**GSAR Journal of Agriculture and Veterinary Sciences**
ISSN: 3048-9075 (Online)
Abbreviated key title: Glob.J. Agri.Vet.Sci.
Frequency: Monthly
Published By GSAR Publishers
Journal Homepage Link- https://gsarpublishers.com/journal-gjavs-home/

# Effect of inclusion level of selected enzymes and enzyme combinations on *in vitro* digestibility of maize (*Zea mays*) stover

**By**

**Mdziniso M. P.[1,2*], Tuitoek J. K.[2], Ondiek J. O.[2] and Muliro P. S.[3]**

[1]Department of Animal Science, University of Eswatini, P.O. Luyengo, Luyengo, Eswatini https://orcid.org/0000-0002-1417-1463
[2]Department of Animal Science, Egerton University, P.O. Box 536, 20115 Egerton, Kenya
[3]Department of Food and Dairy Science, Egerton University, P.O. Box 536, 20115 Egerton, Kenya

**Article History**

*Received: 15/09/2025*
*Accepted: 22/09/2025*
*Published: 24/09/2025*

**Vol – 2 Issue –9**

*PP: -78-86*

***Abstract***

*This study examined the impact of varying dosages of cellulase, xylanase, and cellulase-xylanase (CX) combinations on in vitro dry matter digestibility (IVDMD), organic matter digestibility (IVOMD), and neutral detergent fibre digestibility (IVNDFD) of maize stover. In the first in vitro trial, cellulase and xylanase were added individually to ground maize stover at increasing rates of 0 (control), 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, and 6.5 g/kg DM. The best (based on IVNDFD) individual doses of 1.5 and 2.5 g/kg DM for cellulase and 2.5, 3.5, 4.5, 5.5 g/kg DM for xylanase were selected for the second in vitro trial to study the effect of combining the different levels of cellulase and xylanase on in vitro digestibility of maize stover. An increase ($p<0.05$) in IVNDFD was observed when increasing the dosage of cellulase from 0.5 to 2.5 g/kg DM (34.58 to 40.4%, respectively), thereafter, a decrease ($p<0.05$) from 38.0 to 33.2% was observed at 3.5 to 6.5 g/kg DM, respectively. Supplementing with cellulase in smaller doses of 1.5 and 2.5 g/kg DM yielded higher IVNDFD of 39.46 and 40.40%, respectively. Xylanase improved ($p<0.05$) IVOMD of maize stover across all graded levels compared to the control. There were no differences ($p>0.05$) in IVNDF digestibility when xylanase was applied at lower doses of 0.5 to 2.5 g/kg DM (ranging from 30.48% to 32.36%) and the highest dose of 6.5 g/kg DM (30.74%). The combination of different doses of cellulase and xylanase (CX) improved ($p<0.05$) IVDMD when compared to the control. In vitro dry matter digestibility (IVDMD) ranged from 51.18 % at CX 2.5 x 5.5 g/kg DM to 60.04% at CX 1.5 x 2.5 g/kg DM, with the negative control at 42.87%. The combination of cellulase and xylanase at lower levels of 1.5 g/kg DM and 2.5 g/kg DM, respectively, improved ($p<0.05$) the digestibility of DM, OM and NDF compared to when the enzymes were applied singly. In light of this investigation, it can be concluded that the enzyme, enzyme dosage, and enzyme combination all affect the digestibility of maize stover, with effective digestibility of maize stover achieved when cellulase and xylanase were combined at 1.5 and 2.5 g/kg DM, respectively.*

***Key words**: maize stover, exogenous fibrolytic enzymes, enzyme dose, synergistic effect*

## INTRODUCTION

Feed constraints remain the major limiting factor in livestock production. Developing countries in Africa are faced with feed scarcity, especially during the dry season, because the supply of grass and the quality of natural grazing vary significantly according to the season (FAO and IGAD, 2019). This leads to the utilization of low-quality roughages that are deficient in energy, protein, vitamins, and minerals.

Rademaker et al. (2016) identified the main constraint to the performance of the Kenyan dairy sub-sector to include inadequate quantity and quality of feed for dairy animals, exacerbated by the limited use of supplementary feeds.

In Kenya, the Nakuru and Nyandarua counties rely mainly on fresh natural pastures for their dairy animals, with the addition of fodder including mainly maize stalks, rice straws and a minimum of sorghum and millet stalks (Otieno et al., 2020).

*Corresponding Author: **Mdziniso M. P***

During the peak of the long dry season, the main livestock feed (used by >80% of farmers) and principal crop residue for dairy feeding in semi-arid regions of Kenya is maize stover (Njarui et al., 2011). Maize stover is a crop residue after the harvesting of maize cobs (grain) for human consumption (Heuzé et al., 2019) and is available and abundant since maize is a staple food and top cereal crop produced in most of sub-Saharan Africa (SSA) (Depetris-Chauvin et al., 2017). Maize stover is commonly left on the field to be grazed on by livestock, buried to provide organic matter to the soil (Heuzé et al., 2019) or simply burnt. Due to its availability and abundance, maize stover is a potential source of roughage for ruminants. However, for optimal utilization, nutritional improvement of maize stover is required.

Enzyme supplementation has proven to be an effective strategy to improve the utilisation of forages in livestock production (Beauchemin et al., 2003; Arriola et al., 2011; Azzaz et al., 2013; Salem et al., 2013). The supplementation of ruminant diets with exogenous fibrolytic enzymes has led to significant increases in DM digestibility (Gado et al., 2011; Azzaz et al., 2013; López-Aguirre et al., 2016; Aboul-Fotouh et al., 2017), NDF and ADF digestibility (Arriola et al., 2011; López-Aguirre et al., 2016), and OM digestibility (Sutton et al., 2003; López-Aguirre et al., 2016). Exogenous fibrolytic enzymes have also improved the *in vitro* digestibility of maize stover (Carreón et al., 2010; Gallardo et al., 2010; Bhasker et al., 2013; Fon & Nsahlai, 2013). A recent study reported that the use of exogenous fibrolytic enzymes, namely cellulase, xylanase, pectinase and their combinations, improved the *in vitro* digestibility of maize stover (Mdziniso et al., 2025). Nevertheless, the main variables influencing the effects of exogenous fibrolytic enzymes include the cellulase-to-xylanase ratio (Bhasker et al., 2013), the kind and dosage of the enzyme (Gallardo et al., 2010; van de Vyver & Cruywagen, 2011; Azzaz et al., 2013;) and the forage-to-concentrate ratio (Schingoethe et al., 1999; Pinos-Rodríguez et al., 2008; Arriola et al., 2011), among others.

