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Digestibility and rumen fermentation of a high forage diet pre-treated with a mixture of cellulase and xylanase enzymes

K. Selzer[1], A. Hassen[1#], A.M. Akanmu[1] & A.Z.M. Salem[2]
[1]Department of Animal Sciences, University of Pretoria, Pretoria, South Africa
[2]Depto. de Nutrición Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del, Estado de México, Toluca, Edo de México, México

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Abstract

Forages play an important role in ruminant animal production worldwide. Unlocking the nutritional potential of poor-quality tropical forages with fibrolytic enzymes would improve forage digestibility and utilization. Using in vitro and in vivo methods this study investigated the effect of pre-treating Smutsfinger hay for 24 hours with a mixture of fibrolytic enzyme (100% cellulase; 75% cellulase: 25% xylanase; 50% cellulase: 50% xylanase; 25% cellulase: 75% xylanase; 100% xylanase and a control with no enzyme) on ruminal fermentation and digestibility of nutrients by sheep. For in vitro fermentation, dry matter, neutral detergent fibre (NDF) degradability and volatile fatty acids (VFA) were determined with standard procedures. The same treatments were used for an in vivo digestibility trial using Merino sheep in a 6 x 6 Latin square design. Feed intake and total tract digestibility were recorded. Rumen fluid samples were collected daily, preserved, and analysed for VFA. The addition of 100% cellulase enzyme to Smutsfinger hay in vitro increased (P <0.05) NDF degradability and gas production compared with the control and inclusion of 100% xylanase enzyme. Both 100% cellulase and xylanase enzymes significantly reduced in vitro end time fermentation pH. A 50:50 mixture of cellulase and xylanase plus enzyme in vivo, increased acetate, total VFA concentration, and higher NDF and ADF digestibility of the test feed compared with the control. Inclusion of a 50-75% mixture of cellulase and 50-25% xylanase enzymes treatment led to higher gas production and butyrate concentration, decreased ruminal pH and improved nutrient digestibility.

Keywords: fibrolytic enzymes, ruminants, sheep, volatile fatty acids
[#]Corresponding author: Abubeker.hassen@up.ac.za

Introduction

Forages comprise the biggest part of feeding cost. During the dry season, crop residues and poor quality grasses from rangelands are basal feeds for ruminants in developing countries with tropical climates. Tropical pastures and crop residue are often of variable quality, and are of low nutritional value at maturity because the high rate of lignification (Meissner, 1997) leads to poorer degradation and lower feed utilization efficiency (Krueger *et al.*, 2008) and result in increased cost of production owing to the elongated period for animals to reach their growth potential.

Ruminants, however, have adapted to feed of low quality for growth and production owing to the diverse microbial community in their rumen, but production efficiency is not optimal. Less than 50% of the fibre fraction is readily digested and utilized by the animal (Hatfield *et al.*, 1999). This inefficiency can result in an increase in the quantity of feed needed to maintain the required levels of animal performance. Thus, any improvement in the degradation of the forage cell wall could be of great benefit. Hence this presents a logical area of research for the improvement of forage utilization.

As additives to ruminant nutrition, exogenous fibrolytic enzymes are of growing interest as a way of improving digestibility of fibrous feeds. For instance, cellulase, xylanase and pectinase are used in ruminant feed biotechnology to improve feed utilization, to affect the production of milk and meat and to improve the digestibility of certain feed components. They have been studied extensively in the last couple of decades as a viable means of improving the digestibility of forages typically used in ruminant nutrition. Enzymes such as cellulase and xylanase, which degrade the cell wall, could hydrolyse forage fibre (Feng *et al.*, 1996). Thus,

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supplementation with fibrolytic enzymes may have a positive role in improving the digestibility (Bala *et al.*, 2009; Feng *et al.*, 1996; Geraldo *et al.*, 2008) of the roughage proportion, thereby decreasing the retention time in the rumen and perhaps increasing feed intake. Most research on fibrolytic enzyme additives in ruminants has been conducted on beef and dairy cattle. Research on its effects on small ruminants such as sheep would be beneficial and was the focus of this study. The current in vivo and in vitro study had the objective of establishing the effects of various proportions of cellulase and xylanase enzyme mixtures on the gas production, and dry matter, organic matter, neutral detergent fibre and acid detergent fibre digestibility in sheep fed a high roughage total mixed ration, and the effect of the supplementation on VFA production in sheep.

