulase doses ranging between 0 to 3,200 IU/g DM. An improvement ($p<0.01$) in IVDMD was observed from cellulase supplementation of 6,400 IU/g DM. The digestibility was highest at cellulase concentration of 25,600 IU/g DM and beyond this dose (32,000 to 448,000 IU/g DM), the IVDMD gradually decreased. Supplementing xylanase up to concentration of 800 IU/g DM had no effect on IVDMD and was comparable to control. The IVDMD increased ($p<0.01$) when xylanase was added at 1,600 IU/g DM and highest IVDMD was observed for doses of 25,600 and 32,000 IU/g DM and no further increase in IVDMD was recorded at higher doses of

Table 1. *In vitro* DM digestibility (%) and total sugar release (mg/g DM) from maize stover supplemented with cellulase and xylanase at various concentrations

Enzyme concentration (IU/g DM)	*In vitro* DM digestibility (%)		*In vitro* sugar release (mg/g DM)	
	Cellulase	Xylanase	Cellulase	Xylanase
0	18.32^{defg}	18.32^{ef}	3.08 ^{h}	3.08^{k}
100	17.22^{g}	18.15^{f}	5.59^{fg}	5.63^{j}
200	17.48^{fg}	18.57^{ef}	4.80 ^{g}	9.92^{i}
400	17.90^{efg}	19.10^{ef}	6.06^{fg}	11.28^{h}
800	18.13^{efg}	19.98^{de}	7.36 ^{de}	12.01^{fg}
1,600	18.81^{def}	20.82^{cd}	6.81^{f}	12.54^{f}
3,200	19.17^{de}	21.70^{c}	8.76^{de}	13.07^{f}
6,400	20.79^{bc}	22.49^{bc}	9.16^{d}	13.08^{f}
12,800	21.99^{b}	23.49^{ab}	10.23^{d}	14.58^{e}
25,600	23.74^{a}	24.48^{a}	12.74^{c}	15.70^{d}
32,000	21.16^{b}	25.14^{a}	14.38^{bc}	17.82^{c}
38,400	19.30^{de}	23.77^{ab}	15.76^{b}	18.20^{b}
44,800	19.70^{cd}	23.97^{ab}	19.35^{a}	19.05^{a}
SEM	0.379	0.497	0.658	0.561
p value	0.001	0.001	0.001	0.001

$^{a-g}$ Means with different superscripts in a column differ significantly: $p<0.01$. Each value is average of triplicate; SEM = Standard error of means.

38,400 and 44,800 IU/g DM. Yu et al. (2005) reported that *in vitro* ruminal fluid degradability was improved ($p<0.01$) by 12% in oat hulls, 5% in wheat straw and 2% in alfalfa hay with supplementation by an enzyme mixture (Ferulic acid esterase, 6,500; xylanase, 2,048,000; cellulase, 512,000; β-glucanase, 32,000 and Endo-glucanase, 128,000 IU /g substrate). In the present study *in vitro* sugar release increased ($p<0.01$) in a dose related manner for cellulase and xylanase doses from 0 to 44,800 IU/g DM and highest sugar release was observed at 44,800 IU/g DM for both the enzymes.

The esterified bond between cellulose, hemicellulose and lignin restricts the digestion of recalcitrant cereal straws by ruminal microorganisms (Waghorn and McNabb, 2003). Supplementing cellulase and xylanase at concentrations ranging from 6,400 to 25,600 IU/g and 1,600 to 32,000 IU/g respectively, might have acted on β 1-4 linkages of cellulose and hemicellulose (xylan), to release soluble sugars and thus facilitating the growth of microbes (Bhat and Hazelwood, 2001). Also the synergistic action of these enzymes with endogenous ruminal microbial enzymes (Morgavi et al., 2000) might have resulted in a higher IVDMD. No effect of EFE observed at lower doses (100 to 3,200 IU/g DM) for cellulase and (100 to 800 IU/g DM stover) for Xylanase, which indicated that such low doses of enzymes was unable to degrade the core structure of lignin-cellulosic complexes (Nakashima and Orskov, 1989; McAllister et al., 2000).

