ISSN 1819-1878

# Asian Journal of Animal Sciences

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OPEN ACCESS Asian Journal of Animal Sciences

ISSN 1819-1878
DOI: 10.3923/ajas.2017.171.176

# Research Article
# Cellulase Enzyme Enhanced the Diet Digestibility and Growth Performance of Ewes

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## Abstract

**Background and Objective:** Though the exogenous fibrolytic enzyme, cellulase has been experimented with temperate feed stuffs, experiments on tropical wild grasses are very limited. Enzyme feed specificity is one of the crucial point that should be investigated deeply for the better utilization of enzymes in ruminant feeding. Hence, this study was carried out to investigate the effect of supplementation of cellulase enzyme on diet digestibility and growth performance of female goats (ewes) fed with wild guinea grass (*Panicum maximum* ecotype A). **Methodology:** Eight, 18 months old ewes (initial body weight = 19.40±3.03 kg) were used. The ewes were blocked into two groups based on body weight and randomized within the block for the treatment with Exogenous Fibrolytic Enzyme (EFE) and for the control. The whole experimental period was 180 days, consisted with two 90 days trial. In both trials, measurements of body weight were recorded in 2 week intervals and total feces and spot urine sampling were done for 7 days. All data were analyzed using IBM SPSS 20.0 Statistics software through one way analysis of variance (ANOVA) and all pairwise (Least significance difference ($p<0.05$)) comparisons. **Results:** Average Daily Gain (ADG) gram per day per animal in EFE group was significantly higher ($p<0.05$) than the control group where no difference was observed when ADG is expressed in terms of metabolic body weight. Intake (gram per day per animal) of Dry Matter (DM), Organic Matter (OM) and Neutral Detergent Fiber (NDF) (approximately by 8, 10 and 10%, respectively) were improved significantly ($p<0.05$) where no difference was observed in Crude Protein (CP) intake. Apparent DM and NDF digestibility enhanced (both by around 11%) significantly ($p<0.05$) while there was no difference observed in OM and CP digestibility. Both urinary creatinine and allantoin expressed either as mmol $L^{-1}$ or mg $L^{-1}$ significantly improved ($p<0.05$) with EFE supplementation. **Conclusion:** It is concluded that the supplementation with EFE for ewes is beneficial in means of ADG, intake and digestibility of DM and NDF and urinary allantoin production.

Key words: Average daily gain, fiber digestibility, forage intake, *Panicum maximum*

Received: April 17, 2017 Accepted: June 09, 2017 Published: June 15, 2017

Citation: Rupanjanie Thakshala Seresinhe, Sathya Sujani, Indunil Nishantha Pathirana and Chandima Jayanath Gajaweera, 2017. Cellulase enzyme enhanced the diet digestibility and growth performance of ewes. Asian J. Anim. Sci., 11: 171-176.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Guinea grass ecotype 'A' (*Panicum maximum*) is the most widely distributed fodder grass and due to its plentiful production and year around availability it serves as one of the major feed source in small scale ruminant production systems in Sri Lanka[1]. However the lower digestibility of fiber fraction have limited the optimum utilization by ruminants and ultimately resulted in low nutrient availability to animal[2]. Therefore enhancing the digestibility of fiber component of ruminant feedstuffs has been a huge research interest among ruminant nutritionists; thus various chemical, physical and biological methods have been introduced and some are being practically implemented. Among these approaches administration of microbial enzymes such as cellulases and xylanases have drawn a considerable research attention. With the subsequent positive results such as improved rumen fermentation parameters achieved by researches both *in vitro*[3] and *in vivo* exogenous enzymes are now on the verge of delivering its practical benefits to the ruminant industry. However, most of the previous experiments have focused on large ruminants fed with temperate feed stuffs, whereas this study will focus on the effects of cellulase enzyme on goats, small ruminant fed with a freely available tropical fodder grass. As enzyme feed specificity is a major underlying reason for variations in results, it is crucial to evaluate enzyme activity on different feedstuffs. In the view of the above, this study was conducted to evaluate the digestibility and growth performance of ewes fed with guinea grass ecotype 'A' based diet.

