



Original research article

## Monitoring the fibrolytic potential of microbial ecosystems from domestic and wild ruminants browsing tanniferous forages



Nokwethemba Nqobile Philile Msimango, Fabian Nde Fon\*

Department of Agriculture, Faculty of Science and Agriculture, University of Zululand, KwaDlangezwa 3886, South Africa

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## ABSTRACT

Although the rumen microbiome has been reported to synthesize a rich source of symbiotic enzymes (exocellulase, endocellulase, hemicellulase and cellobiase), the digestion of tropical C<sub>4</sub> grasses and browses by ruminants is still limited. Therefore, this study aimed to unveil potential fibrolytic microbial ecosystems from giraffe, kudu, impala and consortia (A1 [giraffe + kudu], A2 [giraffe + impala], A3 [kudu + impala], and A4 [giraffe + kudu + impala]) browsing tanniferous plants, which can be used to improve forage utilization in domesticated goat. Crude protein enzyme extracts (CPZ) from fresh faecal samples were precipitated by 60% ammonium sulphate and assayed for exocellulase, endocellulase and hemicellulase by incubating with crystalline cellulose, carboxymethyl cellulose and xylan at 38 °C with optimum pH of 5.5 to 6.5 for 1, 2, and 48 h, respectively. Enzyme specific activities were defined as µg of reducing sugar/mg CPZ. *In vitro* fermentation study was done by transferring 33 mL of fresh faecal inoculum into 67 mL of salivary buffer containing 1 g *Acacia sieberiana* and incubating for 72 h at 38 °C. Apparent degradability (APDeg), true degradability (TD), neutral detergent fibre degradability (NDFdeg), acid detergent fibre degradability (ADFdeg), microbial yield (MY), metabolizable energy (ME) and total gas emitted (Gas) were measured. Exocellulase activities were higher ( $P < 0.05$ ) in all wild animals and consortia than those in goat except for A4. Minimal differences in hemicellulase activities ( $P < 0.05$ ) were observed among goat and wild animals and consortia, while endocellulase activity was generally higher ( $P < 0.05$ ) in goat than that in the rest of the systems. Apart from A3, TDeg, NDFdeg and ADFdeg were higher ( $P < 0.05$ ) in all microbial ecosystems from wild animals and consortia than those in goat. Apparent degradability, MY and ME also varied ( $P < 0.05$ ) among these systems. Giraffe, Kudu and A3 produced lower ( $P < 0.05$ ) gas than the goat system. This study showed that microbial ecosystems from wild browsers (especially impala) and consortia possess a higher potential to digest tanniferous forage with less enteric gas production compared with domesticated goat, hence those microbiome could be exploited as microbial feed additives for improving digestibility and reducing enteric gas production in domesticated goat. Improvements of goat's digestibility will depend on the survival and establishment of microbial species in the rumen as well as their fibrolytic and symbiotic potential including tannin tolerance.

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### 1. Introduction

Ruminants derive a large quantity of nutritional requirements (energy) from the breakdown of plants cell wall polysaccharides (Natsir, 2012). The primarily constituent of plants cell wall is cellulose and it is also known to be the most abundant polysaccharide in nature (Bielecki et al., 2005). Often, these complex polysaccharides are bound to lignin, tannin and pectin rendering it inaccessible for digestion. Ruminants are considered as cellulose degrading animals but these complex polysaccharides are degraded by the symbiotic rumen microorganisms which they harbour (Wilson, 2008). These microorganisms include bacteria,

\* Corresponding author.

E-mail address: [FonF@unizulu.ac.za](mailto:FonF@unizulu.ac.za) (F.N. Fon).

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protozoa and fungi (Salem et al., 2015; Santra and Karim, 2003). They inhabit mostly in rumen, caecum and sometimes in the colon of some mammalian herbivores where they ferment forages into volatile fatty acids, methane and carbon dioxide. These microbes produce a collection of highly active plant cell-wall degrading enzymes such as cellulase, hemicellulase and cellobiase (Dashtban et al., 2010; Koike et al., 2003; Zhang and Zhang, 2013). Cellulases hydrolyse the  $\beta$ -1, 4 linkages into cellulose molecules and are very different from the majority as they degrade insoluble substrate (Wilson, 2011). They are produced as a multiple component enzyme system consisting of three enzymes which include endoglucanases, exoglucanases and  $\beta$ -glucosidases (Horn et al., 2012). During cellulose degradation, enzymes secreted by microbes diffuse through rumen liquor to substrate liberating free glucose molecules (Wilson, 2011). However, some enzyme complexes consisting of endoglucanases, exoglucanases and  $\beta$ -glucosidase may also be attached to the substrates and acting symbiotically and liberating free glucose molecules (Wang and McAllister, 2002). Endoglucanases are thought to be non-active against crystalline cellulose but they hydrolyse amorphous cellulose and soluble substrates such as carboxymethyl cellulose (Sona and Mukundan, 2004). This enzyme randomly cut  $\beta$ -glycosidic bonds of cellulose chains and yield new end products (glucose and cellobiose) that are beneficial for both microorganisms and host animal (Zhang and Zhang, 2013). Exoglucanases is also described as cellobiohydrolases, and plays a significant role in hydrolysing crystalline cellulose to either oligosaccharides, cellobiose or glucose (Horn et al., 2012). The third enzyme  $\beta$ -glucosidase convert cellobiose (the main product of the endo- and exoglucanases mixture) to glucose but its activity on insoluble cellulose is insignificant (Linton and Greenaway, 2004).