Eun and Beauchemin (2007) reported that supplementation of EFE at of 1.4 mg/g DM improved *in vitro* NDF degradability up to 20.6% for alfalfa hay against control (18.4%) and up to 60.3% for corn silage against control (13.3%). Similarly, Wang et al. (2004) reported that spraying with enzyme mix (xylanase, β-glucanase, carboxymethylcellulase and amylase) at 1.5 mg/gm DM of wheat straw increased ($p<0.05$) digestibilities of DM, OM and total N, compared to ammoniated wheat straw (5% NaOH treated). The potentially degradable fraction of NDF and ADF increased for alfalfa hay while no differences were observed in corn stover when an *in sacco* trial was performed on Holstein steers to evaluate EFE having 31 cellulase units and 43.4 xylanase units supplemented at 3 g/kg DM (Gallardo et al., 2010).

Based on the *in vitro* results for IVDMD and amount of the total sugars released from maize stover with supplementation of cellulase and xylanase at various concentrations, the best doses selected for cellulase were 6,400, 12,800, 25,600 and 32,000 IU/g and for xylanase were 12,800, 25,600, 32,000, 38,400 and 44,800 IU/g. With the above concentration of enzymes, thirty combinations (5×6) were formulated for maize stover inclusive of un-supplemented (0 IU/g DM for cellulase and xylanase) and tested by *in vitro* studies (Table 2).

Significant interaction of cellulase and xylanase was observed on *in vitro* DM digestibility and sugar release. At a cellulase concentration of 6400, IVDMD % was higher ($p< 0.01$) at higher concentration of xylanase (25,600 to 44,800 IU/g), while at cellulase concentration of 12,800, 25,600 and 32,000 IU/g, IVDMD % was higher at lower xylanase doses (12,800 and 25,600 IU/g) compared to higher xylanase doses (32,000, 38,400 and 44,800 IU/g). Bhat and Hazelwood (2001) reported a synergistic effect between cellulase and xylanase to hydrolyze forage cell wall. A similar synergistic effect between cellulase and xylanase in

Table 2. *In vitro* DM digestibility (%) and total sugar release (mg/100 g DM) from maize stover supplemented with various combinations of cellulase and xylanase

Enzyme combination (IU/g DM)		*In vitro* DM digestibility (%)	*In vitro* sugar release (mg/g DM)
Cellulase	Xylanase		
0	0	18.88^{i}	3.08 ^{t}
0	12,800	22.29^{hi}	14.58^{pq}
0	25,600	22.85^{ghi}	15.70^{op}
0	32,000	25.06^{ghi}	17.82^{mno}
0	38,400	25.07^{ghi}	18.20^{lmno}
0	44,800	24.18^{ghi}	19.05^{lmn}
6,400	0	20.61^{hi}	9.16^{s}
6,400	12,800	25.98^{gh}	16^{nop}
6,400	25,600	29.03^{fg}	25.59^{fgh}
6,400	32,000	32.38^{ef}	26.68^{ef}
6,400	38,400	33.08^{def}	26.4^{ef}
6,400	44,800	32.29^{ef}	23.10^{ghij}
12,800	0	21.31^{hi}	10.23^{rs}
12,800	12,800	49.98^{a}	20.84^{jkl}
12,800	25,600	38.63^{cde}	21.05^{jkl}
12,800	32,000	32.74^{def}	22.23^{ijk}
12,800	38,400	39.03^{cde}	22.59^{ijk}
12,800	44,800	37.09^{def}	19.95^{klm}
25,600	0	24.35^{ghi}	12.74^{qr}
25,600	12,800	45.89^{bc}	24.73^{fghi}
25,600	25,600	48.55^{ab}	25.92^{efg}
25,600	32,000	32.03^{ef}	27.19^{ef}
25,600	38,400	32.77^{def}	31.9^{c}
25,600	44,800	33.23^{def}	22.88^{hijk}
32,000	0	20.47^{hi}	14.38^{pq}
32,000	12,800	41.9^{cd}	28.57^{de}
32,000	25,600	46.00^{ab}	30.40^{cd}
32,000	32,000	21.25^{hi}	36.14^{b}
32,000	38,400	22.59^{ghi}	41.56 ^{a}
32,000	44,800	11.57 ^{j}	36.81 ^{b}
SEM		1.057	0.805
p value		0.001	0.001
Main factors			
Cellulase			
0		22.82^{d}	14.74^{e}
6,400		29.14^{e}	21.21^{c}
12,800		36.46^{a}	19.48^{d}
25,600		35.07^{ab}	24.23^{b}
32,000		29.60^{bc}	31.31^{a}
p value		0.001	0.001
Xylanase			
0		20.72^{c}	9.92^{f}
12,800		35.56^{a}	21.08^{e}
25,600		35.30^{b}	23.73^{d}
32,000		30.11^{b}	26.01^{b}
38,400		32.47^{ab}	28.06^{a}
44,800		29.54^{b}	24.36^{c}
p value		0.001	0.0010

$^{a-t}$ Means with different superscripts in a column differ significantly: p<0.01.