## MATERIALS AND METHODS

The experimental procedure was approved by the Ethical Committee of the University of Ruhuna and the experiment was carried out at the Faculty of Agriculture, University of Ruhuna, Kamburupitiya, Sri Lanka (low country wet zone with a mean temperature of 28°C and a relative humidity of 78%) from March-September, 2014.

**Animals, management and enzyme:** As per the limited availability of experimental animals and other resources same experiment was repeated twice to increase the accuracy.

Eight ewes (Jamnapari×non descriptive) of about 18 months of age weighing 19.40±3.03 kg (for the 1st experimental trial) were selected for this study. At the end of first experimental trial of 3 months goats were provided with 2 weeks of non-experimental time period before commencing the second experimental trial. During this non-experimental period of time goats were fed with a uniform mixture of fodder but different from the experimental feed. Same animals with average weight of 21.375±2.57 kg were used for the second trial. In both trials subsequent procedures were followed. Goats were blocked into two groups on the basis of body weight. Animals were confined to individual metabolic crates with dimensions of 1×0.45×1 $m^3$ and placed in a well-ventilated shed under uniform management conditions for the whole experimental period. All the animals were vaccinated for tetanus with adsorbed tetanus (0.5 mL per animal intramuscularly, Serum Institute of India Ltd., Hadaspar, Pune) and endo and ecto parasites with ivermectin (0.6 mL per animal subcutaneously, FarmChemie Pvt. Ltd., Sri Lanka).

Six weeks regrowth of fresh guinea grass ecotype 'A' (*Panicum maximum*) (unfertilized) was harvested daily from the faculty research farm. Goats were fed with fresh grass (*ad-libitum* intake with at least 10% of daily feed refusal) and restricted amount of commercial concentrate (200 g per animal $day^{-1}$, Milk Plus, CIC Holdings, Sri Lanka) along with commercial salt (2 g per animal $day^{-1}$, Common salt, Dumindu Productions, Sri Lanka) and a commercial mineral mixture (5 g per animal $day^{-1}$, FarmChemie Pvt. Ltd., Sri Lanka). The diet was formulated to provide adequate daily Total Digestible Nutrient (TDN) and Digestible Crude Protein (DCP) requirement for an ewe weighing 20 kg for maintenance and growth of 50 g $day^{-1}$ [4]. All goats were individually fed two times daily and animals were provided with fresh clean water throughout the day. Exogenousfibrolytic enzyme (EFE) namely cellulase (E.C. 3.2.1.4, Dyadic International, Inc., Jupiter, FL, USA) with activity of 115,000-140,000 cellulase U $g^{-1}$ and side activity (typical) of β-glucanase 30,000-36,000 U $g^{-1}$ was utilized. Additional side activities were reported as xylanase, pectinase, mannanase, xyloglucanase, laminarinase, β-glucosidase, β-xylosidase, α-L-arabinofuranosidase, amylase and protease. Initially, the activity of these enzymes under rumen conditions (pH and temperature) was measured.

**Experimental procedure, measurements and sampling:** A completely randomized block design was used and each experiment consisted of 90 days trial, with each trial of 10 days adaptation period, 73 days feeding period and 7 days urine and feces sampling period. Within the block goats were randomly assigned to treatments. Dietary treatments of exogenous fibrolytic enzyme cellulase were either without enzyme (control) or with enzyme; 1380000-1680000 U of cellulase and 36000-43200 U of

β-glucanase per animal per day (EFE). In a preliminary study activity of enzyme was assayed at 39°C and pH 6.5 using carboxymethyl cellulose (Sigma Chemical Co., St. Louis, MO) following the procedures of Wood and Bhatt[5]. Enzyme was sprayed in equal two portions to the chopped guinea grass one hour before feeding.

Goats were weighed at the start of each experimental period and changes in body weight of each goat were recorded each 14 days during the experimental period. Guinea grass and commercial concentrate were sampled weekly to determine Dry Matter (DM) content and diets were adjusted to account for changes in DM content. During sampling period daily offered feed, refusals and total feces for individual goats were quantitatively collected and sub samples (about 10% from total measured) were composited across sampling times for each goat. Spot urine samples from each goat were collected at 4 h intervals and acidified with 10% $H_2SO_4$ to keep the final pH below 3 and then volume up to 100 mL with tap water and stored at -20°C until analyzed for allantoin and creatinine.

