

## Full Length Research Paper

## Extraction and comparison of fibrolytic enzyme additives from gut of 11 ungulates

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Accepted 16 January, 2014

**Microbial populations in herbivores gut attack, degrade and ferment structural carbohydrates in forage cell walls, producing volatile fatty acids and microbial proteins for the host. Exocellulases, endocellulases and cellobiases are the major cellulolytic enzymes while hemicellulase (xylanase) exposes cellulose for fermentation. This study aimed to isolate proteins that hydrolyse fibre or assist fibrolysis in any way from 11 herbivores gut microbial ecosystems (cow, sheep, horse, camel, elephant, zebra, llama, wildebeest, giraffe, impala and buffalo), optimize their working conditions and compare fibrolytic activities as potential feed additives. Exocellulase, endocellulase, xylanase and cellobiase in rumen and faecal crude enzyme extracts were assayed and compared. A broad *in vitro* pH range (4.5 to 8.0) of endocellulase activity was observed for all ecosystems. Enzymes from horse, zebra, impala, wildebeest and elephant showed the highest potential for degrading fibre encouraging further investigation as feed additives.**

**Key words:** Crude cellulase extraction, hemicellulase, rumen and faecal inoculum, ungulates.

### INTRODUCTION

Cellulolytic microbes possess three types of enzymes which enable partial or complete solubilisation of plant cell walls. These enzymes are exocellulases (EC 3.2.1.91), endocellulases (EC 3.2.1.4) and cellobiases (EC 3.2.1.21). These complex enzyme consortia function synergistically to hydrolyse plant cell walls. Exocellulases remove cellobiose units from the non-reducing ends of the cellulose chain, endocellulases act in a random fashion on the region of low crystallinity on the cellulose chain and  $\beta$ -glucosidase produces glucose units from cellobiose (Lynd et al., 2002). Although glucosidase is not strictly necessary for cell wall solubilisation, its presence enhances cellulose hydrolysis because these three enzymes function synergistically and inhibition of endocellulase and exocellulase by cellobiose is avoided. Solubilisation of plant cell walls does not solely depend on cellulases because of the complexity of its association with other macromolecules (Lynd et al., 2002). Apart

from celluloses, hemicelluloses are the second most abundant plant fraction available in nature with xylan being the most abundant of all the hemicelluloses (Petzold et al., 2008). Lignin, pectin and tannins are examples of macromolecules that are often found in close association with cellulose (Mussatto et al., 2008). Therefore for solubilisation of cellulose in roughages to be successful the enzymes hemicellulase, ligninase, tannase and pectinase are required (Fon and Nsahlai, 2012).

Not all the microbes (bacteria, fungi and protozoa) that are found in both the fore- and hindguts have fibrolytic or hemicellulolytic properties but can assist in the fermentation process either directly or indirectly. Fermentation yields volatile fatty acids which can reduce the pH of the rumen fluid to less than 6, thereby halting the activity of most rumen microbes which function optimally at pH 6.2 and above (Russell and Dombrowski 1980). The presence of

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non-fibrolitic microbes that utilise acetic acid as a source of energy reduces its concentration hence maintaining the optimal rumen pH.

Different studies have shown that adding exogenous enzymes to herbivore diet increases milk production due to an increase in feed digestion (Schingoethe et al., 1999; Kung et al., 2000; Yang et al., 2000). In a similar study on fibre rich forages, Feng et al. (1996), Krause et al. (1998), Yang et al. (1999) and Beauchemin et al. (2000) observed only small increments in fibre digestion. *In vitro* increases in dry matter (DM) digestibility have also been reported by Nakashima et al. (1988), Feng et al. (1996), and Yang et al. (1999).

It is possible that microbial enzymes from herbivorous species consuming different food items in the pasture can vary in plant cell wall hydrolysis (Antonio et al., 2011). The major limitations in investigating the fibrolitic potential of the different microbial ecosystems especially that from the wild includes; animals being expensive to kill for rumen fluid, expensive extraction and purification techniques. Several methods have been applied to isolate proteins from the rumen fluid or faecal samples including ammonium sulfate precipitation, three phase partitioning precipitation, gel filtration and acetone precipitation. Ammonium sulphate is one of the most used methods in precipitating crude enzymes because of its simplicity, availability and cost effectiveness (Henry et al., 1974). The main objective of this study was to identify potential fibrolitic enzyme additives secreted by microbes from gut of 11 ungulates.