Each value is average of triplicate; SEM = Standard error of means.

improving IVDMD from maize stover was observed in the present study when the ratio of cellulase and xylanase ranged between 1:1 and 1.25:1.

At all concentrations of cellulase (6,400 to 32,000 IU/g DM) the *in vitro* sugar release increased (p<0.01) with xylanase concentrations (12,800 to 38,400 IU/g DM) but decreased at a xylanase concentration of 44,800 IU/g DM. Earlier studies also revealed that there was improvement in *in vitro* sugar (monosaccharide) release from paddy straw with increasing concentrations of cellulase (40, 60 and 80 IU/g DM) but not with increasing concentrations of xylanase (67, 100 and 133 IU/g DM) in cellulase-xylanase combination, and maximum monosaccharide release was observed with cellulase and xylanase doses of 80 and 100 IU/g DM, respectively when incubated for 24 h (Senthil kumar et al., 2007).

The cellulose-xylanase combinations IU/g selected for further studies with β-D glucanase were 12,800 to 12,800 followed by 25,600 to 25,600 and 25,600 to 12,800. The β-D glucanase at concentrations of 100, 200 and 300 IU/g was supplemented to the ground maize stover along with above combinations to study the synergistic effect of β-D glucanase and the results are presented in Table 3.

Supplementation of β-D glucanase from 100 to 300 IU/g had no beneficial effect on IVDMD and the values were comparable to the combination having no β-D glucanase. While for the combination (25,600 IU cellulase – 12,800 IU xylanase/g, 25,600 IU cellulase – 25,600 IU xylanase/g), supplementation of β-D glucanase at 300 IU/g depressed IVDMD. The IVDMD was highest for cellulase-xylanase-β-D glucanase IU/g combination 25,600-25,600-0, followed by 25,600-12,800-0 and 12,800-12,800-0 with values of 52.15, 51.21 and 49.80%, respectively. On the other hand, Eun et al. (2007) reported increased NDF degradability of both alfalfa hay and corn silage with addition of endoglucanase (301 IU/g DM) to xylanase (693 IU/g DM).

The reason for the lack of response with addition of β-D glucanase in the present study was unclear. This might be due to a sub-optimal dose or to the cell wall structure of the maize stover which would be in agreement with Jalilvand et al. (2008) who observed that responses to level of enzyme addition (12,600 IU cellulase, 7,500 IU xylanase, 1,500 IU β-D glucanase/g) differed with forage type, the level of enzyme application and reported that addition of high levels was less effective than low levels. Based on the *in vitro* studies, the combination cellulase-xylanase-β-D glucanase 25,600-25,600-0 IU/g, had highest IVDMD (52.15%) and was comparable to the combination 12,800-12,800-0 and therefore the latter combination was selected to study the effect of this enzyme cocktail on rumen metabolites in sheep fed 50% maize stover based TMR.

Table 3. *In vitro* DM digestibility (%) and total sugar release (mg/g DM) maize stover supplemented with various combinations of cellulase and xylanase

Enzyme combination (IU/g DM)			*In vitro* DM digestibility (%)	*In vitro* sugar release (mg/g DM)
Cellulase	Xylanase	β-D Glucanase		
0	0	0	18.91 ^{e}	3.08 ^{e}
12,800	12,800	0	49.80^{ab}	20.84 ^{c}
12,800	12,800	100	47.73^{abcd}	29.37 ^{c}
12,800	12,800	200	49.11^{abc}	32.60^{bc}
12,800	12,800	300	47.88^{abcd}	38.23^{a}
25,600	12,800	0	51.21^{ab}	24.73^{d}
25,600	12,800	100	46.85^{abcd}	31.18^{bc}
25,600	12,800	200	45.95^{abcd}	32.09^{bc}
25,600	12,800	300	42.54^{cd}	34.42^{b}
25,600	25,600	0	52.15^{a}	25.92^{d}
25,600	25,600	100	46.07^{abcd}	33.54^{b}
25,600	25,600	200	44.15^{bcd}	33.29^{b}
25,600	25,600	300	41.73^{d}	37.78^{a}
SEM			1.667	0.782
p value			0.001	0.001

[a-d] Means with different superscripts in a column differ significantly: $p<0.01$.
Each value is average of triplicate; SEM = Standard error of means.