**Laboratory analyses:** Composited feed samples, feed refusals and feces were measured for DM contents by drying at 55°C for 48 h. Ground samples (1 mm) were analyzed for ash (525°C, 3 ½ h in muffle furnace), CP [(Kjeltec System 1002, Tecator AB, Hoganas, Sweden)[6]], NDF[7]. Preserved urine samples were thawed and distilled water was added to get samples with concentrations fall within the range of standards (10-50 mg $L^{-1}$) for the analysis of both allantoin and creatinine. Urinary allantoin and creatinine were determined colorimetrically following the procedures described in IAEA TECHDOC 945[8].

**Calculations and statistical analyses:** All the data from two experimental trials were subjected to standard one way analysis of variance (ANOVA) using the general linear model of IBM SPSS Statistics 20.0[9] (IBM Cooperation, Somers, NY, USA) separately. Data presented here are average values of two experimental trials. Significance between individual means was identified using LSD test. The significance of means was considered at $p<0.05$. Descriptive analysis was done using Microsoft Excel 2013 version.

## RESULTS

Referring to the Table 1, the major component of the diet, guinea grass was consisted with approximately 24% of dry matter and 9.64% protein content. Commercial concentrate was consisted with higher dry matter and protein percentage similar to the manufacturer given values. Both ingredients had a higher percentage of NDF of approximately 67 and 56% in guinea grass and concentrate, respectively.

Supplementation of EFE resulted in significantly higher ($p = 0.04$) ADG values (control: 60.26 g $day^{-1}$, EFE: 74.04 g $day^{-1}$) though the numerically enhanced ADG in reference to metabolic body weight observed was statistically insignificant ($p = 0.20$).

Intake of DM, OM and DMD significantly improved ($p<0.05$) with the EFE while there was no significant effect on OMD even though the OMD of FEE supplemented goats reported 5.5% increment compared with the Control group.

However intake and digestibility of CP were not significantly affected with EFE supplementation and observed values were very similar for both groups.

Feeding efficiency expressed as kg DMI $kg^{-1}$ b.wt., gain was not affected significantly in EFE addition groups compared to control (Table 2).

Neutral detergent fiber intake and digestibility increased significantly ($p<0.05$) with EFE supplementation nearly by 10 and 9%, respectively.

Excretion of allantoin and creatinine expressed either in mmol $L^{-1}$ or mg $L^{-1}$ (Table 3) were significantly higher ($p<0.05$) with the EFE supplementation.

## DISCUSSION

Present findings indicate that EFE has improved ADG significantly ($p<0.05$) (Table 2) approximately by 23% when

Table 1: Ingredients and chemical composition of the diet fed to goat

| | | DM basis (%) | | |
|---|---|---|---|---|
| Ingredients | DM (%) | OM | CP | NDF |
| Guinea (*Panicum maximum* 'A') | 23.83±1.73 | 90.64±0.20 | 9.64±0.14 | 66.93±0.983 |
| Commercial concentrate* | 92.91±0.37 | 88.76±0.17 | 15.69±0.23 | 56.23±0.497 |

DM: Dry matter, CP: Crude protein, NDF: Neutral detergent fiber, *Ingredients: Cereals and cereal by-products, oilseed meals, vegetable oil, minerals, vitamins and permitted additives, chemical composition (g/100 g of DM); Fat: 6%, calcium: 1.0%, Phosphorous: 0.75-1.0%, Metabolizable energy: 2500 kcal $kg^{-1}$, Salt (2 g per animal $day^{-1}$) and mineral mixture (5 g per animal $day^{-1}$) (Composition per kilogram, Calcium: 185.2 g, Phosphorous: 79.0 g, Sodium: 92.8 g, Magnesium: 13.6 g, Copper: 1.1 g, Zinc: 61.0 g, Manganese: 5.3 g, Iodine: 68.0 mg, Cobalt: 21.0 mg, Selenium: 45.0 mg) supplied along with the diet, Values are means of 3 replicates±SEM (36 samples were analyzed over 12 weeks)