## MATERIALS AND METHODS

### Experimental animals and nutrition

Animals were chosen with no preference to sex or species. *Bostaurus* (fistulated Jersey cows), *Equus caballus* (horse) and *Ovis aries* (fistulated sheep) were from Ukilinga Research farm, University of KwaZulu Natal (UKZN), Pietermaritzburg. *Llama glama* (llama), *Loxodonta africana* (elephant) and *Camelus dromedarius* (camel) were provided by Brian Boswell Circus, Pietermaritzburg. *Equus quagga* (zebra), *Connochaetes taurinus* (wildebeest), *Aepyceros melampus* (impala), *Syncerus caffer* (buffalo) and *Giraffa camelopardalis* (giraffe) were provided by the Tala Game Reserve, Umbumbulu, KwaZulu-Natal (SA).

This study was conducted in winter (April – August). Camel, elephant and llama were kept in an enclosed area and fed with grass hay (*Eragrostis* dominant) on a regular basis. Game animals such as the impala, wildebeest, zebra and buffalo were grazing on a dry land in an open field where *Pennisetum clandestinum* (Kikuyu grass) dominated, with other grasses. The giraffe browsed on a variety of tree leaves (various acacia species). At the Ukilinga research farm, horse, sheep and cow were fed entirely on grass hay (*Eragrostis* dominant).

### Faecal and rumen fluid collection

A modified procedure previously described by Smith et al. (1974) was employed when collecting rumen samples from fistulated cows and sheep. Rumen digesta (200 ml) was collected through a fistula, strained through four layers of cheese cloth, and treated immediately with 150  $\mu$ l of phenylmethylsulfonyl fluoride (0.1 mmol/L PMSF) to inhibit proteases from lysing enzymes of interest (Owolabi et al., 1888;

Vercoe et al., 2003). On the other hand, faeces were collected *in situ* from the horse, giraffe, buffalo, impala, wildebeest, zebra, elephant, llama and camel and transported in an airtight insulated flask maintained at 38°C. All experimental animals sample collection was governed by the UKZN ethical roles (083/10/animal).

### Protein exclusion and concentration measurements

Protein isolation was done using a modified procedure described by Henry et al. (1974). Both rumen fluid (100 ml) and faeces were used for protein isolation. In the case of faeces, 20 g was dissolved in 80 ml of a homogenization buffer (50 mmol/L sodium-acetate buffer, 0.02% (m/v)  $\text{NaN}_3$ , 0.1 mmol/L PMSF and 0.1 mmol/L EDTA at pH 5.0) before topping to 100 ml. Sample solutions in sealed centrifuge tubes were placed on a shaker for 30 min to facilitate bacteria detachment from fibres before centrifuging (7500 xg, 30min at 4°C) to sediment particulate matter (Figure 1). The supernatant was centrifuged (30 000 xg, 15 min at 4°C) to sediment bacteria cells. The sedimented particles and bacteria cells were dissolved in 10 ml and 5 ml of homogenisation buffer, respectively, sonicated for 3 min at 1 min intervals using a Q125 Ultrasonic Processor (to lyse bacteria cells) and centrifuged (30 000 xg, 15 min at 4°C). The supernatants of the three different steps were pooled and centrifuged (30 000 xg, 15 min at 4°C) to sediment any unlysed cells. Ammonium sulfate (60% (m/v)  $(\text{NH}_4)_2\text{SO}_4$ ) was dissolved in the sample solution to facilitate protein precipitation before centrifuging (7000 xg, 15 min at 4°C). The precipitate was dissolved in 10 ml of storage buffer (20 mmol/L sodium acetate, 0.02 % (m/v)  $\text{NaN}_3$ , and 0.1 mmol/L EDTA at pH 5.0) before dialyzing (12 h in storage buffer). Dialyzed sample solutions were concentrated using polyethylene glycol 20 000.

