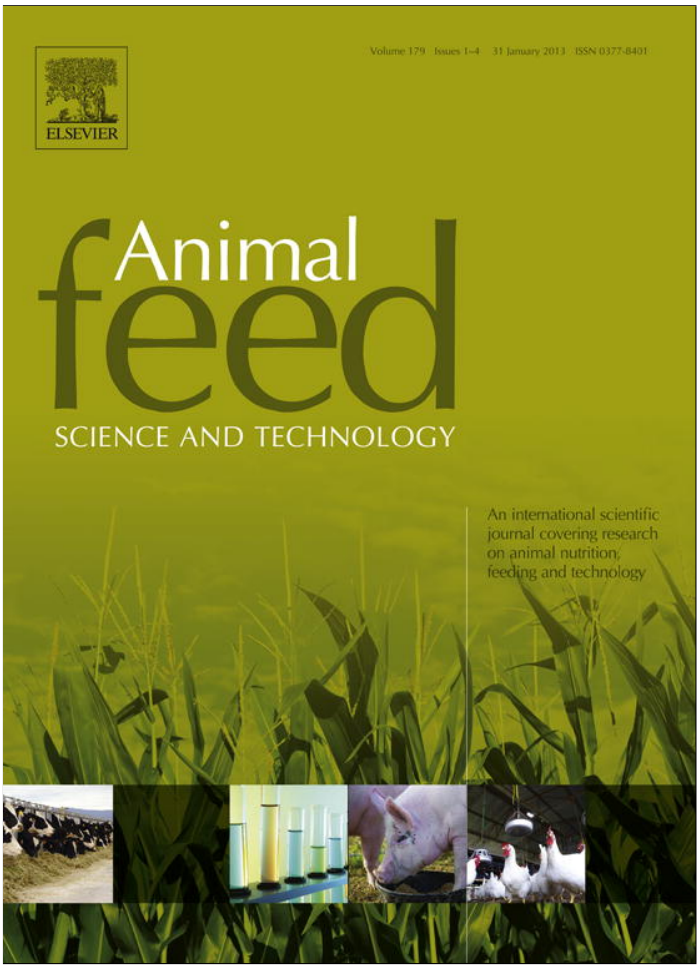

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Effects of exogenous enzymes on *in vitro* gas production kinetics and ruminal fermentation of four fibrous feeds

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ABSTRACT

This study was conducted to investigate effects of increasing doses: 0 (control), 6 (low), 12 (medium) and 24 (high) mg/g DM of ZADO® enzyme preparation mixture (ENZ) on *in vitro* gas production (GP) and some ruminal fermentation parameters of the fibrous feeds *Saccharum officinarum* (leaves), *Andropogon gayanus* (leaves), *Pennisetum purpureum* (leaves) and *Sorghum vulgare* (straw). Rumen liquor was obtained from two Brown Swiss cows fitted with permanent rumen cannulae fed a total mixed ration of a 500:500 commercial concentrate and alfalfa hay *ad libitum*. The GP was recorded at 2, 4, 6, 8, 10, 12, 24, 48, 72 and 96 h of incubation. After 96 h, the incubation was stopped and the pH of the mixture was determined and filtrate used to determine dry matter degradability (DMD), partitioning factor (PF_{96}), gas yield (GY_{24}), *in vitro* organic matter digestibility (OMD), metabolizable energy (ME), short chain fatty acids (SCFA), and microbial crude protien production (MCP). In general, the crude protein (CP) content of the fibrous feeds was low and ranged from 23 g/kg DM (*S. officinarum)* to 44 (*A. gayanus*). The fibre contents (*i.e.*, NDFom and ADFom) were highest ($P<0.05$) in *S. officinarum*. Increasing ENZ dose linearly increased ($P<0.05$) GP of all fibrous feeds and had a quadratically increased ($P<0.05$) asymptotic gas production in *P. purpureum* and *S. vulgare* and rate of gas production in *S. officinarum* and *S. vulgare*. Addition of ENZ also quadratically increased ($P<0.05$) GP at all incubation times in *S. officinarum* and *S. vulgare*, and *A. gayanus*, but only at 72 h in *A. gayanus*. The parameters of ruminal fermentation of OMD, ME, GY_{24} and SCFA linearly increased ($P<0.05$) and MCP linearly decreased ($P<0.05$) with the ENZ addition. Addition of enzyme affected ruminal fermentation of our feeds differently, mainly dependent on their fibre content, although dosage of enzyme was also important as impacts generally increased at higher dosages of ENZ.