As yet, the appropriate concentration of cellulase, xylanase and cellulase-xylanase combination for efficient digestibility of maize stover is unknown. Consequently, this study sought to evaluate the effect of graded levels of cellulase and xylanase applied singly and in combination (CX) on *in vitro* dry matter, organic matter, and neutral detergent fibre digestibility of maize stover.

## MATERIALS AND METHODS

**Experimental site and material**

The Animal Nutrition laboratory at Egerton University, Njoro Main Campus in Nakuru, Kenya, served as the study's site. Following the harvest of maize cobs from the same field at Egerton University's Ngongongeri farm, the same variety (H6213) of maize stover was gathered. In Tatton Agriculture Park (TAP), Egerton University, it was chaffed to a length of 1-2 cm using a chaff cutter and then placed in bags in a dry, clean, and aerated place. Husks, leaves, and stalks made up the maize stover. The maize stover used in this study consisted of 96.2% dry matter, 93.7% organic matter, 4.7% crude protein, 36.6% crude fibre, 60.6% neutral detergent fibre, 42.7% acid detergent fibre, and 17.5 MJ/kg DM gross energy (Mdziniso et al., 2025). Xylanase and cellulase, the fibrolytic enzymes being studied, were acquired from Beijing Smile Feed Sci. & Tech. Co., LTD, Beijing, China. Table 1 displays the enzyme specifications according to the manufacturer's report.

**Table 1: Enzyme specification**

| | Enzyme product name | |
|---|---|---|
| **Item** | **Smizyme Xylanase** | **Smizyme Cellulase** |
| Enzyme concentration (U/g) | 10,000 | 5,000 |
| Enzyme activity (U/g) | 11,850 | 6,352 |
| Source organism | *Aspergillus oryzea* | *Trichoderma longibrachiatum* |
| Optimum temperature ($^oC$) | 37 | 37.5 |
| Optimum pH | 5 - 6.6 | 5 |
| Recommended dosage | 100g/ton complete feed | 100g/ton complete feed |

**Experimental procedure**

***In vitro* procedure**

Using the two-stage method described by Tilley & Terry (1963), the impact of cellulase, xylanase, and cellulase-xylanase combinations on the digestibility of maize stover was assessed. To make sure that the microbial population in the rumen fluid was made up of fibrolytic as well as amylolytic and proteolytic bacteria, three rumen liquor donor bucks were acclimated to a 65% forage: 35% concentrate diet for four weeks. The bucks were given unlimited access to fresh water and mineral lick (Soliva & Hess, 2007). There was only maize stover for roughage. Weighed samples of 0.5 g DM ground stover were placed in glass tubes that held 80–90 ml. Following precise weighing, the experimental enzymes (xylanase and cellulase) were introduced to the tubes in powdered form as follows:

1. In the first *in vitro* trial aimed at evaluating the effect of different inclusion levels of cellulase and xylanase on the *in vitro* digestibility of maize stover, cellulase and xylanase were added individually to ground maize stover at different inclusion levels of 0 (control), 0.5, 1.5, 2.5, 3.5, 4.5, 5.5 and 6.5 g/kg DM maize stover.
2. In the second *in vitro* experiment, 1.5 and 2.5 g/kg DM for cellulase and 2.5, 3.5, 4.5 and 5.5 g/kg DM for xylanase were selected for studying the effect of cellulase-xylanase (CX) combinations on maize stover digestibility. Positive controls of cellulase at

*Corresponding Author: **Mdziniso M. P**

1.5 and 2.5 g/kg DM and xylanase at 2.5, 3.5, 4.5 and 5.5 g/kg DM were also prepared.

A blank with the appropriate enzyme or enzymes, a buffer blank, and a control (maize stover without enzyme supplementation) were made for each experiment. When the enzymes were added, they were not solubilised in water. The rumen content of the three bucks was taken with a stomach tube before the morning feeding (Yáñez-Ruiz et al., 2016) and placed in thermos flasks that had been pre-warmed to 38 $^{o}$C. After being combined at a 50:50 ratio, the rumen content was immediately taken to the laboratory, gassed with $CO_2$, then kept at 38 $^{o}$C. Four layers of cheesecloth were used to squeeze the rumen content while gassing with $CO_2$, and a pre-warmed (38 $^{o}$C) $CO_2$-filled thermos flask was used to collect the rumen fluid. A buffer solution and the filtered rumen fluid were combined at a ratio of 4:1 (1 rumen liquor : 4 buffer solution – v/v) in accordance with McDougall (1948).

The mixture was stirred and gassed with $CO_2$, then 50 ml was pipetted to the digestion tubes containing maize stover samples and enzymes kept in a water bath maintained at 38 $^{o}$C. The tube was sealed with a rubber cork fitted with a sealed rubber tube. The 4 mm slit in the rubber tube on the valve was cut with a sharp knife; the slit normally remained closed, opening only to release gas from inside the tube. After sealing, the tubes were incubated at 38 $^{o}$C in the dark for 48 hours, being shaken 4 times a day by hand. After 48 hours of incubation, the tubes were stored at 4 $^{o}$C for 2 h to stop fermentation. Then pre-weighed glass fibre filter crucibles (porosity 1 crucibles, 70 mL capacity) were used to separate supernatants from residual plant particles and adherent microbial biomass by vacuum filtration. The particles were washed with 100 mL of distilled water and then oven-dried at 65 $^{o}$C to a constant weight to determine the potential DM disappearance at 48 hours. Thereafter, the dried residues per treatment were used for the determination of *in vitro* organic matter digestibility by ashing at 550 $^{o}$C overnight and *in vitro* NDF digestibility, in which the residues were subjected to fibre analyses following procedures of Van Soest et al. (1991).