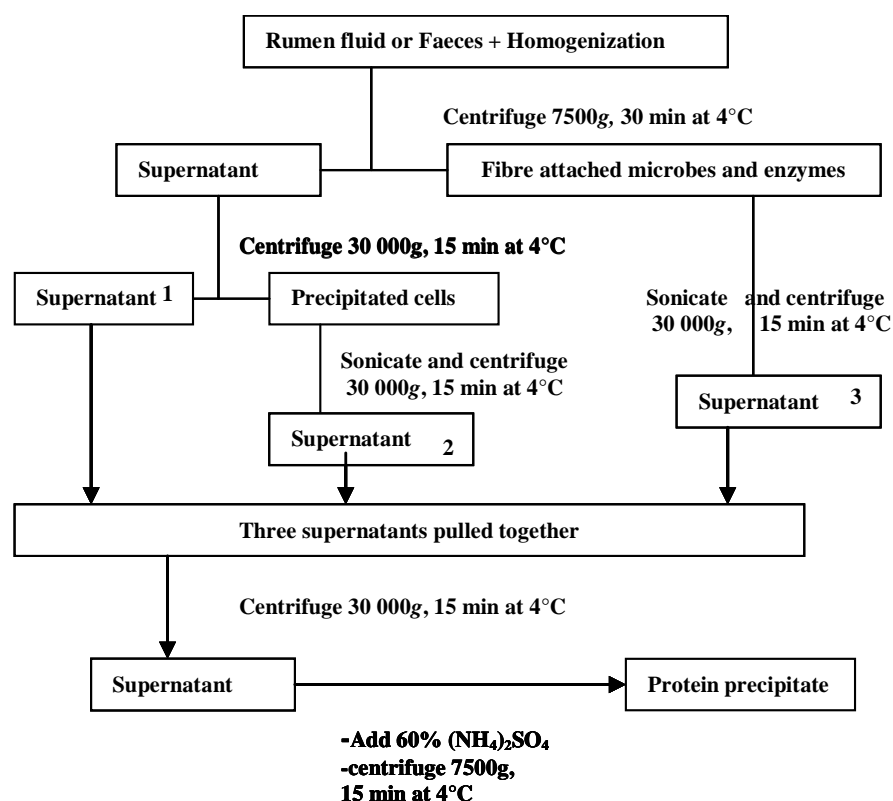
Bradford (1976) dye-binding assay was used to determine crude enzyme concentrations. A standard curve for a micro assay was prepared with bovine serum albumin concentrations of 0, 5, 10, 20, 30 and 40  $\mu$ g/100  $\mu$ l of reaction buffer (20 mmol/L sodium acetate, 0.02% (m/v) $\text{NaN}_3$  and 0.1 mmol/L EDTA at pH 5.0; Goeser et al., 2009). Bradford reagent (900  $\mu$ l of 600 mg Coomassie Brilliant Blue G-250 dissolved in 1 L of 2% perchloric acid and filtered through Whatman number 1 filter paper) was pipetted onto the standard solution (100  $\mu$ l) and absorbance read at 595 nm after 3 min. For the unknown protein sample solutions, 5  $\mu$ l was diluted into 100  $\mu$ l of the reaction buffer before adding Bradford reagent (900  $\mu$ l) and allowed to stand for 3 min. Each assay was performed in triplicates and absorbance was measured at 595 nm. Unknown protein concentrations were determined from the standard curve.

### Activities of crude enzyme samples and pH optimization

The method described by Seyis and Aksoz (2005) for the optimisation of xylanase activity from *Trichoderma* 1073 D3 was modified slightly and used in this assay. Enzyme stability within a pH range of 4.0 to 8.0 was determined by pre-incubating the crude enzyme extracts in 500  $\mu$ l sodium-acetate buffer (400  $\mu$ g/500  $\mu$ l 20 mmol/L sodium-acetate buffer) at different pH values for 30 min before adding 500  $\mu$ l of carboxymethyl cellulose (1% (m/v) CMC in 20 mmol/L sodium acetate, 0.02% (m/v)  $\text{NaN}_3$  and 0.1 mmol/L EDTA) and incubating for 2 h at 39°C. The substrate concentrations as well as the crude enzyme concentration were constant throughout the experiment. Enzyme reactions were stopped by heating at 100°C. Incubated samples were centrifuged at maximum speed (12875 xg) on a desktop centrifuge for 5 min and 400  $\mu$ l of the sample solution was used for reducing sugar analysis.