Abbreviations: ADFom, acid detergent fibre; CP, crude protein; DM, dry matter; DMD, DM degradability; ENZ, the ZADO® enzyme preparation mixture; GP, gas production; GY_{24}, gas yield at 24 h of incubation; OM, organic matter; OMD, *in vitro* OM digestibility; MCP, microbial CP production; ME, metabolizable energy; NDFom, neutral detergent fibre; PF_{96}, partitioning factor at 96 h of incubation; SCFA, short chain fatty acids.

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1. Introduction

Digestion of plant cell walls in fibrous feeds by ruminants is possible mainly due to the enzymes produced by ruminal bacteria, protozoa and fungi. Several studies have focused on improving degradation of fibrous feeds in ruminants using feed additives; ionophores, direct fed microbials and cell wall degrading enzymes, or by using exogenous fibre degrading enzymes to stimulate rumen digestive microorganism's activities (Newbold, 1997; Kung et al., 2000; Nsereko et al., 2002; Giraldo et al., 2004). Fibrous feeds have high cellulose and hemicellulose concentrations that can create a complex of structural carbohydrates and lignin to reduce the digestibility of the carbohydrates and reduce efficient utilization of forages by ruminants. Exogenous fibrolytic enzymes may improve the nutritive value of fibrous feeds due to enhanced attachment by rumen microorganisms (Nsereko et al., 2002), creation of a stable enzyme feed complexes (Kung et al., 2000), and/or the possibility of alteration in the fibre structure, which could stimulate microbial colonization (Newbold, 1997; Giraldo et al., 2004).

However, effect of exogenous enzyme addition is influenced by factors such as diet composition, type of enzyme preparation, enzyme stability, specific enzyme activities, method of application and amount of enzyme added (Yang et al., 2000; Morgavi et al., 2001; Wallace et al., 2001). Non-linear impacts of enzyme addition has been well established *in vivo* (Lewis et al., 1999; Kung et al., 2000) and *in vitro* (Colombatto et al., 2003a,b) with higher levels of enzyme addition having reducing impacts that depend on the substrate used (Yang et al., 2000). The ZADO® enzyme mixture contains 7.1 units/g of endoglucanase, 2.3 units/g of xylanase, 61.5 units/g of α-amylase and 29.2 units/g of protease activity, and has been used in *in vivo* experiments with goats (Gado, 1997), lambs (Gado et al., 2011) and dairy cows (Gado et al., 2009) to improve nutrient digestibility and ruminal fermentation parameters (Gado et al., 2009, 2011), microbial crude protein synthesis and milk production in cows by 23% (Gado et al., 2009), and average daily gain in lambs by 92% (Gado et al., 2011). However no information is available on use of ZADO® *in vitro*.

The aim of this study was to determine effects of increasing doses of the ZADO® enzyme mixture on *in vitro* gas production and some ruminal fermentation parameters of the fibrous feeds of *Saccharum officinarum*, *Andropogon gayanus*, *Pennisetum purpureum* and *Sorghum vulgare*.