Hemicellulose are also considered as secondary factors affecting fibre hydrolysis (de Souza, 2013). Xylan is the most common hemicellulose component of grass and wood that contains up to 45% of the polysaccharide constituent of ruminant feed (Malherbe and Cloete, 2002). Xylan structure is composed of  $\beta$ -1, 4-linked xylose residues (Dunne, 2010). Effective degradation of xylan in the rumen also involves a mutual relationship of rumen microbial population (Wang and McAllister, 2002). These microorganisms have a capability to produce highly active fibrolytic enzyme (xylanase) that degrade xylan into xylose (Wang and McAllister, 2002). Xylanase catalyse  $\beta$ -linkages of xylan to produce xylose as energy substrates for rumen microbes releasing by-products that can be used by ruminants.

The ability of tannin complexing cellulose, forage proteins and even microbial protein (enzymes and microbes cell wall proteins) has been a major concern as it decreases forage degradability and increases production cost. This is even more prominent in browsers as browses are relatively higher in tannin concentrations. These imply that the microbes are faced with a daunting task of first annihilating the effect of tannin before hydrolysing the fibre. Because of the huge diversity of tannin structures, their effects on rumen microbes will also vary widely (Gemed and Hassen, 2015). If rumen microbiome is as diverse as that of tannin, it is imply that microbes will adapt and evolved accordingly with tannin type and concentrations in browses in a browsing niche. However, consuming tannin herbivores are also evolving in their ability to negate the effects of tannin by secreting tannin binding protein in saliva to decrease its effect of rumen microbial protein. Enteric gas production (methane) has been shown to decrease or vary with tannin type and concentration in plant species (Gemed and Hassen, 2015). There are also suggestions that some rumen microbes may have a higher ability of synthesizing tannin binding proteins as well as a thicker glycocalyx for protection. Therefore, the aim of this research was to investigate the potential of fibrolytic

microbial ecosystems from wild browsers (giraffe, kudu, impala or their consortia: A1 [giraffe + kudu], A2 [giraffe + impala], A3 [kudu + impala], A4 [giraffe + kudu + impala]), which can potentially be used to improve browse digestibility in domesticated goat. Consortia were formed to investigate the symbiotic activity of potential fibrolytic and tannin tolerant microbes coming from the different animal species. To achieve this objective, we hypothesized that fibrolytic potential of microbial ecosystems from wild herbivores (impala, giraffe, and kudu) will not differ from that of domesticated goat.

## 2. Materials and methods

### 2.1. Faecal sample collection and inoculum preparation

Faecal inoculum was preferred in this study because it has been previously investigated as an alternative inoculum for rumen fluid and secondly rumen cannulated wild animals and domestic goat were not available and was very expensive to cannulate and manage (Ramin et al., 2015). Fresh faecal samples were collected (within 5 min) after defecation from giraffe, kudu and impala browsing thorn veldt at Tala Game Reserve (KwaZulu-Natal Province, South Africa) and a goat from KwaMthethwa village, Empangeni browsing the communal fields in winter. Faeces were transferred into a pre-warmed thermo flask that has been flushed with CO<sub>2</sub> (Getachew et al., 2004; Posada et al., 2012) and taken to the laboratory of the Department of Agriculture at University of Zululand. Inocula were prepared by mixing 60 g faecal sample with 250 mL of warm salivary buffer solution (2 L of warm Solution A [NaHCO<sub>3</sub> 19.60 g, Na<sub>2</sub>HPO<sub>4</sub> 7.40 g, KCl 1.14 g and MgCl<sub>2</sub>·6H<sub>2</sub>O 0.26 g]) containing 2 mL of Solution B (5.3 g CaCl<sub>2</sub>·2H<sub>2</sub>O in 100 mL distilled water) that was pre-warmed at 39 °C prior use before squeezing through four layers of cheese cloth to get a filtrate (inoculum).