Dry matter intake and rumen fermentation pattern

There was no significant difference in the initial and final body weights in each group, during the experiment. The dry matter intake of un-supplemented and enzyme supplemented maize stover based TMR was 632.69 g/d and 602.71 g/d, respectively. There was no significant difference in the average feed intake of dry matter g/kg $W^{0.75}$) by supplementing the enzyme cocktail to 50% maize stover based TMR, indicating that the sheep of both groups were maintained on same plane of nutrition (Table 4).

Supplementing the above selected enzyme cocktail had a significant effect on TVFA and NH_3-N concentration in sheep fed 50% maize stover based TMR, though no interaction of enzyme supplementation and period of rumen liquor collection was observed. The higher TVFA with enzyme addition could be result of higher availability of fermentable soluble carbohydrates due to increased fibrolytic activity in rumen. Hristov et al. (2000) reported

Table 4. Rumen fermentation pattern in lambs fed maize stover based TMR supplemented with EFE

Attribute	TMR-MS	TMR-MS+EFE		TMR-MS	TMR-MS+EFE
Body weights (kg)			Total nitrogen (mg/100 ml)		
Initial	20.07±3.447	20.30±3.637	0 h	173.88±29.181	186.88±14.879
Final	20.22±3.431	20.47±3.595	2 h	234.88±21.877	193.50±12.553
Average intake (g/kg $W^{0.75}$)			4 h	288.50±21.154	284.63±13.677
Dry matter	71.46±8.53	68.36±8.36	6 h	266.13±30.681	233.75±14.833
Ruminal pH			Mean (p<0.280)	240.84±14.609	224.69±9.663
0 h	6.39±0.370	6.18±0.366	Ammonia nitrogen (mg/100 ml)		
2 h	5.99±0.293	5.73±0.32	0 h	14.90±0.770	16.50±1.614
4 h	5.24±0.148	4.99±0.10	2 h	21.60±1.190	22.00±1.301
6 h	5.00±0.087	4.77±0.054	4 h	25.70±2.463	31.30±1.37
Mean (p<0.185)	5.65±0.156	5.42±0.156	6 h	19.20±1.710	25.00±2.049
Total volatile fatty acids (meq/100 ml)			Mean (p<0.005)	20.35±1.056^{b}	23.70±1.288^{a}
0 h	14.63±0.844	18.00±1.150			
2 h	21.00±0.802	21.75±1.16			
4 h	26.88±0.875	30.25±0.940			
6 h	24.38±0.822	26.25±0.959			
Mean (p<0.001)	21.72±0.916^{b}	24.06±0.969^{a}			

[ab] Means bearing different superscripts in a row and sub-column differ significantly: $p<0.01$; $p<0.05$.
SEM = Standard error of means; MS = Maize stover; EFE = Exogenous fibrolytic enzyme; TMR = Total mixed ration.

an increase in TVFA concentration in heifers fed on a barley silage based diet supplemented with EFE. Similarly, increased TVFA concentration in rumen of lambs with intra-ruminal supplementation of 5 g fibrozyme was reported by Pinos Rodriguez et al. (2008). The average values of ruminal pH, irrespective of ration fell after 2 h post feeding and continued to decline until 6 h after feeding. The decline in the ruminal pH values with EFE supplementation was due to increased TVFA concentration. The peak TVFA, ammonia nitrogen and total nitrogen concentrations in the ruminal fluid ($p<0.01$) was observed at 4 h post feeding irrespective of rations and fell at 6 h post feeding (Table 4). Avellaneda et al. (2009) reported a significant increase ($p<0.01$) in ammonia nitrogen concentration in rumen liquor of lambs fed on guinea grass supplemented with enzyme (having 100 xylose units/g) at 3 g/d/lamb corroborating the present findings.

CONCLUSION

The exogenous fibrolytic enzymes cellulase and xylanase supplemented singly or in combinations increased *in vitro* DM digestibility and *in vitro* sugar release but supplementation of β-D glucanase along with cellulase and xylanase had no synergistic effect. Based on the *in vitro* studies, the fibrolytic enzymes combination cellulase-xylanase-β-D-glucanase 25,600-25,600-0 IU/g was optimum for enhancing nutrient utilization from maize stover and supplementation of cellulase-xylanase-β-D-glucanase 12,800-12,800-0 IU/g to sheep fed 50% maize stover based TMR increased the TVFA and NH_3-N concentration in rumen.

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