Material and methods

This study was completed at the experimental farm of the University of Pretoria, with ethical clearance from the Animal Ethics Committee (project number EC113-13). Dry matter and ash contents of the Smutsfinger hay and TMR were determined according to methods 934.01 and 942.05, respectively (AOAC, 2019). Methods 12 and 13 of ANKOM Technology (Macedon, New York, USA) were used to determine ADF and NDF. The nitrogen (N) concentration of the TMR was determined with TruMac N (Leco Corp. St. Joseph, Michigan, USA) following the manufacturer's recommended procedure for implementing method 968.06 (AOAC, 2019). Crude protein concentration was calculated by multiplying the N percentage by a factor of 6.25. Tables 1 and 2 show the nutrient composition of Smutsfinger hay used in the in vitro trial and of the TMR, which was formulated at maintenance level for the in vivo study.

Table 1 Nutrient composition of Smutsfinger hay (g/kg)

Chemical components	As is basis	Dry matter basis
Dry matter	881.0	1000
Organic matter	891.6	876.9
Ash	108.4	123.1
Crude protein	53.9	61.2
Neutral detergent fibre	651.8	739.8
Acid detergent fibre	417.4	473.8

Table 2 Ingredient and nutrient composition of total mixed ration to be fed to mature sheep

Ingredients	% composition
Yellow maize	33.9
Wheat middling	6.2
Molasses	8.0
Limestone powder - hand add	0.47
Salt	1.22
Urea	0.08
Vitamin/mineral supplement	0.15
Chemical components	DM (g/kg)
Organic matter	906.9
Ash	93.1
Crude protein	77.2
Neutral detergent fibre	402.2
Acid detergent fibre	233.7

For both experiments, six treatments were tested, including the control, which consisted of various proportions of the exogenous fibrolytic enzymes cellulase plus and xylanase plus. The cellulase plus and xylanase plus enzymes were obtained from Dyadic International Inc. (Jupiter, Florida, USA) as concentrated liquids of acid cellulase (E.C. 3.2.1.4) and acid-neutral endo-1, 4-β-D-xylanase (E.C. 3.2.1.8), respectively. They were produced by the fermentation of non-genetically modified *Trichoderma longibrachiatum* (formerly *Trichoderma reesei*). The level of addition was based on the outcome of a previous study (Gemeda *et al.*, 2014). About 20 *u*l of each enzyme treatment was added to 100 ml distilled water. Smutsfinger hay was dried at 55 °C for 24 hours and milled through a 1-mm screen. Approximately 0.5 g of the milled sample was weighed into incubation vials. All enzyme treatments were applied 24 hours prior to incubation to allow for the enzyme-substrate interaction (Beauchemin *et al.*, 2003). Approximately 1 mL of the appropriate enzyme treatment was pipetted directly onto the substrate already in the incubation vials 24 hours before the start of in vitro incubation and left at room temperature. These treatments that were applied to the Smutsfinger hay in both the in vitro and in vivo experiments:

T1: TMR plus hay treated with 0.4 ml/kg cellulase plus (100%) and 0.0 ml/kg xylanase plus (0%)
T2: TMR plus hay treated with 0.3 ml/kg cellulase plus (75%) and 0.1 ml/kg xylanase plus (25%)
T3: TMR plus hay treated with 0.2 ml/kg cellulase plus (50%) and 0.2 ml/kg xylanase plus (50%)
T4: TMR plus hay treated with 0.1 ml/kg cellulase plus (25%) and 0.3 ml/kg xylanase plus (75%)
T5: TMR plus hay treated with 0.0 ml/kg cellulase plus (0%) and 0.4/kg xylanase plus (100%)
T6: Control without enzyme

Rumen fluid was collected from three Merino sheep at the small stock section, University of Pretoria Experimental Farm. The animals were fed lucerne ad libitum as basal diet with two feedings a day to ensure constant availability of fresh feed and consistency of the rumen fluid. Rumen liquor was collected from at least two animals and squeezed through two layers of cheesecloth. At the laboratory, the rumen fluid was further strained through a single layer of cheesecloth to ensure that any large feed particles were removed before it was transferred to a large glass beaker that had been pre-warmed in a water bath at 39 °°C and purged continuously with CO_2.