### 2.2. Crude protein enzyme extraction, dialysis and concentration

Protein extraction for enzyme analysis was done as described by Byrne et al. (1975) with minor changes in quantities and volumes (Fon et al., 2014b). Before crude protein enzyme extraction, 200 mL of inoculum was treated with 1,500  $\mu$ L of phenylmethylsulfonyl fluoride (PMSF) (0.1 mmol/L PMSF 1:100 faecal fluid) to inhibit proteases from lysing enzymes of interest (Owolabi et al., 1988). Faecal fluid (100 mL) from each inoculum was used for enzyme extraction after cell disruption by sonication to release proteins. After sonication, the samples were centrifuged at 10,000  $\times$  g for 15 min at 4 °C until a clear supernatant was obtained. Ammonium sulphate (60%) was dissolved in the supernatant before centrifuging at 7,500  $\times$  g for 15 min at 4 °C to precipitate proteins. After centrifugation, the supernatant was discarded and the remaining precipitate was dissolved in 7 mL storage buffer (20 mmol/L sodium acetate, 0.02% NaN<sub>3</sub> and 0.1 mmol/L EDTA at pH 5.01) and transferred into a dialysis membrane (10,000 molecular weight cut-off, from Sigma–Aldrich). The membrane was immersed in 2 L homogenization buffer (50 mmol/L sodium acetate, 0.02% NaN<sub>3</sub> and 0.1 mmol/L EDTA at pH 5.02) for 24 h to completely remove the salt before concentrating with polyethylene glycol 20000 and stored for enzyme assays. Bradford dye binding assay (Bradford, 1976) was used to determine protein concentrations. The unknown crude protein enzyme concentrations were determined from a standard plot of known concentrations of bovine serum albumin (0.5 to 40  $\mu$ g bovine serum albumin/250  $\mu$ L reaction buffer) read at 595 nm absorbance.

### 2.3. Enzyme assay

Crude protein enzymes from extracts were analysed for different cellulase and hemicellulase activities as described by Fon et al. (2014b). Exocellulase was assayed by pipetting 0.5 mL of 1% (mass/vol) crystalline cellulose in a reaction buffer (20 mmol/L sodium acetate, 0.02% [mass/vol]  $\text{NaN}_3$  and 0.1 mmol/L EDTA at pH 5.0) into 0.5 mL of crude protein enzymes solution (giraffe, impala, kudu, goat or consortia: A1 [giraffe + kudu, 1:1], A2 [giraffe + impala, 1:1], A3 [kudu + impala, 1:1] and A4 [giraffe + kudu + impala, 1:1:1]) obtained from inoculum and incubated for 72 h at 38 °C. The control was same as the enzyme treatment but for the absence of substrate. The specific activity of endocellulase was determined by reacting 0.5 mL of 0.5% (mass/vol) carboxymethyl cellulose in the reaction buffer with 0.5 mL of crude protein enzymes solution and incubated for 2 h at 38 °C. Xylan (0.6 mL of 0.1% [mass/vol] xylan solution in the reaction buffer) was pipetted into 0.4 mL of crude protein solution and incubated at 38 °C for 2 h to determine hemicellulase activity. The concentration of reducing sugar (glucose or xylose) released in the reaction was determined using both glucose and xylose standards as described by the Dinitrosalicylic method (Miller, 1959). All enzyme reaction mixtures were centrifuged ( $6,000 \times g$  for 5 min) before analysing for reducing sugars. Each enzyme assay was replicated three times with three pseudo repeat for each run. Enzyme specific activity was defined as  $\mu\text{g}$  of reducing sugar/mg crude protein.

### 2.4. Chemical analysis of *acacia sieberiana*

*Acacia sieberiana* was used for *in vitro* digestibility in this study. Dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and condensed tannin (CT) were analysed for *Acacia sieberiana* to ascertain its nutrient composition. Nitrogen (N) concentration was measured by Kjeldahl method described by Basha (2012) using nitrogen analyser system. The chemical components (NDF, ADF and ADL), cellulose and hemicellulose were determined according to Van Soest et al. (1991) principles using ANKOM fibre method. Acid-butanol proanthocyanidins assay was used to determine CT (Makkar and Goodchild, 1995).