### Quantification of reducing sugars

Dinitrosalicylic (DNS) method (Miller, 1959) was chosen for analysis because of its sensitivity, simplicity and availability. A standard curve



**Figure 1.** A flow chart for the extraction of fibrolytic enzymes from rumen fluid and faeces.

of micrograms of reducing sugars (glucose or xylose) against absorbencies at 540 nm was plotted as described by Wood and Bhat (1988). DNS reagent, 600  $\mu$ l (0.001 M sodium metabisulfate, 0.708 M potassium sodium tetrates, 0.25 M sodium hydroxide, and 0.021 M phenol) was pipetted into 400  $\mu$ l of the sample solution and boiled for 5 min. The reaction mixture was then cooled under running water and the absorbance measured at 540 nm. The absorbance values were translated into concentration of reducing sugars using the standard curve.

### Enzyme assays

Exocellulase activity was assayed following a modified version of the method described by Gerrit et al. (1985). Exocellulase activity was measured by pipetting 0.5 ml of 1% (m/v) crystalline cellulose in the reaction buffer into 0.5 ml of crude enzyme solution obtained from different herbivores and incubating for 48 h at 39°C and pH 5.5 (standard assay condition). The enzyme reaction was stopped by boiling at 100°C, and the reaction mixture was centrifuged (6000 xg, 5 min at 4°C) and 400  $\mu$ l sample analysed for reducing sugars using DNS method. Each ecosystem was represented by three samples each of which was analyzed in triplicates. Specific enzyme activity was measured in  $\mu$ g of glucose/ $\mu$ g protein.

For endocellulase activity, the reaction mixture contained 0.5 ml of 0.5% (m/v) carboxymethyl cellulose (CMC) in the reaction buffer at pH 5.5 and 0.5 ml of crude enzyme. The enzyme reaction was stopped as described for exocellulases. Samples were centrifuged at 6000 xg at RT for 5 min, and then 400  $\mu$ l of the reaction mixture was analyzed for reducing sugars using DNS method.

Cellobiase activity was measured in a mixture of 0.5 ml of 0.1%

(m/v) *p*-nitrophenyl  $\beta$ -D-glucopyranoside (*p*NP-G) in the reaction buffer and 0.5 ml of crude enzyme solution. The mixture was incubated in a water bath for 1 h at 39°C. Each enzyme reaction was stopped with 100  $\mu$ l of sodium carbonate solution (200 mmol/L NaHCO<sub>3</sub>) and the reaction mixture centrifuged (6000 xg for 5 min), following which *p*-nitrophenol (*p*NP) liberated was analysed by reading the absorbance at 407 nm. The molar absorption coefficient ( $\epsilon$ ) of *p*NP at 407 nm was taken to be 18300 mol<sup>-1</sup> cm<sup>-1</sup> from similar calculations done by Frutos et al. (2002). The number of moles of *p*NP released was assumed to be directly proportional to the amount of glucose.

Xylanase hydrolyses xylan into xylose and oligosaccharides (Chivero et al., 2001). A modified procedure described by Khanna (1993) was used to assay xylanase activity. The assay was carried out by pipetting 0.6 ml of 0.1% (m/v) xylan solution in the reaction buffer (pH 5.0) into 0.4 ml of crude enzyme solution and incubating at 39°C for 1 h. The reaction was stopped with 100  $\mu$ l of 200 mmol/L NaHCO<sub>3</sub>, centrifuged (at 6000 xg for 5 min) and analysed for reducing sugars using the DNS method. Each ecosystem was represented by three samples each of which was analysed in triplicate. Specific activities of the above enzymes were defined as  $\mu$ g of xylose/mg crude enzyme.

### Statistical analysis

The results from exocellulase, endocellulase, cellobiase and hemicellulase specific activities as well as total protein, purification fold and yields were subjected to analysis of variance (ANOVA) using the general linear model of SAS. The model was:  $Y_{ij} = \mu + T_i + e_{ij}$ , where  $Y_{ij}$  is the individual observation,  $\mu$  is the overall mean,  $T_i$