The incubation media was prepared according to Menke and Steingass (1988). A few hours before in vitro incubation, 2.5 g tryptone was dissolved completely in 500 mL water. Then, 0.125 mL micro-mineral solution, 250 mL buffer solution, 250 mL macro-mineral solution, and 1.25 mL 0.1% resazurin solution were added. The container with the media was then placed in a water bath at 39 °C and purged continuously with CO_2 for 45 minutes. To reduce oxygen, approximately 0.313 g L-cysteine hydrochloride and 0.313 g sodium sulphide was added and purged with CO_2 continuously for another 15 minutes or until the solution turned from grey to clear. The medium was then kept in the water bath with continuous CO_2 bubble. One part of rumen fluid was added to four parts buffer solution. The vials containing the enzyme-feed complex were placed in the incubation oven at 39 °C to warm them and prevent cold shock to the rumen fluid micro-organisms. Approximately 42 mL (84 mL/g substrate) of rumen fluid plus medium was then added to the enzyme-feed complex under a stream of CO_2 to each vial and the vials were closed immediately with rubber stoppers and crimp sealed. The bottles were returned to the oven and set to rotate at 120 rpm. When all the bottles had been filled, a needle was inserted through the rubber stopper of each vial for about five seconds to release built-up gas and to create the starting point for the vials. Three independent runs were performed with four replications per treatment in each run.

A semi-automated system was used for the gas pressure readings. It consisted of a digital pressure gauge with a Luer lock adapter and disposable needle. Gas pressure and methane concentration were measured and cumulated at 2, 4, 8, 12, 24, and 48 hrs to avoid pressure build up that exceeded 7 psi, which could be re-absorbed into the liquid phase. Digital gas pressure readings were converted to gas volumes produced in mL (Akanmu & Hassen, 2018). Incubation was terminated after 48 hr by placing the incubation vials on ice to stop fermentation. The vials were opened as soon as possible to sample the supernatant aliquots for VFA. The pH of the fluid was then measured using a pH meter. To determine VFAs, 4 mL 25% phosphoric acid was added to 20 mL filtered rumen sample. This was mixed and stored at -20 °C until required. The samples were analysed for VFAs by defrosting and centrifuged at 4500 rpm for 20 minutes before being filtered through Cameo 30 (0.45 μm) filters (Webb, 1994). In vitro dry matter digestibility and fibre analysis were carried out on the fermented residue following standard procedures. Dry matter of Smutsfinger hay was also determined. All the results were converted to a 100% DM basis and the degradability of nutrients calculated as follows.

$$\% \text{ Dry matter degradability} = \frac{\text{g DM in 0.5 g Smutsfinger hay - g DM in residue after incubation}}{\text{g DM in 0.5 g Smutsfinger}} \times 100$$

The in vivo experiment was a 6 x 6 Latin square design. Six sheep were allocated to one of the six treatments at six periods. The first adaptation and experimental period consisted of 24 days, of which 14 days were for adaptation and the other 10 days for data collection on feed intake, faecal output, rumen digestibility and rumen sampling. The sheep were fed a TMR formulated for maintenance level throughout the experimental period. Feeding took place ad libitum twice a day at 06h00 and 16h00, and fresh clean water was made available at all times. Amounts of feed offered and refused were recorded daily and the bodyweights were recorded at the beginning of each experimental period and at the end of each cycle. Smutsfinger hay was pre-incubated for 24 hrs with the prepared enzyme solution separately for each treatment. Initially, animals were fed untreated TMR for 14 days before the start of adaptation. On the morning of feeding the pre-treated Smutsfinger hay was mixed with the appropriate amount of concentrate to form the TMR. The animals were housed in single metabolic cages with one sheep assigned to each treatment. The sheep were fed ad libitum and were fitted with a faecal collection bag during the last three days of adaptation to allow them to adapt to the faecal bags before the 10 days of data collection, during which the faecal bags stayed on. The amounts of feed offered and refusals were weighed, recorded and sub-sampled daily, and faecal output was weighed, recorded, sub-sampled and frozen each day. The sub-sampled feed and faeces were later dried and analysed to determine DM, ash, OM, CP, NDF, and ADF. Rumen liquor had been collected twice a day for four consecutive days by extending the collection time by three hours in the subsequent days. Rumen content was squeezed through two layers of cheesecloth into containers and a small amount of inoculum was added. The rumen fluid was prepared for the gas chromatographic method to determine VFAs as described. The data were analysed using the general linear model procedure of SAS 9.4 (SAS Institute, Inc., Cary, North Carolina). Means were compared using Duncan's test when the treatment effect was significant.