Table 2: Body weight change, intake and digestibility of nutrients in response to the exogenous fibrolytic enzyme supplementation

| Items | EFE | Control | SEM | Significance | p-value |
|---|---|---|---|---|---|
| ADG (g day$^{-1}$) | 74.04 | 60.26 | 4.78 | * | 0.037 |
| ADG (g kg$^{-1}$) | 4.46 | 3.78 | 0.53 | NS | 0.203 |
| **Intake (g day$^{-1}$)** | | | | | |
| DM | 662.87 | 611.30 | 23.61 | * | 0.029 |
| OM | 602.88 | 548.76 | 22.38 | * | 0.016 |
| CP | 98.32 | 96.55 | 4.37 | NS | 0.686 |
| NDF | 425.42 | 385.49 | 15.73 | * | 0.012 |
| **Apparent digestibility (%)** | | | | | |
| DM | 61.25 | 55.59 | 2.871 | * | 0.049 |
| OM | 63.04 | 57.78 | 3.04 | NS | 0.085 |
| CP | 78.01 | 77.76 | 0.97 | NS | 0.887 |
| NDF | 64.74 | 58.37 | 2.65 | * | 0.016 |
| Feed efficiency$^{\dagger}$ | 9.14 | 10.65 | 1.03 | NS | 0.148 |

EFE: Exogenous fibrolytic enzyme, SEM: Standard error mean, ADG: Average daily gain, DM: Dry matter, OM: Organic matter, CP: Crude protein, NDF: Neutral detergent fiber, $^{\dagger}$Total kilograms of dry matter intake (DMI)/Total kilograms of live weight gain, Values are means of 8 replicates, *Significance at 0.05, NS: Not significant

Table 3: Allantoin and creatinine excretion of goats in response to the supplementation of exogenous fibrolytic enzyme

| Items (g day$^{-1}$) | EFE | Control | SEM | Significance | p-value |
|---|---|---|---|---|---|
| Creatinine (mmol L$^{-1}$) | 4.45 | 3.67 | 0.26 | * | 0.025 |
| Creatinine (mg L$^{-1}$) | 502.66 | 414.42 | 29.70 | * | 0.025 |
| Allantoin (mmol L$^{-1}$) | 7.54 | 4.95 | 0.25 | ** | 0 |
| Allantoin (mg L$^{-1}$) | 1192.55 | 781.26 | 40.40 | ** | 0 |

EFE: Exogenous fibrolytic enzyme, SEM: Standard error mean, Values are means of 8 replicates. *Significance at 0.05, **Significance at 0.01

compared with the control group. This could be attributed to the improved ADG mainly to the significantly enhanced DMI by 8.43% with EFE supplementation. Further, it can also be assumed that improved performance may have been due to the increased OMI and DMD. Improved ADG in goats could be also explained by an increase in the available nutrients to animals for deposition and growth. Improved DMD in goats could be also referred to the improvement in ruminal fermentation activities, by supplementing EFE with guinea grass[10]. In the current study DMI of goat ranged between 2.51-2.56% b.wt., which is less than the required DMI of 3.0-3.1% b.wt., which was reported by Devendra and McLeroy[11] for tropical goats. As stated by Kearl [4], DMI of a goat (average weight 25 kg) for growth (for a 50-75 g day$^{-1}$ ADG) and maintenance should be ranged between 710-730 g day$^{-1}$. Despite the slight reduction of DMI, which have been due to the quality of forage, both groups have attained the targeted ADG of 50 g day$^{-1}$ and this could be explained as a result of significantly enhanced ($p<0.05$) DMD with EFE supplement.

In agreement with the present results exogenous enzymes have been reported to increase ADG, DMI and DMD in several past studies where small ruminants have been used, by Gado *et al.*[10], where they have observed only significantly improved ($p<0.05$)weight gains.

In contrary to present findings, some studies have shown that fibrolytic enzymes have no significant influence ($p<0.05$) on feed intake in goats and lambs respectively, ADG and nutrient digestibility[12].

In spite of significantly higher ($p<0.05$) ADG and DMD, feed efficiency did not enhance significantly, even so EFE supplementation triggered a numerically higher feed efficiency.