**Table 1.** Purification of crude enzyme extracts from herbivores using ammonium sulphate.

Sample source	Sample fraction	Total protein (mg)	Total activity (nmol glucose)	Specific activity <sup>1</sup>	Purification (fold)	Yield (%)
Cow	-Supernatant	310 <sup>ab</sup>	2928 <sup>f</sup>	9.4 <sup>f</sup>	1	100
	-(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> precipitate	123 <sup>b</sup>	1982 <sup>g</sup>	16.1 <sup>b</sup>	1.706	39.7
Sheep	-Supernatant	313 <sup>ab</sup>	2783 <sup>f</sup>	8.9 <sup>g</sup>	1	100
	-(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> precipitate	138 <sup>b</sup>	1378 <sup>g</sup>	10.0 <sup>f</sup>	1.125	43.9
Horse	-Supernatant	588 <sup>a</sup>	5553 <sup>c</sup>	9.4 <sup>f</sup>	1	100
	-(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> precipitate	233 <sup>b</sup>	8263 <sup>a</sup>	35.0 <sup>a</sup>	3.765	39.6
Camel	-Supernatant	396 <sup>ab</sup>	3300 <sup>e</sup>	8.3 <sup>g</sup>	1	100
	-(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> precipitate	333 <sup>ab</sup>	2960 <sup>f</sup>	8.9 <sup>g</sup>	1.067	84.1
Elephant	-Supernatant	462 <sup>ab</sup>	5390 <sup>c</sup>	11.7 <sup>d</sup>	1	100
	-(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> precipitate	146 <sup>b</sup>	4536 <sup>d</sup>	31.1 <sup>a</sup>	2.667	31.6
Zebra	-Supernatant	876 <sup>a</sup>	7787 <sup>b</sup>	8.9 <sup>g</sup>	1	100
	-(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> precipitate	159 <sup>b</sup>	5231 <sup>c</sup>	33 <sup>a</sup>	3.688	18.2
Llama	-Supernatant	518 <sup>ab</sup>	4317 <sup>d</sup>	8.3 <sup>g</sup>	1	100
	-(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> precipitate	201 <sup>b</sup>	2010 <sup>g</sup>	10.0 <sup>f</sup>	1.2	38.8
Wilbebeest	-Supernatant	938 <sup>ab</sup>	8337 <sup>a</sup>	8.9 <sup>g</sup>	1	100
	-(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> precipitate	182 <sup>b</sup>	3855 <sup>e</sup>	21.1 <sup>e</sup>	2.375	19.5
Giraffe	-Supernatant	336 <sup>ab</sup>	2800 <sup>f</sup>	8.3 <sup>g</sup>	1	100
	-(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> precipitate	84 <sup>b</sup>	747 <sup>h</sup>	8.9 <sup>g</sup>	1.067	25
Impala	-Supernatant	451 <sup>ab</sup>	375 <sup>i</sup>	8.3 <sup>b</sup>	1	100
	-(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> precipitate	135 <sup>b</sup>	2025 <sup>g</sup>	15.0 <sup>c</sup>	1.8	29.9
Buffalo	-Supernatant	486 <sup>ab</sup>	4320 <sup>d</sup>	8.9 <sup>g</sup>	1	100
	-(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> precipitate	170 <sup>b</sup>	2742 <sup>f</sup>	16.1 <sup>b</sup>	1.813	35.0
SEM		52.1	290.1	1.51	0.13	6.81
P-value		*	**	**	*	**

Substrate = Carboxymethyl cellulose, \*P<0.05, \*\*P < 0.01, values with different superscripts letters in the same column and animal differ at P<0.05 (a, b, c, d, e, f, g), <sup>1</sup> = nmol glucose/mg/min.

is the effect of the treatment (enzyme sources, pH or crude enzyme concentrations) and  $e_{ij}$  is the random variation.