*In vitro* DM digestibility was calculated as follows (Tilley & Terry, 1963):

*In vitro* DM digestibility (%) = [A – (B – C)/A] * 100

where: A = dry weight of the sample; B = dry weight of residue after digestion; C = dry weight of reagent blank
*In vitro* organic matter digestibility (%) was calculated as
[(A x (%OM/100)) – (B – C) x 100] / A
where: A = Dry weight of sample; B = weight of ash of sample residue; C = weight of ash of blank
*In vitro* neutral detergent fibre digestibility was calculated as follows:
NDFD (%) = [($NDF_{feed}$ – $NDF_{residue}$)/$NDF_{feed}$] x 100

where: $NDF_{feed}$ is the content of NDF (g/kg DM) of feed incubated
$NDF_{residue}$ is the amount of NDF (g/kg DM) in the residue

**Statistical analysis**
The experiment was undertaken in a completely randomized design (CRD). The data were normally distributed as per the normality test done using Box plot and Shapiro-Wilk test and a homogeneity test done using Leven's test. The data were subjected to analysis of variance (ANOVA) of Statistical Analysis Software (Version 9.4 SAS Institute Inc., Cary, NC, USA) using the following model:
$Y_{ij} = \mu + T_i + e_{ij}$

where: $Y_{ij}$ response variable, $\mu$ = overall mean, $T_i$ = treatment effect, $e_{ij}$ = random error

Significance was declared at $p < 0.05$, and mean separation was done using Duncan's multiple range test (Duncan, 1955).

## RESULTS

**Effect of cellulase on *in vitro* digestibility of maize stover**
Supplementation of maize stover with cellulase improved ($p<0.05$) *in vitro* dry matter, organic matter, and neutral detergent fibre digestibility. There was no observed improvement ($p<0.05$) in IVDMD among cellulase doses, ranging from 47.83 to 53.41%. However, an increase ($p<0.05$) in *in vitro* organic matter digestibility (IVOMD) was observed when cellulase was applied at increasing rates from 0.5 g/kg DM to 2.5 g/kg DM, ranging from 44.39 to 49.41%, respectively, after which IVOMD declined ($p<0.05$) reaching 39.68% at a dose of 6.5g/kg DM. A similar trend was observed in IVNDFD, where an increase ($p<0.05$) in IVNDFD was observed when increasing the dosage of cellulase from 0.5 to 2.5 g/kg DM (34.58 to 40.4%, respectively), thereafter, a decrease ($p<0.05$) from 38.0 to 33.2% was observed. Supplementing with cellulase in a smaller dose of up to 2.5 g/kg DM maize stover increased ($p<0.05$) OM and NDF digestibility compared to higher doses. A dose of 2.5 g/kg DM yielded the highest ($p<0.05$) IVOMD of 49.41% while 1.5 and 2.5 g/kg DM doses yielded higher IVNDFD of 39.46 and 40.40%, respectively.

**Table 2: Influence of cellulase at different inclusion levels on in vitro digestibility of maize stover**

| Enzyme dose (g/kg DM) | IVDMD (%) | IVOMD (%) | IVNDFD (%) |
|---|---|---|---|
| 0 (Control) | 41.76$^{b}$ | 30.82$^{e}$ | 28.26$^{d}$ |
| 0.5 | 49.63$^{a}$ | 44.39$^{bc}$ | 34.58$^{bc}$ |
| 1.5 | 52.23$^{a}$ | 47.43$^{ab}$ | 39.46$^{a}$ |
| 2.5 | 53.41$^{a}$ | 49.41$^{a}$ | 40.40$^{a}$ |
| 3.5 | 50.06$^{a}$ | 45.32$^{ab}$ | 38.00$^{ab}$ |
| 4.5 | 48.48$^{a}$ | 41.48$^{cd}$ | 36.81$^{abc}$ |
| 5.5 | 47.83$^{a}$ | 40.67$^{cd}$ | 34.41$^{bc}$ |
| 6.5 | 47.89$^{a}$ | 39.68$^{d}$ | 33.22$^{c}$ |
| SEM | 1.15197 | 0.8644 | 0.8296 |
| Probability | p<0.0001 | p<0.0001 | p<0.0001 |

*Corresponding Author: **Mdziniso M. P**

Sample size (N) = 3; [a,b,c,d,e] Means in the same column with different superscripts differ significantly (p<0.05)

**Effect of xylanase on *in vitro* digestibility of maize stover**

Supplementing maize stover with xylanase at 0.5, 3.5 and 4.5 g/kg DM improved (p<0.05) IVDMD, with the highest digestibility ranging from 52.91 to 53.41%. No improvements (p>0.05) observed in IVDMD of supplemented maize stover and the control when xylanase was applied at 1.5, 2.5, 5.5 and 6.5 g/kg DM. Application of xylanase improved (p<0.05) IVOMD of maize stover across all graded levels compared to the control. The highest IVOMD of 48.66% was observed with a xylanase dose of 3.5 g/kg DM. Compared to the control, similar (p>0.05) IVNDF digestibility of maize stover were observed when xylanase was applied at lower doses of 0.5 to 2.5 g/kg DM, (ranging from 30.48% to 32.36%) and highest dose of 6.5 g/kg DM (30.74%). A higher (p<0.05) IVNDFD of maize stover was observed when xylanase was applied at higher doses of 3.5 to 5.5 g/kg DM, yielding 32.7% to 33.3% digestibility compared to the control (27.57%). Xylanase doses of 3.5, 4.5 and 5.5 g/kg DM improved (p<0.05) IVNDFD compared the smaller doses and the highest dose of 6.5 g/kg DM, which was comparable (p>0.05) to the control.

**Table 3: Influence of different doses of xylanase on maize stover digestibility**

| Enzyme dose (g/kg DM) | IVDMD (%) | IVOMD (%) | IVNDFD (%) |
|---|---|---|---|
| Control | 44.48[b] | 32.68[c] | 27.57[b] |
| 0.5 | 52.91[a] | 46.43[ab] | 30.74[ab] |
| 1.5 | 48.14[ab] | 41.29[b] | 30.48[ab] |
| 2.5 | 50.31[ab] | 43.40[ab] | 32.36[ab] |
| 3.5 | 53.22[a] | 48.66[a] | 33.22[a] |
| 4.5 | 53.41[a] | 45.44[ab] | 33.30[a] |
| 5.5 | 51.36[ab] | 43.96[ab] | 32.70[a] |
| 6.5 | 49.81[ab] | 41.60[b] | 30.74[ab] |
| SEM | 1.5271 | 1.12065457 | 1.017198 |
| Probability | p<0.0001 | p<0.0001 | p<0.0001 |

Sample size (N) = 3; [a,b,c] Means in the same column with different superscripts differ significantly (p<0.05)

**Effect of cellulase-xylanase combinations on *in vitro* digestibility of maize stover**

In the second experiment, cellulase and xylanase doses applied singly on maize stover were prepared as positive controls (Table 4). Applying xylanase alone (xylanase positive control) at 3.5 g/kg DM was comparable (p>0.05) to cellulase at 2.5 g/kg DM (53.16 and 53.10 g/kg DM, respectively). Overall, when applied singly, cellulase and xylanase had a similar (p>0.05) effect on IVDMD and IVOMD, with both enzymes being superior (p<0.05) to the control. However, higher (p<0.05) IVNDFD was obtained with cellulase applied at 2.5 g/kg DM (36.38%) compared to xylanase applied at 2.5 g/kg DM (31.08%) and CX applied at 2.5 x 5.5 g/kg DM (31.16%). This improvement in IVNDFD was comparable (p>0.05) to when xylanase was applied at a higher dose of 5.5 g/kg DM (34.07%).