### 2.5. *In vitro* digestibility and gas production

Gas production was measured using the computerized pressure transducer system as described by Nsahlai et al. (2011). Approximately 1 g of ground (pass through 1 mm sieve) *Acacia sieberiana* dry matter was transferred into 250 mL Duran bottles containing 67 mL salivary buffer. Faecal inoculum (33 mL for giraffe, kudu, impala or goat) was then added into the bottle while flushing with  $\text{CO}_2$  to maintain anaerobiosis. Microbial consortia the 33 mL inoculum was prepared as follows; A1 (15 mL giraffe + 15 mL kudu), A2 (15 mL giraffe + 15 mL impala), A3 (15 mL kudu + 15 mL impala) and A4 (11 mL giraffe + 11 mL kudu + 11 mL impala). The bottles were tightly closed with stoppers and placed in an incubator for 72 h at 39 °C with blanks lacking substrate only. After incubation, the contents of each reach was centrifuged at  $8,000 \times g$  for 15 min at 4 °C and dried for 72 h at 60 °C. The control incubation was same as the treatment mixture but for the absence of substrates. Apparent digestibility (APD), true digestibility (TD), neutral and acid detergent fibre digestibility (NDFdeg and ADFdeg) and cellulose digestibility (HEMdeg) were measured. Metabolizable energy was calculated using a formula described by (Afshar et al., 2011) using the gas produced and microbial yield. *In vitro* digestibility was replicated thrice for each animal with five pseudo repeats for each run.

### 2.6. Statistic analysis

Statistically Analysis System (SAS 9.3, 2013) was used to determine gas production from the regression equation (Campos et al., 2004). The treatment effects for APD, TD, NDFdeg, ADFdeg, HEMdeg, microbial yield, and gas parameters obtained from the regression equation were evaluated using linear analysis of Variance (ANOVA).

## 3. Results

The chemical compositions of *Acacia sieberiana* were successfully determined and are shown in Table 1.

Enzymes specific activities are shown in Table 2 and the results showed that enzyme activities varied ( $P < 0.05$ ) among microbial ecosystems. According to the results, exocellulase activities were higher ( $P < 0.05$ ) in all wild animals and consortia than those in goat except for A4. Minimal differences in hemicellulase activities ( $P < 0.05$ ) were observed between goat and wild animals or consortia. Hemicellulase activity was the highest in A4 followed by impala, A1, A2 and giraffe. Endocellulase activity was generally higher ( $P < 0.05$ ) in goat than that in the rest of the microbial ecosystems.

The results obtained from *in vitro* digestibility of *Acacia sieberiana* showed that APD, TD, NDFdeg, ADFdeg, HEMdeg, MY, ME and total gas production varied ( $P < 0.05$ ) among the different microbial ecosystems (Table 3). Apparent degradability in impala was the highest among all the systems. True degradability and NDFdeg were highest ( $P < 0.05$ ) in impala and A1, moderate in A2, A4, giraffe and kudu and the lowest in goat and A3. For HEMdeg, impala and kudu showed the highest activity. Interestingly, MY was the lowest in impala, followed by goat while the rest were relatively moderate with the highest observed in A1. The ME was the highest in impala while the rest of the microbial ecosystems seem to have similar quantities of ME. The gas production from goat was the highest ( $P < 0.05$ ), followed by A1, A4 and A2 while the rest of the microbial ecosystems showed relatively lower values compared with goat.

## 4. Discussion

Most microbes in the rumen can utilize the monomeric units of cellulose or hemicelluloses or their by-products after fermentation, but only a few of them produces enzymes that have the potential to degrade these complex polysaccharides (Zhang and Zhang, 2013). That is why to efficiently digest cellulose requires a combination of symbiotic hydrolytic enzymes (Pérez et al., 2002). Despite the complexity of these polysaccharides, tannin and lignin complexing with these polysaccharides render them inaccessible and difficult to degrade (Mlambo and Mapiye, 2015; Ndagurwa and Dube, 2013) especially in browse species with high levels. Many studies have demonstrated that tannin play a huge part in influencing cellulose

**Table 1**  
Chemical composition of *Acacia sieberiana*.<sup>1</sup>

Chemical composition	<i>Acacia sieberiana</i> , g/kg
Dry matter	947 ± 57.7
Crude protein	127 ± 15.3
Neutral detergent fibre	658 ± 26.9
Acid detergent fibre	515 ± 28.8
Acid detergent lignin	393 ± 38.9
Condensed tannin	68.7 ± 4.3
Cellulose	122 ± 54.9
Hemicellulose	142 ± 38.9

<sup>1</sup> Each chemical component extraction was replicated three times with three pseudo repeats in each run.