## RESULTS

### Crude enzymes (endocellulases) activities

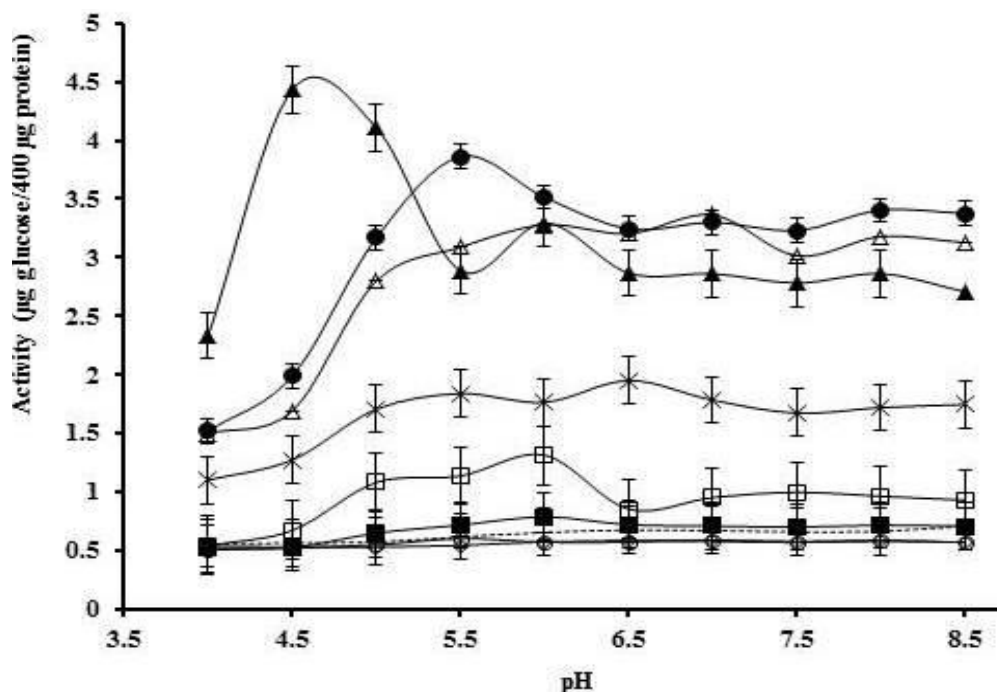
The colour of the protein precipitates consisted of variable shades of brown and green. The horse, wilbebeest, zebra, buffalo and impala were characterized by a light reddish brown precipitate while those from the elephant, giraffe, llama and camel were characterised by a whitish-brown precipitate. Precipitates from the cow and sheep were characterized by a greenish colour. The crude enzyme extracts were active to the extent that their enzyme specific activities on carboxymethyl cellulose increased (P<0.05) as protein concentration decreased following purification (Table 1). The activity recovered (purification fold) was highest (P<0.05) in the horse and zebras, intermediate in the wilbebeest, impala and buffalo, and lowest in the camel, sheep and giraffe. It was interesting to note that the camel with the highest percentage yield (84.1%) had the lowest recovery (1.06 fold).

### pH optimization

Endocellulase had a broad (P<0.05) pH range of activity for all the experimental microbial ecosystems (Figure 2). Despite the broad pH range, some distinct (P<0.05) peaks of endocellulase activities were observed within the pH range 4.5–5.0 for the elephant, at pH 5 for the horse and the zebra, within pH 5.0-6.0 for the sheep, and within pH 5.0–7.0 for the wilbebeest. Endocellulase activity for the rest of the animals (cow, llama, giraffe and camel) responded minimally within pH 4.0 to 8.0 range.

### Optimization of crude enzyme concentrations for enzyme assay

Generally, product formation increased (P < 0.05) with increasing crude enzyme concentration from the sampled herbivore microbial ecosystems. Glucose liberated from crystalline cellulose, CMC and pNP-G following incubation with crude extracts, increased (P < 0.05) non-linearly (Figure 3). Xylose liberated from xylan by xylanase from crude enzyme extracts also increased (P < 0.05) non-



**Figure 2.** The effect of pH on endocellulase activity. Product formation was monitored for samples from the reaction mixture of cow (■), sheep (□), horse (●), camel (○), elephant (▲), zebra (Δ), llama (◇), wildebeest (-), giraffe (+), impala (--) and buffalo (•).

linearly (Figure 3D). In Figure 3A, the highest increase was obtained in the zebra, horse and wildebeest while the elephant and impala showed moderate increases. High ( $P < 0.05$ ) levels of product formation were also observed in horse, impala and wildebeest when endocellulase concentrations were varied (Figure 3C). A similar situation was also observed when cellobiase was incubated with pNP-G (Figure 3B). Product formation from the reaction mixtures of the horse, zebra, impala, wildebeest and sheep were dominant while the rest were crowded together at low product formation. The result was not very different when hemicellulases were incubated with xylan (Figure 3D). The product formed in the sheep was as high as those observed from the horse and zebra, while the cow, camel and giraffe activities tended to be high. An enzyme concentration of 340  $\mu\text{g/ml}$  for optimal activity was established as suitable for exocellulase and endocellulase.