The combination of different doses of cellulase and xylanase (CX) improved (p<0.05) IVDMD of maize stover when compared to maize stover without enzyme supplementation (negative control). *In vitro* dry matter digestibility (IVDMD) ranged from 51.18 % at CX 2.5 x 5.5 g/kg DM to 60.04% at CX 1.5 x 2.5 g/kg DM with the negative control at 42.87%. The combination of cellulase and xylanase at lower levels of 1.5 g/kg DM and 2.5 g/kg DM, respectively, improved (p<0.05) the digestibility of DM, OM and NDF compared to when the enzymes were applied singly. Among the combinations, lower doses of cellulase and xylanase combinations of 1.5 x 2.5 and 1.5 x 3.5 g/kg DM had higher (p<0.05) IVDMD of 60.04% and 57.99%, respectively, compared to the other CX combinations (with IVDMD ranging from 51.18 to 54.83%.

IVOMD was positively influenced (p<0.05) by all CX combinations except CX 2.5 x 2.5 and 2.5 x 5.5 g/kg DM. These improvements (ranging from 42.22 to 47.86g/kg DM), however, were comparable (p>0.05) to when cellulase and xylanase were applied singly (ranging from 42.53 to 44.89%). When cellulase was kept at 1.5 g/kg DM, increasing xylanase gradually decreased (p<0.05) IVDMD and IVOMD. When cellulase was kept at 2.5 g/kg DM, increasing xylanase did not (p>0.05) improve IVDMD and IVOMD, though slight (p>0.05) increases were observed up to 2.5 x 4.5 g/kg DM. On the contrary, an improvement (p<0.05) was observed in IVNDFD, peaking at CX 2.5 x 3.5 g/kg DM then declining significantly (p<0.05) at 2.5 x 5.5 g/kg DM (31.16%). This declined IVNDFD was comparable (p<0.05) to maize stover without enzyme supplementation (26.29%).

Overall, increasing the cellulase level in the CX combination from 1.5 g/kg DM to 2.5 g/kg DM lowered (p<0.05) DM, OM and NDF digestibility. Though xylanase applied singly at 2.5 g/kg DM (positive control) failed to improve IVNDFD (31.08%), when combined with cellulase at CX 1.5 x 2.5 g/kg DM, an improvement (p<0.05) was observed (39.12%) and was higher (p<0.05) than when 2.5 g/kg DM xylanase was combined with 2.5 g/kg DM cellulase (31.51%). IVNDFD was improved (p<0.05) by CX 1.5 x 2.5 g/kg DM (39.12%) and CX 2.5 x 3.5 g/kg DM (39.20%), and these values are comparable (p>0.05). However, the smaller dose of CX 1.5 x 2.5 g/kg DM further improved (p<0.05) IVDMD compared to CX 2.5 x 3.5 g/kg DM.

**Table 4: *In vitro* digestibility of maize stover treated with cellulase-xylanase combinations**

| Enzyme dose (g/kg DM) | IVDMD (%) | IVOMD (%) | IVNDFD (%) |
|---|---|---|---|
| 0 (negative control) | 42.87[d] | 35.78[c] | 26.29[e] |
| **Individual enzymes (positive control)** | | | |

*Corresponding Author: **Mdziniso M. P**

| | | | |
|---|---|---|---|
| Xylanase 2.5 | 51.05$^{c}$ | 44.02$^{ab}$ | 31.08$^{de}$ |
| Xylanase 3.5 | 53.16$^{bc}$ | 42.59$^{ab}$ | 32.10$^{cd}$ |
| Xylanase 4.5 | 51.98$^{c}$ | 42.84$^{ab}$ | 32.19$^{cd}$ |
| Xylanase 5.5 | 52.54$^{bc}$ | 42.96$^{ab}$ | 34.07$^{bcd}$ |
| Cellulase 1.5 | 50.56$^{c}$ | 44.89$^{ab}$ | 35.70$^{abcd}$ |
| Cellulase 2.5 | 53.10$^{bc}$ | 42.53$^{ab}$ | 36.38$^{abc}$ |
| **Cellulase-Xylanase combinations** | | | |
| 1.5 x 2.5 | 60.04$^{a}$ | 47.86$^{a}$ | 39.12$^{a}$ |
| 1.5 x 3.5 | 57.99$^{ab}$ | 44.76$^{ab}$ | 38.60$^{ab}$ |
| 1.5 x 4.5 | 54.83$^{abc}$ | 42.34$^{ab}$ | 37.75$^{ab}$ |
| 1.5 x 5.5 | 53.59$^{bc}$ | 42.22$^{ab}$ | 36.38$^{abc}$ |
| 2.5 x 2.5 | 51.42$^{c}$ | 40.55$^{bc}$ | 31.51$^{cd}$ |
| 2.5 x 3.5 | 53.90$^{bc}$ | 42.90$^{ab}$ | 39.20$^{a}$ |
| 2.5 x 4.5 | 54.21$^{bc}$ | 45.32$^{ab}$ | 37.66$^{ab}$ |
| 2.5 x 5.5 | 51.18$^{c}$ | 41.17$^{bc}$ | 31.16$^{de}$ |
| SEM | 1.0972771 | 1.0835049 | 0.9674792 |
| Probability | p<0.0001 | p<0.0001 | p<0.0001 |

Sample size (N) = 3; $^{a,b,c,d,e}$ Means in the same column with different superscripts differ significantly (p<0.05)