2. Materials and methods

2.1. Fibrous feed species and enzyme mixture preparation

Three individual samples of each of the fibrous feeds *Saccharum officinarum* (leaves), *Andropogon gayanus* (leaves), *Pennisetum purpureum* (leaves) and *Sorghum vulgare* (straw) were randomly and manually harvested in triplicate from different sites in the State of Mexico. Samples were dried at 60 °C for 48 h in a forced air oven to constant weight, ground in a Wiley mill to pass a 1 mm sieve and stored in plastic bags for subsequent determination of chemical components and *in vitro* gas production. Three levels of ZADO® (ENZ) a powdered multi-enzyme commercially available feed additive produced from *Ruminococcus flavefaciens* by the Academy of Scientific Research and Technology in Egypt (Patent No.: 22155, Cairo, Egypt) were used. Doses of ENZ were (/gDM): control (0 mg), low (6 mg), medium (12 mg) and high (24 mg). Prior to the study, the enzyme mixture was assayed for several enzymatic activities and was found to contain (/g of enzyme preparation) 7.1 units of endoglucanase, 2.3 units of xylanase, 61.5 units of α-amylase and 29.2 units of protease activity.

2.2. In vitro incubations

Rumen inoculum was collected from two Brown Swiss cows (400–450 kg body weight) fitted with permanent rumen cannula and fed *ad libitum* a total mixed ration made up of 500:500 commercial concentrate (PURINA®, Toluca, Mexico) and alfalfa hay formulated to meet all of their nutrient requirements (NRC, 2001). Fresh water was available to cows at all times during the rumen inoculum collection phase.

Ruminal contents from each cow was obtained before the morning feeding, mixed and strained through four layers of cheesecloth into a flask with O_2 free headspace. Samples of each feed were weighed into 120 ml serum bottles with appropriate addition of ENZ doses/g DM. Consequently, 10 ml of particle free ruminal fluid was added to each bottle followed by 40 ml of the buffer solution according to Goering and Van Soest (1970), with no trypticase added, in a 1:4 (v/v) proportion.

A total of 432 bottles (3 bottles of each triplicate sample for each of the four feeds in three runs in different weeks with each dose of ENZ (*i.e.*, 0, 6, 12, and 24 mg ENZ/g DM) plus three bottles as blanks (*i.e.*, rumen fluid only), were incubated for 96 h. Once all bottles were filled, they were immediately closed with rubber stoppers, shaken and placed in the incubator at 39 °C. The volume of gas produced was recorded at times of 2, 4, 6, 8, 10, 12, 24, 48, 72 and 96 h of incubation using the pressure reading technique (Extech Instruments, Waltham, USA) of Theodorou et al. (1994). At the end of incubation (*i.e.*, 96 h), bottles were uncapped, pH was measured using a pH meter (Conductronic pH15, Puebla, Mexico) and the contents of each bottle were filtered to obtain the non-fermented residue for the determination of degraded substrate.

2.3. Dry matter degradability

At the end of incubation (*i.e.*, 96 h), the contents of each serum bottle were filtered under vacuum through glass crucibles with a sintered filter (coarse porosity no. 1, pore size 100–160 μm, Pyrex, Stone, UK). Fermentation residues were dried at 105 °C overnight to estimate DM disappearance with loss in weight after drying being the measure of undegradable DM.

2.4. Chemical analyses and assay of enzymatic activity

Samples of the feeds were analyzed for DM (#934.01), ash (#942.05), N (#954.01) and EE (#920.39) according to AOAC (1997). The neutral detergent fibre (NDFom, Van Soest et al., 1991), acid detergent fibre (ADFom) and lignin(sa) (AOAC, 1997; #973.18) analyses used an ANKOM200 Fiber Analyzer Unit (ANKOM Technology Corp., Macedon, NY, USA). NDFom was assayed without use of an alpha amylase but with sodium sulfite in the NDFom. Both NDFom and ADFom are expressed without residual ash.

Enzyme activities in the enzyme preparation (*i.e.*, ENZ) were determined. Endoglucanase was determined according to Robyt and Whelan (1972), α-amylase was according to Bernfeld (1955), protease by Lin et al. (1969), and xylanase activity by Robyt and Whelan (1972) by catalyzing the hydrolysis of xylan from oat spelt, with the reducing groups liberated determined using alkaline copper reagent.