**Table 2**  
Hemicellulase, endocellulase and exocellulase specific activities of crude protein extracts.<sup>1</sup>

Item	Enzyme specific activities		
	Hemicellulase, µg xylose/mg	Endocellulase, µg glucose/mg	Exocellulase, µg glucose/mg
Goat	29.98 <sup>c</sup>	99.93 <sup>a</sup>	65.87 <sup>d</sup>
Giraffe	30.05 <sup>c</sup>	95.63 <sup>c</sup>	72.69 <sup>bc</sup>
Kudu	29.77 <sup>d</sup>	95.83 <sup>c</sup>	72.91 <sup>bc</sup>
Impala	30.88 <sup>a</sup>	93.10 <sup>d</sup>	80.23 <sup>a</sup>
A1	30.37 <sup>b</sup>	95.18 <sup>c</sup>	76.03 <sup>ab</sup>
A2	30.11 <sup>c</sup>	96.03 <sup>c</sup>	70.34 <sup>bc</sup>
A3	29.67 <sup>d</sup>	96.88 <sup>b</sup>	70.97 <sup>bc</sup>
A4	31.06 <sup>a</sup>	95.67 <sup>c</sup>	61.80 <sup>d</sup>
SEM	0.03	0.12	0.85
P-value	0.05	0.05	0.05

<sup>1</sup> A1 = giraffe + kudu (1:1), A2 = giraffe + impala (1:1), A3 = kudu + impala (1:1), A4 = giraffe + kudu + impala (1:1:1). Each enzyme specific activity was replicated three times with five pseudo repeats for each run.

SEM = standard error of the means.

a,b,c,d Numbers in a column with different superscript letters are significantly different ( $P < 0.05$ ).

digestibility either by binding to cellulose, cellulases, rumen microbes or to rumen symbiotic enzymes (Horner et al., 1988; Ximenes et al., 2010). Most goats in communal systems as well as in commercial farms suffer nutrient deficiency (often seen in poor body condition scores and weight loss) especially in winter (dry season), yet some of them browse the thorn veldt or are supplemented with browses. Often, more than 50% of forages browsed are eliminated through faeces. Therefore, this study looked at microbes from impala, goat and kudu which are browsing the thorn veldt at Tala Game Reserve hoping to find microbes that have not only evolved in their fibrolytic potential but also with ability to resist and manage variable tannin conditions with minimal effect on fibrolytic enzymes. The results obtained from crude enzyme extracts showed that domesticated goats microbial ecosystem can digest or ferment amorphous or soluble polysaccharides (endocellulase activity) as good as wild ruminants and their consortia. However, this was not true for exocellulase activities which are responsible for complex fibre degradation as it varied. The impala showed the highest potential in hydrolysing cellulose which was 20% greater than that of goat. This high activity was associated to microbes that have evolved with their fibrolytic potential influenced by diet type browsed in the fields (Pitta et al., 2014) as impala was observed browsing *Lantana camara* during winter. *Lantana camara* at that time of the year appeared to be high in fibre, tannin and lignin as demonstrated by its dark blue green colour. Both

giraffe and kudu had a 10% increase in cellulose digestibility compared with that of goat. Their activities were also influenced by their browsing habit. However, they were seen browsing leaves from tall acacia plants with relatively lower content of cellulose, tannin and fibre favoured by their height. Although the statistical analysis of hemicellulase activity showed that it varied among the different microbial ecosystems, the differences were really small. The hemicellulolytic activity of impala and A1 microbial ecosystems were the highest with 3% and 1.2%, respectively, different from that of goat. The impala seems to be showing greater efficiency in digesting both cellulose and hemicellulose which is a bit higher than observed in a previous study by Fon et al. (2014a). A similar study by Nagpal et al. (2011) also demonstrated a higher fibrolytic activity from wild deer browsing as well.

The observed chemical composition of *Acacia sieberiana* leaves used in this study is similar to that previously reported by Nsahlai et al. (2011) on *Acacia sieberiana*. For *in vitro* digestibility studies, impala, giraffe, kudu and consortia showed greater potential in fermenting *Acacia sieberiana* than goat except for A3. Microbial ecosystems in impala and A1 showed the highest percentage difference in TD (16.3% and 15.2%), NDFdeg (34.9% and 33.2%) and ADFdeg (54.0% and 56.4%, respectively) when compared with goat. This result was consistent with the higher exocellulase activity observed from crude enzyme extracts for impala and A1. Therefore, we have reasons to believe that impala may be harbouring microbes that might have evolved with their ability to ferment forages in the presence of tannin. It is worth mentioning that the relatively high tannin tolerance of the impala microbial ecosystem will also depend on type of tannin as well as concentrations. Although TD (6.6% and 10.2%), NDFdeg (14.1% and 21.6%) and ADFdeg (24.2% and 28.9%, respectively) for giraffe and kudu were higher than those of goat, their consortia (A1) were even greater for TD (15.5%), NDFdeg (33.2%) and ADFdeg (56.4%). The increase in A1 digestibility was associated to a positive symbiotic microbial activity. Interestingly, impala with the highest ability to ferment *Acacia sieberiana* produced the least microbial yield as one of the products of fermentation (Rymer et al., 2005). It was suggested that the highest digestibility values observed for impala was associated to microbial efficiency rather than population. It was logical to assume that high carbohydrates degradation and protein will provide both energy (ATP) and nitrogen for microbial growth (Pathak, 2008) but this was not the case. It was also very interesting to find out that goat produced more gas than microbial ecosystems from wild animals and consortia. This confirms the adaptability of goat microbial ecosystem in digesting soluble polysaccharides (with high gas as a characteristic by-product) than high fibre forages.