## DISCUSSION

This study found that adding enzymes increased the digestion of maize stover. It was observed that across all doses, cellulase enhanced (p<0.05) IVNDFD when compared with maize stover without enzyme supplementation, while xylanase improved (p<0.05) NDF digestibility at certain doses. Supplementing with cellulase and xylanase has also been shown to improve fodder digestibility in the past Arriola et al., 2011; Azzaz et al., 2013; Salem et al., 2013) (Ribeiro et al., 2018; Mdziniso et al., 2025). Worth noting is that as cellulase level increased from 0.5 g/kg DM there was an increase in maize stover digestibility reaching a peak at 2.5 g/kg DM. This increase in digestibility was significant (p<0.05) in OM and NDF digestibility among the enzyme doses. Increasing xylanase doses to 3.5, 4.5 and 5.5 g/kg DM improved (p<0.05) IVNDFD compared to the control. The increase in maize stover digestibility with cellulase supplementation was due to the ability of cellulase to cleave the beta 1,4-D-glucan linkages of cellulose, the major component of plant cell walls, producing glucose, cellobiose and cello-oligosaccharides (COS) as primary products (Sukumaran et al., 2005; Jayasekara & Ratnayake, 2019) while xylanase target xylan, a major (about 70%) component of hemicellulose, hydrolysing the xylan to oligomers, beta-xylosidase degrades the oligomers to xylose, a fermentable sugar (Zhang et al., 2019; Basit et al., 2021). Therefore, enzyme supplementation released more nutrients, stimulating colonisation of maize stover by microbes.

Exogenous fibrolytic enzymes increase the solubility of DM and NDF, which may be attributed to increased pre-digestion of ingredients (Pinos-Rodríguez et al., 2008), thus releasing more nutrients. This provides readily fermentable carbohydrates due to the increased amount of readily fermentable carbohydrates released by EFE acting on cellulose and pectin (Azzaz et al., 2013; Qiu et al., 2024). These nutrients may have supported the production of glycocalyx, which permits adhesion between bacteria or between the substrate and bacteria (Yang et al., 1999). This may partially protect them from degradation by proteases, as reviewed by Beauchemin et al. (2004), thus increasing fibre digestion. Supplementing with exogenous fibrolytic enzymes, therefore, encourage rumen microorganisms' attachment and colonization to the plant cell wall, thus increasing fibrolytic activity (Yang et al., 1999; Arriola et al., 2011), improving their capability of hydrolysing plant cell walls and hemicellulose concentrations in plants (Romero et al., 2016), and they provide synergistic enhancement of microbial enzyme activity in the rumen (Beauchemin et al., 2004; Hu et al., 2013).

When evaluating the effect of recombinant fibrolytic enzymes, Ribeiro et al. (2018) concluded that XYL10A, a xylanase enzyme, may have promoted a shift in the microbial population colonising feed particles when they observed an increase in endoglucanase activity with the addition of the enzyme. When investigating the synergy between ruminal fibrolytic enzymes and exogenous enzymes, Morgavi et al. (2000) concluded that an improvement in fibre digestion with enzyme supplementation may be partly attributed to the synergy between endogenous enzymes produced by rumen microbes and the exogenous enzymes added in cattle diets. Pre-digestion of feed ingredients with EFE high in xylanolytic activity increased the rate of degradation or the potentially degradable fraction of DM and NDF (Pinos-Rodríguez et al., 2008).

Cellulase-xylanase (CX) combinations in most doses improved (p<0.05) IVDMD, IVOMD and NDFD when compared to maize stover without enzyme supplementation (negative control). Moreover, some CX doses in this study enhanced maize stover digestibility compared to when cellulase and xylanase were added singly (positive control). These findings are in line with Qiu et al. (2024) who reported lower NDF content of the mixed silage of king grass and rice straw with the supplementation of a mixture of cellulase and xylanase. Correspondingly, Mdziniso et al. (2025) reported that IVNDFD was improved (p<0.05) with cellulase-xylanase combination than when cellulase and xylanase were applied singly. The improvement may be attributed to the synergistic effect of cellulase and xylanase (Song et al., 2016). Xylanase is considered an accessory enzyme (Hu et al., 2013; Dias et al., 2022). Zhang et al. (2012) reported that xylan coats cellulose, thus inhibiting the hydrolysis of lignocellulosic material by blocking the access of cellulase to cellulose. Xylanases hydrolyse this xylan (Zhang et al., 2019; Basit et al., 2021) and further alter the fibre structure of lignocellulosic materials by promoting swelling of cellulose

*Corresponding Author: **Mdziniso M. P**

fibres and increasing fibre porosity (Hu et al., 2011; Zhao et al., 2018). All these increase the surface area of cellulose thus increasing the accessibility of cellulases to cellulose and lowering the amount of enzymes required to achieve significant hydrolysis of cellulose (Hu et al., 2013).

Further, in the present study, it was observed that cellulase and xylanase, when applied singly, required higher doses to achieve a similar effect in maize stover digestibility to CX combination of 1.5 x 2.5 g/kg DM (lower dose). These findings concur with Beauchemin et al. (2000) who reported that lactating cows fed diets supplemented with low-dose enzymes (1.22 ml/kg of TMR DM) with beta-glucanase, xylanase and endocellulase activities had a greater intake of digestible nutrients compared to cows fed control diets or diets with a high dose of enzymes (3.67 ml/kg of TMR DM). Similarly, Gallardo et al. (2010) reported that an increase in the inclusion level of an enzyme of cellulase and xylanase activities up to 3 g/kg DM alfalfa hay increased the potentially degradable fraction and degradation rate of NDF and ADF. In an *in vitro* study where sugar beet was supplemented with Tomoko commercial enzyme product and Asperozym enzyme, Azzaz et al. (2013) observed that increasing levels of the fibrolytic enzymes up to 2g/kg DM increased *in vitro* DM digestibility and *in vitro* OM digestibility. Basit et al. (2021) reported that an improvement in the hydrolysis of xylan and cellulose and a reduction of enzyme dosage is achieved when xylanase is used as an accessory enzyme to cellulase. Therefore, when combined, cellulase and xylanase supplementation of maize stover would not only improve maize stover digestibility but also reduce the use of higher quantities of the same when applied singly.

The enhancement in maize stover digestibility with CX combination may be attributed to the synergistic effect of cellulase and xylanase in degrading the fibre component (Hu et al., 2013) of maize stover through cellulose hydrolysis. As previously mentioned, cellulase breaks down cellulose while xylanase targets xylan, a major component of hemicellulose (Zhang et al., 2019). Therefore, the hemicellulose barriers in the plant cell walls may have been removed by xylanase thus exposing cellulose to cellulase. The breakdown of cellulose and hemicellulose releases sugars that serve as substrates for microbial fermentation, thereby promoting microbial growth. Direct-fed enzymes stimulate an increase in ruminal microbes, resulting in higher numbers of rumen microbes, which increase digestion activity of feeds through enhanced microbial colonization of feed particles (Yang et al., 1999; Arriola et al., 2011; Salem et al., 2013) or direct cell wall hydrolysis (Arriola et al., 2011), thus increasing the digestibility of the feed.