Result and Discussion

The cumulative gas reading after 48 hr incubation differed (P <0.05) among treatments. Higher (P <0.05) TGP values were observed for T1 and T2 than for the control and T3 - T5. The corresponding increase in gas production associated with the addition of cellulase was similar to the findings of Gemeda *et al.* (2014). In another study, Kung *et al.* (2002) found higher gas production from forages treated with enzymes than that of untreated forage. Cellulases are used widely in any process that involves processing of plant-based materials. The characteristic enzymatic endo-hydrolysis of β-1,4 D-glycosidic bonds in cellulose and in β--D-glucans (Fernandes, 2008) makes it an important enzyme in animal agriculture and could be responsible for the higher TGP witnessed in this study. Higher inclusion of cellulose led to more TGP. The results of TGP in mL/g DM are shown in Table 3 with the fermentation parameters and feed degradability.

The results showed no difference (P >0.05) in DM degradability, although higher degradability values were obtained for the groups treated with enzyme. However, percentage NDF degradability differed (P <0.05). Compared with the control, T1 and T2 had higher NDF degradability, whereas the other treatments were the same as control statistically. These findings correlate with those of Alvarez *et al.* (2009), Bala *et al.* (2009) and Lewis *et al.* (1996) as these authors reported increased nutrient degradability for feeds treated with cellulase and xylanase enzymes compared with control. According to these results, cellulase and xylanase can improve fibre degradation, and the best enzyme treatment for increased degradation would be a 100% cellulase treatment. The 24-hour pre-incubation of feed sample with enzymes in the present study might have produced an improvement in the attachment of micro-organisms to the plant cell wall components (Nsereko *et al.*, 2000; Wang *et al.*, 2001), leading to increased colonization and alteration in the fibre structure because of enzyme effects (Sutton *et al.*, 2003; Elwakeel *et al.*, 2007; Giraldo *et al.*, 2008). Dry matter and NDF degradability after 48 hr incubation of Smutsfinger hay with various enzyme mixtures are presented in Table 3.

Table 3 In vitro gas and methane production and fermentation characteristics of Smutsfinger hay treated with cellulase and xylanase enzymes

Characteristics	T1	T2	T3	T4	T5	T6	SE
Total gas production, ml/g DM	179.8[a]	172.8[a]	170.4[ab]	153.1[c]	157.4[bc]	155.2[bc]	19.51
Methane production, ml/g DM	69.3[a]	67.4[ab]	63.3[ab]	60.3[ab]	59.7[ab]	57.8[b]	10.17
Dry matter digestibility, %	33.4	32.5	30.6	31.8	29.9	29.6	4.92
NDF degradability, %	36.0[a]	33.7[ab]	31.7[bc]	30.4[bc]	30.0[c]	28.2[bc]	4.46
pH	6.5[c]	6.5[c]	6.7[a]	6.6[b]	6.5[c]	6.7[ab]	0.00
Acetate, mM/L	43.3	42.7	42.1	41.1	43.0	41.9	2.45
Propionate, mM/L	14.8	14.6	14.5	14.4	14.8	14.4	0.23
Iso-Butyrate, mM/L	1.4	1.38	1.34	1.34	1.4	1.35	0.00
Butyrate, mM/L	4.9[a]	4.7[ab]	4.7[ab]	4.6[ab]	4.8[a]	4.6[b]	0.03
Valerate, mM/L	1.9	1.9	1.8	1.9	1.9	1.8	0.01
Total volatile fatty acids, mM/)	66.3	65.2	64.4	63.4	65.9	64	5.01
Acetate to propionate ratio	2.9	2.9	2.9	2.9	2.9	2.9	0.00

[a,b,c] Within a row, means with a similar superscript were not detected as being different with probability P =0.05
NDF: neutral detergent fibre, T1: TMR plus hay treated with 0.4 ml/kg cellulase plus and 0.0 ml/kg xylanase plus, T2: TMR plus hay treated with 0.3 ml/kg cellulase plus and 0.1 ml/kg xylanase plus, T3: TMR plus hay treated with 0.2 ml/kg cellulase plus and 0.2 ml/kg xylanase plus, T4: TMR plus hay treated with 0.1 ml/kg cellulase plus and 0.3 ml/kg xylanase plus, T5: TMR plus hay treated with 0.0 ml/kg cellulase plus, and 0.4/kg xylanase plus, T6: control without enzyme addition