**Table 3**  
*In vitro* digestibility of *Acacia sieberiana* by inoculum from both domestic and wild herbivores and their consortia.<sup>1</sup>

Item	APDeg, g/kg DM	TD, g/kg DM	NDFdeg, g/kg DM	ADFdeg, g/kg DM	HEMdeg, %	MY, g/kg DM	ME, MJ/kg DM	Gas, mL/g DM
Goat	256.0 <sup>b</sup>	453.3 <sup>c</sup>	278.9 <sup>c</sup>	193.4 <sup>b</sup>	64.8 <sup>bc</sup>	197.3 <sup>e</sup>	26.4 <sup>ab</sup>	70.4 <sup>a</sup>
Giraffe	152.3 <sup>cd</sup>	483.9 <sup>ab</sup>	318.5 <sup>ab</sup>	240.3 <sup>a</sup>	65.5 <sup>bc</sup>	330.9 <sup>b</sup>	21.4 <sup>b</sup>	14.1 <sup>c</sup>
Kudu	243.6 <sup>b</sup>	499.1 <sup>ab</sup>	339.3 <sup>ab</sup>	249.3 <sup>a</sup>	72.8 <sup>a</sup>	255.4 <sup>cd</sup>	20.2 <sup>b</sup>	21.0 <sup>c</sup>
Impala	383.3 <sup>a</sup>	527.2 <sup>a</sup>	376.4 <sup>a</sup>	298.0 <sup>a</sup>	71.5 <sup>a</sup>	143.9 <sup>f</sup>	22.2 <sup>b</sup>	51.7 <sup>ab</sup>
A1	116.6 <sup>d</sup>	523.7 <sup>a</sup>	371.7 <sup>ab</sup>	302.6 <sup>a</sup>	67.0 <sup>abc</sup>	407.1 <sup>a</sup>	30.2 <sup>a</sup>	65.9 <sup>ab</sup>
A2	242.8 <sup>b</sup>	485.6 <sup>ab</sup>	321.5 <sup>ab</sup>	255.4 <sup>a</sup>	60.7 <sup>bc</sup>	242.7 <sup>d</sup>	24.0 <sup>ab</sup>	53.0 <sup>ab</sup>
A3	127.0 <sup>d</sup>	435.3 <sup>c</sup>	255.1 <sup>ab</sup>	178.6 <sup>b</sup>	58.5 <sup>c</sup>	308.3 <sup>b</sup>	23.6 <sup>ab</sup>	42.3 <sup>b</sup>
A4	201.4 <sup>cb</sup>	496.0 <sup>b</sup>	335.2 <sup>ab</sup>	259.4 <sup>a</sup>	66.2 <sup>abc</sup>	294.5 <sup>cb</sup>	26.7 <sup>ab</sup>	61.8 <sup>ab</sup>
SEM	8.38	5.41	6.90	6.90	17.70	7.22	9.44	2.96
P-value	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

<sup>1</sup> A1 = giraffe + kudu (1:1), A2 = giraffe + impala (1:1), A3 = kudu + impala (1:1), A4 = giraffe + kudu + impala (1:1:1). Each degradability parameter was replicated three times with five pseudo repeats for each run.

SEM = standard error of the means, APDeg = apparent degradability, TD = true degradability, NDFdeg = neutral detergent fibre degradability, ADFdeg = acid detergent fibre degradability, HEMdeg = hemicellulose degradability (%), MY = microbial yield, ME = metabolizable energy.

a,b,c,d Numbers in a column with different superscript letters are significantly different ( $P < 0.05$ ).