After reaching a peak at 2.5 g/kg DM with cellulase supplementation, IVNDFD decreased ($p<0.05$) with a further increase in cellulase dose. Similarly, lower xylanase doses of 1.5 and 2.5 g/kg DM and a highest dose of 6.5 g/kg DM of xylanase failed to improve ($p>0.05$) IVNDFD of maize stover, and the higher cellulase dose of 2.5 g/kg DM in CX combinations resulted in lower DM and OM digestibility. These findings are in line with results from Bhasker et al. (2013) who reported improvement ($p<0.01$) in IVDMD of maize stover from lower cellulase concentration of 6,400 to 25,600 IU/g DM and higher xylanase concentration of 25,600 and 32,000 IU/g DM. Similarly, Mdziniso et al. (2025) reported no improvement ($p<0.05$) in IVNDFD when cellulase was applied at 3 g/kg DM of maize stover. A higher concentration of exogenous enzymes may have reduced the rumen bacteria's attachment when they competed for available binding sites (Morgavi et al., 2000).

According Beauchemin et al. (2003), a barrier against microbial colonisation in the diet may be created with an excess of enzymes, as these may attach to sites meant for rumen bacteria, thus making them unavailable. A lack of synergistic effects was reported by Ribeiro et al. (2018) when enzyme mixtures of effective fibrolytic enzymes failed to improve the fibre digestion of barley straw. It was suggested that, instead of only having a direct impact on fibre hydrolysis, this lack of synergism may indicate that the mechanisms by which individual enzymes enhance fibre digestion in the rumen may be more indirect, through encouraging microbial attachment to the feed, changes in the microbial population, and corresponding enzymatic activity (Ribeiro et al., 2018).

Notably, the most effective enzyme combination in this study was cellulase-xylanase at 1.5 and 2.5 g/kg DM, with a ratio of 1:1.67. These findings are in line with the ratios of 1:2.5 (Gaafar et al., 2010) and 1:1.7 (Khademi et al., 2022) for cellulase to xylanase, and 1:1.5:1:1.5 for cellulase-xylanase-pectinase and laccase (Zhang et al., 2022) that were reported to be effective in the digestibility of forages.

## CONCLUSION

The synergistic effect of cellulase and xylanase is beneficial to the digestibility of maize stover. This study demonstrated that the digestibility of maize stover using cellulase and xylanase is influenced by enzyme dosage and enzyme combination. When applied singly, cellulase was effective at lower levels (1.5 and 2.5 g/kg DM), and xylanase was effective at higher levels (3.5, 4.5, and 5.5 g/kg DM). However, when cellulase and xylanase were administered together, efficient hydrolysis was accomplished at lower doses than when they were applied separately. This suggests that a combination of cellulase and xylanase may be used to lower the quantity of enzymes to achieve effective digestion in ruminant diets. This study, therefore, established that effective digestibility of maize stover is achieved with cellulase-xylanase doses of 1.5 x 2.5 g/kg DM.

## ETHICAL CONSIDERATION

The Egerton University Institutional Scientific and Ethics Review Committee granted clearance for the experiment's animal handling procedures under approval number EUISERC/APP/204/2022. The National Commission for Science, Technology, and Innovation (NACOSTI) granted a research license, with license number NACOSTI/P/22/21751.

*Corresponding Author: **Mdziniso M. P**

## ACKNOWLEDGMENT

The Centre of Excellence in Sustainable Agriculture and Agribusiness Management (CESAAM) provided funding for this study.