2.5. Calculations

To estimate kinetic parameters of GP, results (ml/g DM) were fitted using the NLIN option of SAS (2002) according to France et al. (2000) as:

$$A = b \times (1 - e^{-c(t-L)})$$

where A is the volume of GP at time t; b is the asymptotic GP (ml/g DM); c is the rate of GP (/h), and L (h) is the discrete lag time prior to gas production.

Metabolizable energy (ME, MJ/kg DM) and *in vitro* organic matter digestibility (OMD, g/kg OM) were estimated according to Menke et al. (1979) as:

$$\text{ME} = 2.20 + 0.136 \quad \text{GP(ml/0.5 g DM)} + 0.057\,\text{CP(g/kg DM)}$$
$$\text{OMD} = 148.8 + 8.89\,\text{GP} + 4.5\,\text{CP(g/kg DM)} + 0.651\,\text{ash(g/kg DM)}$$

where GP is net GP in ml from 200 mg of dry sample after 24 h of incubation.

The partitioning factor at 96 h of incubation (PF_{96}; a measure of fermentation efficiency) was calculated as the ratio of DM degradability *in vitro* (DMD, mg) to the volume (ml) of GP at 96 h (*i.e.*, DMD/total gas production (GP_{96})) according to Blümmel et al. (1997). Gas yield (GY_{24}) was calculated as the volume of gas (ml gas/g DM) produced after 24 h of incubation divided by the amount of DMD (g) as:

$$\text{gas yield}(GY_{24}) = \frac{\text{ml gas/g DM}}{\text{g DMD}}$$

Short chain fatty acid concentrations (SCFA) was calculated according to Getachew et al. (2002) as:

$$\text{SCFA(mmol/200 mg DM)} = 0.0222\,\text{GP} - 0.00425$$

where GP is the 24 h net gas production (ml/200 mg DM).

Microbial CP biomass production was calculated according to Blümmel et al. (1997) as:

$$\text{MCP(mg/g DM)} = \text{mg DMD} - (\text{ml gas} \times 2.2 \quad \text{mg/ml})$$

where 2.2 mg/ml is a stoichiometric factor which expresses mg of C, H and O required for the production of SCFA gas associated with production of 1 ml of gas.

2.6. Statistical analyses

The experimental design for the *in vitro* ruminal GP and fermentation parameter analyses was a completely random design considering, as fixed factors, type of forage (S) and enzyme preparation level (Z) in the linear model (Steel et al., 1997). Data of each of the three runs within the same sample were averaged prior to statistical analysis. Mean values of each individual sample within each species (three samples of each) were used as the experimental unit (Udén et al., 2012). The statistical model was:

$$Y_{ijkl} = \mu + S_j + Z_k + (S \times Z)_{jk} + E_{ijkl}$$

where Y_{ijk} = is every observation of the ith fibrous specie (S_i) when incubated in the jth ENZ doses (Z_j; enzyme preparation); μ is the general mean; S_i (i = 1–4) is the feed effect; Z_j is the enzyme dose effect (j = 1–4); $(SZ)_{ij}$ is the interaction between feed

Table 1
Chemical composition[a] of the four fibrous feeds (g/kg DM).

Species	OM	CP	ADFom	NDFom
Saccharum officinarum (leaves)	948	23b	482a	698a
Andropogon gayanus (leaves)	881	44a	410b	579c
Pennisetum purpureum (leaves)	944	40a	378b	557c
Sorghum vulgare (straw)	930	43a	386b	614b
SEM	52.3	7.3	26.1	35.1

[a] OM: organic matter, CP: crude protein, ADFom, acid detergent fibre, NDFom, neutral detergent fibre.
Different letters (a, b, c) following means within column indicate differences at P<0.05.

and enzyme dose; and E_{ijk} is experimental error. Linear and quadratic polynomial contrasts were used to examine responses of feeds to increasing addition levels of the enzyme preparation.

3. Results

In general, the CP content of the feeds was low (Table 1) ranging from 23 (*S. officinarum)* to 44 (*A. gayanus*) g/kg DM. Fibre content (*i.e.*, NDFom and ADFom) were highest for *S. officinarum*.