High enteric gas production is not cost effective to farmers (energy loss and metabolic disorders like bloat) as well as to environmentalist because it is a major contributor to global warming especially methane. Therefore, giraffe, impala, kudu and consortia had not only harbour microbes with relatively higher fibrolytic potential but also had the potential to decrease the amount of energy lost as enteric gas (Singh et al., 2010). Other studies have shown that microbes respond differently to forages and different types of tannins (Gemed and Hassen, 2015), therefore we cannot absolutely conclude that impala can replicate its efficiency in digesting forages with different types of phenolic compounds without investing its effect in the presence of different types of tannins. The hypothesis was rejected as microbial ecosystems from giraffe, impala, kudu and consortia showed a higher ability in digesting *Acacia sieberiana* than domesticated goat microbial ecosystem.

## 5. Conclusion

The results from exocellulase activity and *in vitro* digestibility of *Acacia sieberiana* showed that giraffe, impala, kudu and consortia can utilize browses forages better than domesticated goat. Microbial ecosystems in impala and A1 showed the highest potential in utilising *Acacia sieberiana* as forage and could be used as a potential inoculum to improve the digestibility of tanniferous forages. The study also showed that wild microbial ecosystem and their consortia produced less enteric gases than domesticated goat. A future study to investigate the effect of microbial ecosystems from giraffe, impala, kudu and consortia on goat both *in vitro* and *in vivo* is important for its application as a feed additive.