## REFERENCES

1. Aboul-Fotouh, G., El-Garhy, G., Abd El-Mola, A., Mousa, G., & Azzaz, H. (2017). Effect of Using Some Fibrolytic Enzymes in the Ration on Lactating Goats Performanc. *Egyptian Journal of Nutrition and Feeds*, *20*(2), 1–9. https://doi.org/10.21608/ejnf.2017.103671
2. Arriola, K. G., Kim, S. C., Staples, C. R., & Adesogan, A. T. (2011). Effect of fibrolytic enzyme application to low- and high-concentrate diets on the performance of lactating dairy cattle. *Journal of Dairy Science*, *94*(2), 832–841. https://doi.org/10.3168/jds.2010-3424
3. Azzaz, H. H., Murad, H. A., Kholif, A. M., Morsy, T. A., Mansour, A. M., & El-Sayed, H. M. (2013). Increasing nutrient bioavailability by using fibrolytic enzymes in dairy buffaloes feeding. *Journal of Biological Sciences*, *13*(4), 234–241. https://doi.org/10.3923/jbs.2013.234.241
4. Basit, A., Jiang, W., & Rahim, K. (2021). Xylanase and Its Industrial Applications. *Biotechnological Applications of Biomass*, 1–22. https://doi.org/10.5772/intechopen.92156
5. Beauchemin, K. A., Colombatto, D., Morgavi, D. P., Yang, W. Z., & Rode, L. M. (2004). Mode of action of exogenous cell wall degrading enzymes for ruminants. *Canadian Journal of Animal Science*, *84*(1), 13–22. https://doi.org/10.4141/A02-102
6. Beauchemin, K. A., Colombatto, D., Morgavi, D., & Yang, W. (2003). Use of Exogenous Fibrolytic Enzymes to Improve Feed Utilization by Ruminants. *Journal of Animal Science*, *81*(14_suppl_2), E37–E47. https://doi.org/10.2527/2003.8114_suppl_2E37x
7. Beauchemin, K. A., Rode, L. M., Maekawa, M., Morgavi, D. P., & Kampen, R. (2000). Evaluation of a nonstarch polysaccharidase feed enzyme in dairy cow diets. *Journal of Dairy Science*, *83*(3), 543–553. https://doi.org/10.3168/jds.S0022-0302(00)74914-9
8. Bhasker, T. V., Nagalakshmi, D., & Srinivasa Rao, D. (2013). Development of appropriate fibrolytic enzyme combination for maize stover and its effect on rumen fermentation in sheep. *Asian-Australasian Journal of Animal Sciences*, *26*(7), 945–951. https://doi.org/10.5713/ajas.2012.12590
9. Carreón, L., Pinos-Rodríguez, J. M., Bárcena, R., González, S. S., & Mendoza, G. (2010). Influence of fibrolytic enzymes on ruminal disappearance and fermentation in steers fed diets with short and long particle length of forage. *Italian Journal of Animal Science*, *9*(1), 83–87. https://doi.org/10.4081/ijas.2010.e17
10. Depetris-Chauvin, N., Porto, G., & Mulangu, F. (2017). Agricultural Supply Chains, Growth and Poverty in Sub-Saharan Africa: Market Structure, Farm Constraints and Grass-Root Institutions. In *Agricultural Supply Chains, Growth and Poverty in Sub-Saharan Africa: Market Structure, Farm Constraints and Grass-Root Institutions* (1st ed.). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-53858-6
11. Dias, I. K. R., Siqueira, G. A., & Arantes, V. (2022). Xylanase increases the selectivity of the enzymatic hydrolysis with endoglucanase to produce cellulose nanocrystals with improved properties. *International Journal of Biological Macromolecules*, *220*(August), 589–600. https://doi.org/10.1016/j.ijbiomac.2022.08.047
12. Duncan, D. B. (1955). Multiple Range and Multiple F Tests. *Biometrics*, *11*(1), 1–42. https://doi.org/https://doi.org/10.2307/3001478
13. FAO and IGAD. (2019). *East Africa Animal Feed Action Plan*. http://www.fao.org/resilience/resources/resources-detail/en/c/1208044/
14. Fon, F. N., & Nsahlai, I. V. (2013). Effect of direct-fed microbial consortia on ruminal fermentation of maize stover in sheep. *Small Ruminant Research*, *111*(1–3), 71–75. https://doi.org/10.1016/j.smallrumres.2012.09.016
15. Gaafar, H. M. A., Abdel-Raouf, E. M., & El-Reidy, K. F. A. (2010). Effect of Fibrolytic Enzyme Supplementation and Fibre Content of Total Mixed Ration on Productive Performance of Lactating Buffaloes. *Slovak Journal of Animal Science*, *43*(3), 147–153.
16. Gado, H. M., Salem, A. Z. M., Odongo, N. E., & Borhami, B. E. (2011). Influence of exogenous enzymes ensiled with orange pulp on digestion and growth performance in lambs. *Animal Feed Science and Technology*, *165*(1–2), 131–136. https://doi.org/10.1016/j.anifeedsci.2011.02.016
17. Gallardo, I., Bárcena, R., Pinos-Rodríguez, J. M., Cobos, M., Carreón, L., & Ortega, M. E. (2010). Influence of exogenous fibrolytic enzymes on in vitro and in sacco degradation of forages for ruminants. *Italian Journal of Animal Science*, *9*(1), 34–38. https://doi.org/10.4081/ijas.2010.e8
18. Heuzé, V., Tran, G., & Lebas, F. (2019). *Maize stover*. Feedipedia, a Programme by NRA, CIRAD, AFZ and FAO. https://www.feedipedia.org/node/16072
19. Hu, J., Arantes, V., Pribowo, A., & Saddler, J. N. (2013). The synergistic action of accessory enzymes enhances the hydrolytic potential of a "cellulase mixture" but is highly substrate specific. *Biotechnology for Biofuels*, *6*(1), 1–12. https://doi.org/10.1186/1754-6834-6-112
20. Hu, J., Arantes, V., & Saddler, J. N. (2011). The enhancement of enzymatic hydrolysis of

*Corresponding Author: **Mdziniso M. P**

lignocellulosic substrates by the addition of accessory enzymes such as xylanase : is it an additive or synergistic effect ? The enhancement of enzymatic hydrolysis of lignocellulosic substrates by the addition o. *Biotechnology for Biofuels*, *4*(1), 36. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3198685&tool=pmcentrez&rendertype=abstract%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3042927&tool=pmcentrez&rendertype=abstract