Further non-significance in both CP intake and digestibility could most probably be due to the fact that the diet consisted with a commercial concentrate which is easily digestible (for the composition of the concentrate refer Table 1). According to the existing body of knowledge, exogenous fibrolytic enzymes appeared to improve digestion only when fiber digestion is restricted, but no effects occur when digestion is high as in commercial concentrate. In the present study CP intake in goats reported to be ranged between 96.55-98.32 g day$^{-1}$ in control and EFE group respectively. The digestibility of CP in the present study (81.19-81.62% in control and EFE groups, respectively) is higher than the values reported by Titi and Lubbadeh[13] who observed 72.1-73.5% of CP digestibility in Awasssi lambs fed with a cellulase supplemented diet. In contrast to the higher CP digestibility values reported by above studies, Gonzalez *et al.*[14] obtained comparatively lower values ranging from 59.6-63% in dairy goats supplemented with fibrolytic enzymes.

Improved NDF digestibility may be the main cause for the significantly enhanced ($p<0.05$) NDF intake and DMI, by reducing the physical rumen fill. The effect of fibrolytic enzyme supplementation on NDF digestibility ofgoat[15] has been studied. In the present study NDF digestibility increased

approximately by 9% and the result is in agreement with the findings of Ganai *et al.*[16]. Oba and Allen[17] found that 1% increment in vitro NDF degradation achieved 0.17 kg increase in DMI and 0.25 kg increase in 4% fat corrected milk yield. Thus, the present results would be very much beneficial in means of DMI and milk production in goats.

In the present study only urinary allantoin was estimated as previously it has been identified as the major purine component[18]. Carro *et al.*[19] reported that allantoin ranged between 87.3 and 90.8% from total purine derivatives in goats. A study by Purwati *et al.*[20] reported that proportion of urinary xanthine and hypoxanthine to total purine derivatives in goats was very low, 0.32 and 0.36%. Therefore the excretion of xanthine and hypoxanthine in the present experiment was not estimated. Peer-reviewed published information on urinary allantoin excretion in small ruminants either in sheep or in goat supplemented with fibrolytic enzymes are extremely limited. As theory suggests the excretion of allantoin is responsive to the high dietary protein intake, even though in the present study CP intake did not significantly vary between treatment and control groups. Therefore authors would like to attribute the elevated allantoin concentration to the enhanced DMI and presumably the improved microbial growth and activity with EFE. Similar to our findings, George *et al.*[21] reported that values for urinary allantois concentrations ranged from 4.68-7.40 mmol $L^{-1}$ in a study where Barberi goats were fed with a 50:50 forage to concentrate diet.

It is generally presumed that excretion rate of creatinine is relatively constant in healthy animals and remains independent of level of feed intake. Creatinine is an indicator of body protein turnover and creatinine in urine is excreted in proportion to live weight. Since a significant improvement ($p<0.05$) of ADG and final body weight in EPE treated group was noticeable, the allied higher creatinine excretion was observed as expected. In the previously mentioned study of George *et al.*[21], creatinine concentrations ranging from 4.02-4.20 mmol $L^{-1}$ were observed, which were slightly higher than the values of the control group in this study. This difference has been due to the use of different breeds.

In contrast to these values Marapana and Seresinhe[18] obtained much lower values ranging from 1.001-2.910 and 1.110-2.590 mmol $L^{-1}$ for allantoin and creatinine, in goats fed with a farm and an experimental diet, respectively. The present findings pave the pathway for efficient utilization of freely available, poor quality wild fodder grass as a ruminant feedstuff. This basic experiment should be expanded, where other tropical feedstuffs will be evaluated with different supplementation levels. Less availability and accessibility to enzyme products and less awareness of farmers can be considered as major limitations in implementing this under field conditions in tropical region where, mostly small scale farms are predominant.

## CONCLUSION

It is concluded that the exogenous fibrolytic enzyme, cellulose enhanced the feed value and efficient utilization of wild guinea grass (*Panicum maximum*) for goats as evidenced by significantly enhanced growth, intake and digestibility parameters and urinary allantoin production.

## SIGNIFICANCE STATEMENT

This study discloses that exogenous fibrolytic enzyme cellulase, a biological approach which has an influence on enhancing feed value of a low quality tropical forage thus emphasizing the potential utilization with tropical feed stuffs and encouraging researchers to conduct experiments with more tropical feed resources.

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