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## References

- Afshar M, Naser M, Hormoz M, Mohammad E, Ali M, Hossein C, et al. Evaluating potential nutritive value of pomegranate processing by-products for ruminants using *in vitro* gas production technique. *J Agric Biol Sci* 2011;6:45–51.
- Basha NAD. Feeding behaviour, diet selection of goats and nutritive value of browse species in sub-humid subtropical savannah, South Africa (vol thesis). Pietermaritzburg: University of KwaZulu-Natal; 2012. <http://researchspace.ukzn.ac.za/>.
- Bielecki S, Krystynowicz A, Turkiewicz M, Kalinowska H. Bacterial cellulose. *Biopolymers online*. 2005. <http://onlinelibrary.wiley.com/>.
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Chem* 1976;72:248–54.
- Byrne H, Christou N, Verma D, Maclachlan G. Purification and characterization of two cellulases from auxin-treated pea epicotyls. *J Biol Chem* 1975;250:1012–8.
- Campos F, Sampaio A, Bose M, Vieira P, Sarmiento P. Evaluation of *in vitro* gas production of roughages and their mixtures using the curves subtraction method. *Anim Feed Sci Technol* 2004;116:161–72.
- Dashbani M, Maki M, Leung KT, Mao C, Qin W. Cellulase activities in biomass conversion: measurement methods and comparison. *Crit Rev Biotechnol* 2010;30:302–9.
- de Souza B. Microbial degradation of lignocellulosic biomass. Sustainable degradation of lignocellulosic biomass-techniques, applications and commercialization. 2013. p. 207–47.
- Dunne JC. A proteomic analysis of fibre degradation and assimilation by butyriovibrio proteoclasticus. Thesis. 2010. <http://hdl.handle.net/10063/1654>.
- Fon FN, Nsahlai IV, Scogings PF. Extraction and comparison of fibrolytic enzyme additives from gut of 11 ungulates. *Afr J Biomed Res* 2014a;8:31–8.
- Fon FN, Nsahlai IV, Scogings PF, Basha NAD. *In vitro* cellulase production from five herbivore microbial ecosystems and consortia. *Ann Anim Sci* 2014b;14:329–40.
- Gemed BS, Hassen A. Effect of tannin and species variation on *in vitro* digestibility, gas, and methane production of tropical browse plants. *Asian-Aust J Anim* 2015;28:188–99.
- Getachew G, Robinson P, DePeters E, Taylor S. Relationships between chemical composition, dry matter degradation and *in vitro* gas production of several ruminant feeds. *Anim Feed Sci Technol* 2004;111:57–71.
- Horn SJ, Vaaje-Kolstad G, Westereng B, Eijsink VG. Novel enzymes for the degradation of cellulose. *Biotechnol Biofuels* 2012;5:1–13.
- Horner JD, Gosz JR, Cates RG. The role of carbon-based plant secondary metabolites in decomposition in terrestrial ecosystems. *Am Nat* 1988;132:869–83.
- Koike S, Pan J, Kobayashi Y, Tanaka K. Kinetics of *in sacco* fiber-attachment of representative ruminal cellulolytic bacteria monitored by competitive pcr. *J Dairy Sci* 2003;86:1429–35.
- Linton SM, Greenaway P. Presence and properties of cellulase and hemicellulase enzymes of the gecarcinid land crabs *gecarcoidea natalis* and *discoplax hirtipes*. *J Exp Biol* 2004;207:4095–104.
- Makkar H, Goodchild A. Quantification of tannins: a laboratory manual. Aleppo Syria: International centre for agricultural research in the dry areas; 1995. <http://www.springer.com/>.
- Malherbe S, Cloete TE. Lignocellulose biodegradation: fundamentals and applications. *Rev Environ Sci Biotechnol* 2002;1:105–14.
- Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chem* 1959;31:426–8.
- Mlambo V, Mapiye C. Towards household food and nutrition security in semi-arid areas: what role for condensed tannin-rich ruminant feedstuffs? *Food Res Int* 2015;76(Part 4):953–61.
- Nagpal R, Puniya AK, Sehgal JP, Singh K. *In vitro* fibrolytic potential of anaerobic rumen fungi from ruminants and non-ruminant herbivores. *Mycoscience* 2011;52:31–8.
- Natsir A. Fibre utilization by ruminants, vol. 2; 2012. p. 1–188. <http://repository.unhas.ac.id/>.
- Ndagurwa HGT, Dube JS. Evaluation of potential and effective rumen digestion of mistletoe species and woody species browsed by goats in a semi-arid savanna, southwest zimbabwe. *Anim Feed Sci Technol* 2013;186:106–11.
- Nsahlai I, Fon F, Basha N. The effect of tannin with and without polyethylene glycol on *in vitro* gas production and microbial enzyme activity. *S Afr J Anim Sci* 2011;41:337–44.
- Owolabi B, Beguin P, Kilburn G, Miller C, Antony J. Expression in *escherichia coli* of the cellulomonas fimi structural gene for endoglucanase b. *Appl Environ Microbiol* 1988;54:518–23.
- Pathak A. Various factors affecting microbial protein synthesis in the rumen. *Vet World* 2008;1:186–9.
- Pérez J, Muñoz-Dorado J, de la Rubia T, Martínez J. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. *Int Microbiol* 2002;5:53–63.
- Pitta DW, Kumar S, Veitcharelli B, Parmar N, Reddy B, Joshi CG. Bacterial diversity associated with feeding dry forage at different dietary concentrations in the rumen contents of mehshana buffalo (*bubalus bubalis*) using 16s pyrotags. *Anaerobe* 2014;25:31–41.
- Posada SL, Noguera RR, Segura JA. Ruminant feces used as inoculum for the *in vitro* gas production technique. *RCCP* 2012;25:592–602.
- Ramin M, Lerose D, Tagliapietra F, Huhtanen P. Comparison of rumen fluid inoculum vs. faecal inoculum on predicted methane production using a fully automated *in vitro* gas production system. *Livest Sci* 2015;181:65–71.
- Rymer C, Huntington J, Williams B, Givens D. *In vitro* cumulative gas production techniques: history, methodological considerations and challenges. *Anim Feed Sci Technol* 2005;123:9–30.
- Salem AZM, Elghandour MMY, Kholif AE, Odongo NE, Jiménez FJP, Montes-de-Oca R, et al. The effect of feeding horses a high fiber diet with or without exogenous fibrolytic enzymes supplementation on nutrient digestion, blood chemistry, fecal coliform count, and *in vitro* fecal fermentation. *JEVS* 2015;35:735–43.
- Santra A, Karim S. Rumen manipulation to improve animal productivity. *ASIAN Australas J Anim Sci* 2003;16:748–63.
- Singh KM, Pandya PR, Parnerkar S, Tripathi AK, Ramani U, Koringa PG, et al. Methanogenic diversity studies within the rumen of surti buffaloes based on methyl coenzyme m reductase a (mcrA) genes point to methanobacteriales. *Pol J Microbiol* 2010;59:175–8.
- Sona A, Mukundan M. Studies on isolation, purification and properties of endoglucanase from the hepatopancreas of perna viridis (PhD Thesis). Central Institute of Fisheries Technology; 2004. <http://dyuthi.cusat.ac.in/>.
- Van Soest Pv, Robertson J, Lewis B. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci* 1991;74:3583–97.
- Wang Y, McAllister T. Rumen microbes, enzymes and feed digestion-a review. *Asian-Aust J Anim Sci* 2002;15:1659–76.
- Wilson DB. Three microbial strategies for plant cell wall degradation. *Ann N. Y Acad Sci* 2008;1125:289–97.
- Wilson DB. Microbial diversity of cellulose hydrolysis. *Curr Opin Microbiol* 2011;14:259–63.
- Ximenes E, Kim Y, Mosier N, Dien B, Ladisch M. Inhibition of cellulases by phenols. *Enzyme Microb Technol* 2010;46:170–6.
- Zhang X-Z, Zhang Y-HP. Cellulases: characteristics, sources, production, and applications. In: Bioprocessing technologies in biorefinery for sustainable production of fuels, chemicals, and polymers; 2013. p. 131–46.