21. Jayasekara, S., & Ratnayake, R. (2019). Microbial Cellulases: An Overview and Applications. *Cellulose*, 1–21. https://doi.org/10.5772/intechopen.84531
22. Khademi, A. R., Hashemzadeh, F., Khorvash, M., Mahdavi, A. H., & Pazoki, A. (2022). Use of exogenous fibrolytic enzymes and probiotic in finely ground starters to improve calf performance. *Scientific Reports*, 1–14. https://doi.org/10.1038/s41598-022-16070-0
23. López-Aguirre, D., Hernández-Meléndez, J., Rojo, R., Sánchez-Dávila, F., López-Villalobos, N., Salem, A. F. Z. M., Martínez-González, J. C., Vázquez-Armijo, J. F., & Ruíz, S. (2016). Effects of exogenous enzymes and application method on nutrient intake, digestibility and growth performance of Pelibuey lambs. *SpringerPlus*, *5*(1). https://doi.org/10.1186/s40064-016-3075-7
24. McDougall, E. I. (1948). Studies on Ruminant Saliva. 1. The composition and output of sheep's saliva. *Biochemical Journal*, *43*, 99–109. https://doi.org/10.1042/bj0430099
25. Mdziniso, M. P., Tuitoek, J. K., Ondiek, J. O., & Muliro, P. S. (2025). Effectiveness of selected fibrolytic enzymes on in vitro digestibility of maize (Zea mays) stover. *GSAR Journal of Agriculture and Veterinary Sciences*, *9075*(July), 69–76.
26. Morgavi, D. P., Beauchemin, K. A., Nsereko, V. L., Rode, L. M., Iwaasa, A. D., Yang, W. Z., McAllister, T. A., & Wang, Y. (2000). Synergy between ruminal fibrolytic enzymes and enzymes from Trichoderma longibrachiatum. *Journal of Dairy Science*, *83*(6), 1310–1321. https://doi.org/10.3168/jds.S0022-0302(00)74997-6
27. Njarui, D. M. G., Gatheru, M., Wambua, J. M., Nguluu, S. N., Mwangi, D. M., & Keya, G. A. (2011). Feeding management for dairy cattle in smallholder farming systems of semi-arid tropical Kenya. *Livestock Research for Rural Development*, *Volume 23*(Article #111). http://www.lrrd.org/lrrd23/5/njar23111.htm
28. Otieno, G. O., Muendo, K., & Mbeche, R. (2020). Smallholder Dairy Production, Motivations, Perceptions and Challenges in Nyandarua and Nakuru Counties, Kenya. *IOSR Journal of Agriculture and Veterinary Science*, *13*, 42–50. https://doi.org/10.9790/2380-1301024250
29. Pinos-Rodríguez, J. M., Moreno, R., González, S. S., Robinson, P. H., Mendoza, G., & Álvarez, G. (2008). Effects of exogenous fibrolytic enzymes on ruminal fermentation and digestibility of total mixed rations fed to lambs. *Animal Feed Science and Technology*, *142*(3–4), 210–219. https://doi.org/10.1016/j.anifeedsci.2007.08.005
30. Qiu, C., Liu, N., Diao, X., He, L., Zhou, H., & Zhang, W. (2024). Effects of Cellulase and Xylanase on Fermentation Characteristics, Chemical Composition and Bacterial Community of the Mixed Silage of King Grass and Rice Straw. *Microorganisms*, *12*(3). https://doi.org/10.3390/microorganisms12030561
31. Rademaker, C. J. C., Bebe, B. O., Lee, J. Van Der, Kilelu, C., Tonui, C., Omedo Bebe, B., van der Lee, J., Kilelu, C., & Tonui, C. (2016). *Sustainable growth of the Kenyan dairy sector : a quick scan of robustness, reliability and resilience. Report 3R Kenya/WLR 979*. https://doi.org/10.18174/391018
32. Ribeiro, G. O., Badhan, A., Huang, J., Beauchemin, K. A., Yang, W., Wang, Y., Tsang, A., & McAllister, T. A. (2018). New recombinant fibrolytic enzymes for improved in vitro ruminal fiber degradability of barley straw. *Journal of Animal Science*, *96*(9), 3928–3942. https://doi.org/10.1093/jas/sky251
33. Salem, A. Z. M., Gado, H. M., Colombatto, D., & Elghandour, M. M. Y. (2013). Effects of exogenous enzymes on nutrient digestibility, ruminal fermentation and growth performance in beef steers. *Livestock Science*, *154*(1–3), 69–73. https://doi.org/10.1016/j.livsci.2013.02.014
34. Schingoethe, D. J., Stegeman, G. A., & Treacher, R. J. (1999). Response of Lactating Dairy Cows to a Cellulase and Xylanase Enzyme Mixture Applied to Forages at the Time of Feeding. *Journal of Dairy Science*, *82*(5), 996–1003. https://doi.org/10.3168/jds.S0022-0302(99)75319-1
35. Soliva, C. R., & Hess, H. D. (2007). Measuring methane emission of ruminants by in vitro and in vivo techniques. In H. P. S. Makkar & P. E. Vercoe (Eds.), *Measuring Methane Production from Ruminants* (pp. 15–31). Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-6133-2_2
36. Song, H. T., Gao, Y., Yang, Y. M., Xiao, W. J., Liu, S. H., Xia, W. C., Liu, Z. L., Yi, L., & Jiang, Z. B. (2016). Synergistic effect of cellulase and xylanase during hydrolysis of natural lignocellulosic substrates. *Bioresource Technology*, *219*, 710–715. https://doi.org/10.1016/j.biortech.2016.08.035
37. Sukumaran, R. K., Singhania, R. R., & Pandey, A. (2005). Microbial cellulases - Production, applications and challenges. *Journal of Scientific and Industrial Research*, *64*(11), 832–844.
38. Sutton, J. D., Phipps, R. H., Beever, D. E., Humphries, D. J., Hartnell, G. F., Vicini, J. L., & Hard, D. L. (2003). Effect of method of application

*Corresponding Author: **Mdziniso M. P**

of a fibrolytic enzyme product on digestive processes and milk production in Holstein-Friesian cows. *Journal of Dairy Science*, *86*(2), 546–556. https://doi.org/10.3168/jds.S0022-0302(03)73633-9

39. Tilley, J. M. A., & Terry, R. A. (1963). A Two-Stage Technique for the in vitro digestion of forage crops. *Journal of the British Grassland Society*, *18*, 104–111. https://doi.org/10.1111/j.1365-2494.1963.tb00335.x
40. Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *Journal of Dairy Science*, *74*(10), 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2
41. Yáñez-Ruiz, D. R., Bannink, A., Dijkstra, J., Kebreab, E., Morgavi, D. P., O'Kiely, P., Reynolds, C. K., Schwarm, A., Shingfield, K. J., Yu, Z., & Hristov, A. N. (2016). Design, implementation and interpretation of in vitro batch culture experiments to assess enteric methane mitigation in ruminants-a review. *Animal Feed Science and Technology*, *216*, 1–18. https://doi.org/10.1016/j.anifeedsci.2016.03.016
42. Yang, W. Z., Beauchemin, K. A., & Rode, L. M. (1999). Effects of an enzyme feed additive on extent of digestion and milk production of lactating dairy cows. *Journal of Dairy Science*, *82*(2), 391–403. https://doi.org/10.3168/jds.S0022-0302(99)75245-8
43. Zhang, F., Bunterngsook, B., Li, J.-X., Zhao, X.-Q., Champreda, V., Liu, C.-G., & Bai, F.-W. (2019). Chapter three - Regulation and production of lignocellulolytic enzymes from Trichoderma reesei for biofuels production. In *Advances in Bioenergy* (1st ed., pp. 79–119). Elsevier Inc. https://doi.org/10.1016/bs.aibe.2019.03.001
44. Zhang, J., Tang, M., & Viikari, L. (2012). Xylans inhibit enzymatic hydrolysis of lignocellulosic materials by cellulases. *Bioresource Technology*, *121*, 8–12. https://doi.org/10.1016/j.biortech.2012.07.010
45. Zhang, J., Wang, C., Liu, Q., Guo, G., Huo, W., Pei, C., & Jiang, Q. (2022). Influence of fibrolytic enzymes mixture on performance, nutrient digestion, rumen fermentation and microbiota in Holstein bulls. *Journal of Animal and Feed Sciences*, *31*(1), 46–54. https://doi.org/10.22358/jafs/147188/2022
46. Zhao, J., Dong, Z., Li, J., Chen, L., Bai, Y., Jia, Y., & Shao, T. (2018). Ensiling as pretreatment of rice straw: The effect of hemicellulase and Lactobacillus plantarum on hemicellulose degradation and cellulose conversion. *Bioresource Technology*, *266*, 158–165. https://doi.org/10.1016/j.biortech.2018.06.058

*Corresponding Author: **Mdziniso M. P**