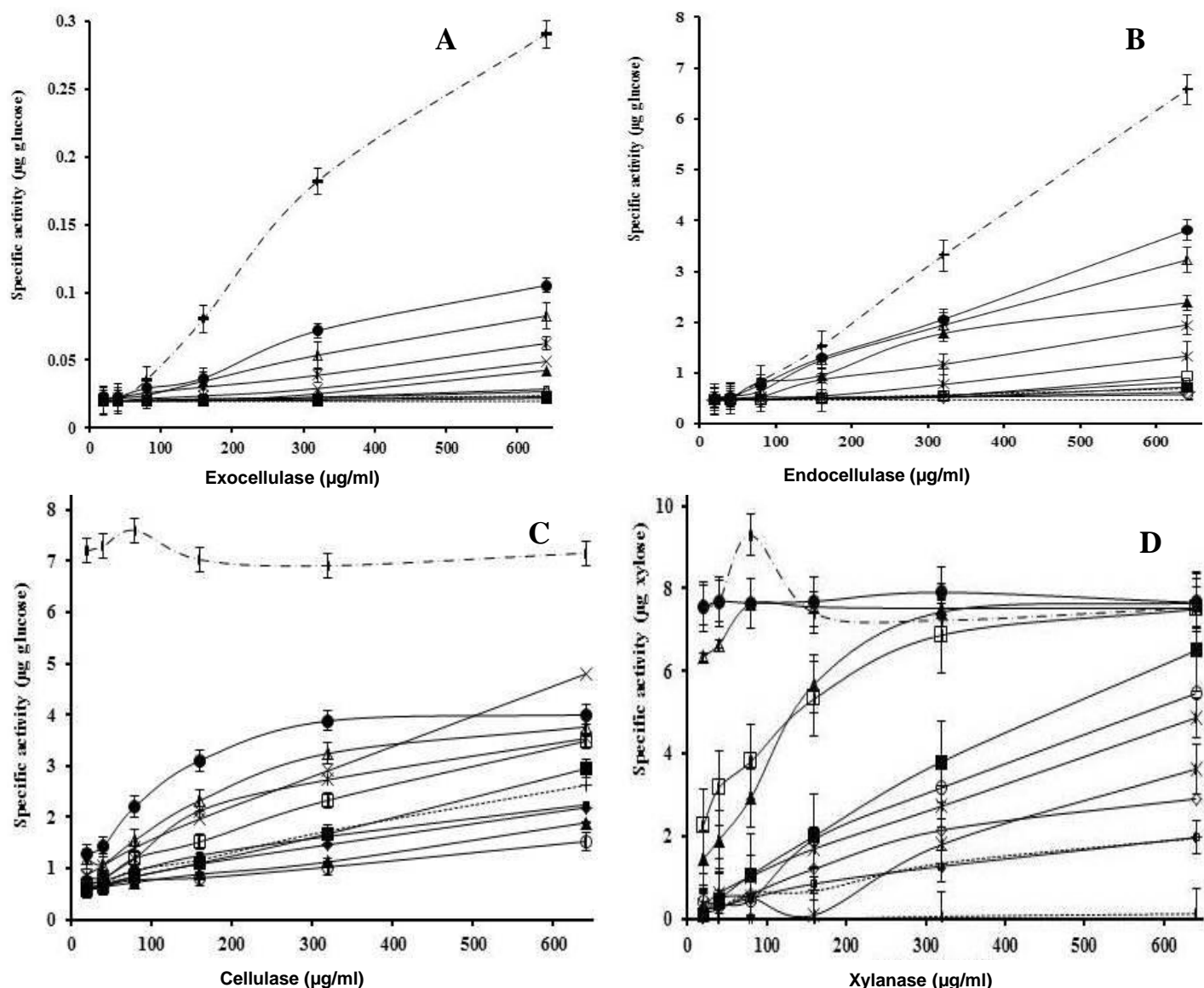
## DISCUSSION

Collecting fresh samples with limited exposure to oxygen maximised the presence of anaerobic cellulolytic and hemicellulolytic microbes as well as their enzymes. Limited exposure to oxygen prevents cell death, which was vital for the release of periplasmic proteins during sonication (Dunn et al., 2000). Ammonium sulfate precipitation

reduced the amount of unwanted proteins in the samples. This was confirmed by the increase in specific activity of crude enzyme samples with a decrease in protein concentration (Groleau and Forsberg 1983). The reddish-brown protein precipitate observed in horse, wildebeest, impala and zebra was due to high cellobiase content while the greenish colour in cow and sheep precipitates was probably due to the plant pigment chlorophyll.