Volatile fatty acid production and end fermentation pH showed no difference (P >0.05) across treatments, except for butyrate. T1 and T5 had higher (P <0.05) butyric acid production compared with the control and other treatments. This agrees with Krueger *et al.* (2008), who reported an increase in butyrate concentrations with the addition of cellulase and xylanase enzymes on Bahia grass hay. In contrast with the findings of this study, Gemeda *et al.* (2014) recorded generally higher acetate and total VFA concentration for the samples treated with cellulase and xylanase compared with the control. Kung *et al.* (2002) recorded that VFA production did not vary among treatments. The results of this study agree with those of Yang et al. (2002), who found that the addition of an enzyme did not affect acetate and total VFA concentrations, but showed increased molar proportions, with T1 and T5 in this study being the most effective compared with control. The reason for the high butyrate concentration and a tendency towards higher acetate and total VFA proportions in T1 and T5 could be an increase in the reactivity of the enzyme after the 24-hour incubation, which could have caused enhanced colonization and digestion of slowly degradable fibre fraction by ruminal micro-organisms. A reduced pH value recorded for T1 and T5 could have influenced the rumen fermentation process. There was no difference (P >0.05) between the pH values of the control and those of T3 and T4 xylanase treatment. However, there was a (P <0.05) difference between the other treatments and the control, indicating a reduction in the end fermentation pH with higher cellulase inclusion. The findings of this study agree with those of Lewis *et al.* (1996), who reported decreased ruminal pH in steers fed grass forage treated with exogenous enzymes, with T1 and T5 being the most effective compared with control.

There was a difference (P <0.05) between the treatments in terms of DM intake per unit metabolic body weight where the addition of cellulose and xylanase increased DM intake compared with T1. The results of this study agree with those of Chen *et al.* (1992), Fredeen *et al.* (1993), and Bala *et al.* (2009), all of whom found an increase in DM intake with the addition of cellulase and xylanase enzyme mixtures. In contrast, Lewis *et al.* (1996) and Rode *et al.* (1999) showed that exogenous fibrolytic enzymes fed directly to the animal or added to the feed did not affect the DM intake of cattle. The lack of response in intake improvement may be because enzymes were being fed directly into the rumen and were not pre-treated for 24 hours before feeding, as in this experiment, which gave the enzymes ample time to pre-act on the feed. Table 4 shows the DM intake (g/head/kg $W^{0.75}$) and nutrient digestibility.

However, other studies found different responses to the addition of enzymes. Rodriguez *et al.* (2002) reported that the addition of cellulose and xylanase increased DM intake, whereas Feng *et al.* (1996) reported that DM intake was increased by fibrolytic enzymes when added to dry forages, but not when fresh forages were used. This study shows that T3 improved DM intake most. This finding agreed with those of

Bala *et al.* (2009), in which the addition of a 50% mixture of cellulase and xylanase enzymes prior to feeding was more effective in small ruminants than feeding these enzymes separately or not at all. The gut fill capacity, in relation to forage characteristics, could be considered a main factor of regulation of voluntary intake (Decruyenaere *et al.,* 2009). Intake appeared to be limited by the maximal volume that the digestive tract can reach (Allison, 1985; Allen, 1996). If the transit rate of digesta could be increased when the quality of forage was poor, intake could increase (Johnson *&* Combs, 1992). The increase in intake thus appeared to be because of increased total tract digestibility of the fibre proportion of the feed, allowing the animal to empty its gut better, making more space for additional intake.

Table 4 Intake and nutrient digestibility of rams fed diets containing cellulase and xylanase enzymes

Variable	T1	T2	T3	T4	T5	T6	SE
Intake, g DM/head/kg $W^{0.75}$	47.6^{d}	62.3^{bc}	78.2^{a}	58.4^{bc}	64.7^{b}	60.9^{bc}	13.2
DM digestibility, g DM/head/d	610	630	680	600	660	600	60.1
Organic matter digestibility, g DM/head/d	620	640	690	610	670	620	51.1
Crude protein digestibility, g DM/head/d	460	510	580	480	540	450	11.4
NDF digestibility, g DM/head/d	490^{ab}	490^{ab}	580^{a}	430^{b}	520^{ab}	430^{b}	10.2
ADF digestibility, g DM/head/d	400^{ab}	440^{ab}	520^{a}	350^{ab}	430^{ab}	240^{b}	36.3

^{a,b,c} Within a row, means with a similar superscript were not detected as being different with probability P =0.05
DM: dry matter, NDF: neutral detergent fibre, ADF: acid detergent fibre, T1: TMR plus hay treated with 0.4 ml/kg cellulase plus and 0.0 ml/kg xylanase plus, T2: TMR plus hay treated with 0.3 ml/kg cellulase plus and 0.1 ml/kg xylanase plus, T3: TMR plus hay treated with 0.2 ml/kg cellulase plus and 0.2 ml/kg xylanase plus, T4: TMR plus hay treated with 0.1 ml/kg cellulase plus and 0.3 ml/kg xylanase plus, T5: TMR plus hay treated with 0.0 ml/kg cellulase plus, and 0.4/kg xylanase plus, T6: control without enzyme addition