Specific activity and enzyme stability were both affected by pH and temperature. The rate of enzyme reactions might be compromised if these factors were neglected. pH optimisation was vital as enzymes were isolated from 11 different ecosystems which might vary greatly. The pH profile showed that activity occurs with a wide range as opposed to the narrow *in vivo* pH range (5.6 to 8) described by many authors (Russell and Dombrowski, 1980). This disparity can be ascribed to the presence of enzymes only in *in vitro* systems unlike *in vivo* where very low or high pH might compromise microbial growth and survival. However, the peaks observed in the horse (pH 5.5) and elephant (pH 5) demonstrated that these ecosystems were harbouring microbes that were active in slightly acidic conditions. Variations can also be attributed to the genetic differences among strains of microbes in various ecosystems in the expression of these enzymes.

Other factors also affecting activity include enzyme crystalline cellulose, CMC and pNP-G following incubation x substrate concentrations can be observed in the work



**Figure 3.** The effect of enzyme concentrations on product formation. Product formation from crystalline cellulose (3A), carboxymethylcellulose (3B), p-nitrophenyl  $\beta$ -1,4-glucopyranoside (3C) or xylan (3D) were monitored from the reaction mixture of cow (■), sheep (□), horse (●), camel (○), elephant (▲), zebra (△), llama (◇), wildebeest (·), giraffe (+), impala (×) and buffalo (◊). The positive controls (- -) were commercial cellulase from *Aspergillusniger* (3A (exocellulase) and 3B (endocellulase)) cellobiase (3C) and xylanase from *Thermomyces ianuginosus* (3D). The negative control (...) was BSA for all experiments. Exocellulase = crystalline cellulase, Endocellulase = carboxymethyl cellulase.

of Henry et al. (1974) but should be optimised for every sample as enzyme concentration and activity vary relatively to its purification method (Jahir et al., 2011). The results arising from incubating different crude enzyme concentrations with the different substrates (crystalline cellulose, CMC, xylan and cellobiose) established that 340  $\mu$ g was the ideal concentration to apply in enzyme assays. It was also noted that as the crude enzyme concentration increased the rate also increased linearly but linearity was lost at high crude enzyme concentrations. This was

expected because at a high crude enzyme concentration, substrate concentration would become rate limiting. This was similar to the results obtained by Groleau and Forsburg (1983) in studies on cellobiase and endocellulase.

In an attempt to optimise extract concentrations for enzyme assays with the different substrates, it was noted that the rate at which protein samples from the different ecosystems hydrolyse specific substrates was not the same. Crude enzyme samples from the horse, zebra and wildebeest were the most active when incubated with all

four substrates but for the elephant which was also very active when incubated with CMC. Interestingly, the hindgut fermenters contained the most active protein samples. Although, many studies have stated that there are no major differences among the microbes in both fore- and hindgut fermenters (Smith and Mackie, 2004; (Smith and Mackie, 2004, Alexander, 1993), the results obtained from this study clearly indicate that there might be some factors influencing activity in these chambers. If time spent by digesta in the hindgut is a major factor (Mould et al., 2005; Schingoethe, 1993) then it is imperative to postulate that microbes in the hindgut will be more efficient in order to extract sufficient nutrients from the food before elimination as faeces. However, not all the foregut fermenters exhibited low activities as the wildebeest; the impala and cow showed intermediate activities with all four substrates. Variation in these activities might have been influenced by differences in ecological niches or composition of microbial species. For herbivores within the same geographical region, the differences in enzyme activity could be attributed to differential genetic evolution of microbes which might have resulted in variable fibrolytic efficiencies.

## Conclusion

An enzyme concentration of 340 µg/ml for optimal activity was established as suitable for exocellulase and endocellulase. Crude enzyme samples from the zebra, horse and wildebeest were the most active when incubated with all four substrates while those from the elephant, impala, cow and giraffe were intermediate. Future studies to confirm high activity among these ecosystems would include determining the affinity of enzyme to substrate, maximum velocity as well as their catalytic efficiencies. These results can assist in establishing cellulases from microbial population of zebra, horse, impala and wildebeest as feed additives.

## ACKNOWLEDGEMENTS

We would like to acknowledge National Research Foundation for funding this project and the University of Zululand and University KwaZulu-Natal for facilitating the project.

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