The total tract digestibility of various nutrients in sheep fed fibrolytic enzymes did not significantly affect the digestibility of the DM, OM and CP components, though there was a tendency towards higher values in the treatments that included enzyme than the control. This agrees with the findings of Burroughs *et al.* (1960) and Theurer *et al.* (1963), who reported no effect of fibrolytic enzymes on the digestibility of DM, OM and CP. In this study, however, the digestibility of the fibre component (NDF and ADF) increased for the 50% cellulase and xylanase mixture compared with control (P <0.05). These findings agree with the reports of other researchers who found an increase in digestibility of various quality forages (Bala *et al.*, 2009; Feng *et al.,* 1996; Geraldo *et al.,* 2008). The mechanism responsible for this increase in digestibility might be the synergistic action of the cellulase and xylanase enzymes, which is believed to come from the ability of xylanase to expose the cellulose microfibril core by removing the hemicellulose or the hemicellulosic side chains (Selig *et al.*, 2008).

Feeds that are high in rapidly fermentable carbohydrates lead to increased microbial populations that favour production of more propionate and butyrate than acetate (Moran, 2005). Higher acetate production comes mainly from slowly fermentable forages. Table 5 shows the VFA production in mM/L. There was a difference (P <0.05) between the control and the 50:50 cellulase and xylanase mixture in acetate and total VFA production. These results agree with the in vitro results reported in Table 3 and those of Gemeda *et al.* (2014), who recorded that acetate and total VFA concentration tended to be higher for the samples treated with enzyme compared with the control. The mechanism responsible for the increase in acetate production might be the synergistic action of the cellulase and xylanase enzymes in the 50% mixture treatment, which resulted in greater NDF and ADF digestibility than the control treatment.

Table 5 Volatile fatty acids (mM/L) of rams fed diets containing cellulase and xylanase enzymes

Volatile fatty acid	T1	T2	T3	T4	T5	T6	SE
Acetate	36.9b	36.1b	42.1a	36.4b	40.0b	39.6b	15.46
Propionate	11.6	12.2	12.9	11.8	13.6	12.0	3.19
Iso-butyrate	1.2bc	1.1c	1.6a	1.1bc	1.5ab	1.3abc	0.07
Butyrate	2.3	2.3	2.6	2.4	2.2	2.4	0.11
Valeric	0.65	0.61	0.71	0.66	0.67	0.66	0.01
Total	52.7b	52.3b	59.9a	52.3b	58.0ab	55.9b	30.6
Acetate propionate ratio	3.34	3.08	3.38	3.2	3.15	3.51	0.17

[a,b,c] Within a row, means with a similar superscript were not detected as being different with probability P =0.05
T1: TMR plus hay treated with 0.4 ml/kg cellulase plus and 0.0 ml/kg xylanase plus, T2: TMR plus hay treated with 0.3 ml/kg cellulase plus and 0.1 ml/kg xylanase plus, T3: TMR plus hay treated with 0.2 ml/kg cellulase plus and 0.2 ml/kg xylanase plus, T4: TMR plus hay treated with 0.1 ml/kg cellulase plus and 0.3 ml/kg xylanase plus, T5: TMR plus hay treated with 0.0 ml/kg cellulase plus, and 0.4/kg xylanase plus, T6: control without enzyme addition

Conclusion

Pre-treating Smutsfinger hay with 50–75% mixture of the cellulase and 50–25% xylanase led to higher gas production and butyrate concentration, decreased ruminal pH, and improved DM and fibre degradation. Similarly, during the in vivo study, pre-treatment of Smutsfinger hay with a mixture of 50% cellulase and xylanase plus enzyme increased that acetate and total VFA concentrations and enhanced the intake and the total tract digestibility of the NDF and ADF components of the feed. This suggests that a 50% cellulase and xylanase plus enzyme mixture is the optimal concentration that could be used for practical application to improve nutrient digestibility, rumen fermentation parameters and fibre digestion in vivo.

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Authors' Contribution

KS and AH designed the study. KS collected data, whereas KS and AH were involved in data analysis. KS, AMA and AH wrote the draft manuscript. AMA, AH and AZMS did the editing and proofreading. AH funded and supervised the study.

Conflict of Interest Declaration

There are no conflicts of interest.

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