Increasing ENZ dose, linearly increased (P<0.05) GP of all feeds, increased (P<0.05, quadratic effect) asymptotic gas production in *P. purpureum* and *S. vulgare*, and increased (P<0.05, quadratic effect) rate of gas production in *S. officinarum* and *S. vulgare* (Table 2 and Fig. 1). The lag was not affected by ENZ addition.

Addition of ENZ increased (P<0.05, quadratic effect) GP at all incubation times in *S. officinarum* and *S. vulgare* and *A. gayanus*, except at 72 h in *A. gayanus* (Table 2).

Table 2
In vitro rumen gas kinetics and cumulative gas production after 96 h of incubation as affected by level of exogenous enzyme preparation (ENZ; mg/g DM).

Substrate (S)	Enzyme dosage (ENZ)	Gas production parameters[a]			*In vitro* gas production (ml/g DM)					
		b	*c*	*L*	GP_6	GP_{12}	GP_{24}	GP_{48}	GP_{72}	GP_{96}
Saccharum officinarum (leaves)	0	139	0.013	2.72	9.8	19.0	35.2	61	80.7	95.1
	6	197	0.011	2.14	12.0	23.1	42.9	75	98.2	116.1
	12	206	0.017	1.14	19.6	37.2	67.5	112	142.2	162.3
	24	220	0.031	0.83	37.1	68.0	114.8	169	195.5	208.0
	P									
	Linear	0.659	0.454	0.544	0.047	0.050	0.056	0.071	0.084	0.072
	Quadratic	0.341	0.001	0.079	<0.001	<0.001	<0.001	0.001	0.002	0.001
Andropogon gayanus (leaves)	0	233	0.010	1.31	13.2	25.7	48.5	87	117.2	141.2
	6	222	0.017	1.32	21.2	40.4	73.2	122	154.2	175.9
	12	239	0.017	1.73	22.7	43.2	78.6	131	166.7	190.5
	24	234	0.051	1.41	47.5	81.2	125.9	175	201.0	215.4
	P									
	Linear	0.819	0.369	0.901	0.077	0.024	0.002	0.031	0.138	0.041
	Quadratic	0.812	0.171	0.621	0.011	0.002	<0.001	0.005	0.050	0.006
Pennisetum purpureum (leaves)	0	152	0.019	1.44	16.7	31.6	56.6	92	114.4	128.3
	6	183	0.019	1.47	19.7	37.2	66.9	109	136.3	153.4
	12	208	0.019	1.66	22.2	42.1	75.7	124	154.6	174.1
	24	257	0.018	1.33	24.2	45.5	81.0	131	162.4	183.4
	P									
	Linear	0.099	0.831	0.807	0.521	0.509	0.483	0.421	0.349	0.574
	Quadratic	0.005	0.873	0.867	0.203	0.192	0.170	0.124	0.080	0.263
Sorghum vulgare (straw)	0	245	0.020	1.75	27.7	52.2	93.3	151	186.8	208.9
	6	240	0.026	1.17	33.8	62.9	109.2	169	201.2	218.9
	12	255	0.027	1.75	38.5	71.0	122.0	185	217.9	235.2
	24	280	0.030	1.58	46.1	84.6	143.5	213	247.3	264.0
	P									
	Linear	0.784	0.050	0.129	0.095	0.091	0.086	0.089	0.112	0.125
	Quadratic	0.016	0.016	0.428	0.006	0.005	0.004	0.003	0.003	0.003
Pooled SEM		21.1	0.0070	0.464	3.77	5.76	8.24	12.2	14.91	13.21
P value										
S		0.01	0.34	0.85	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
ENZ		0.01	0.01	0.49	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
S × ENZ		0.45	0.22	0.38	0.05	0.03	0.04	0.20	0.48	0.16

[a] *b* is the asymptotic gas production (ml/g DM); *c* is the rate of gas production (h^{-1}); *L* is the initial delay before gas production begins (h).

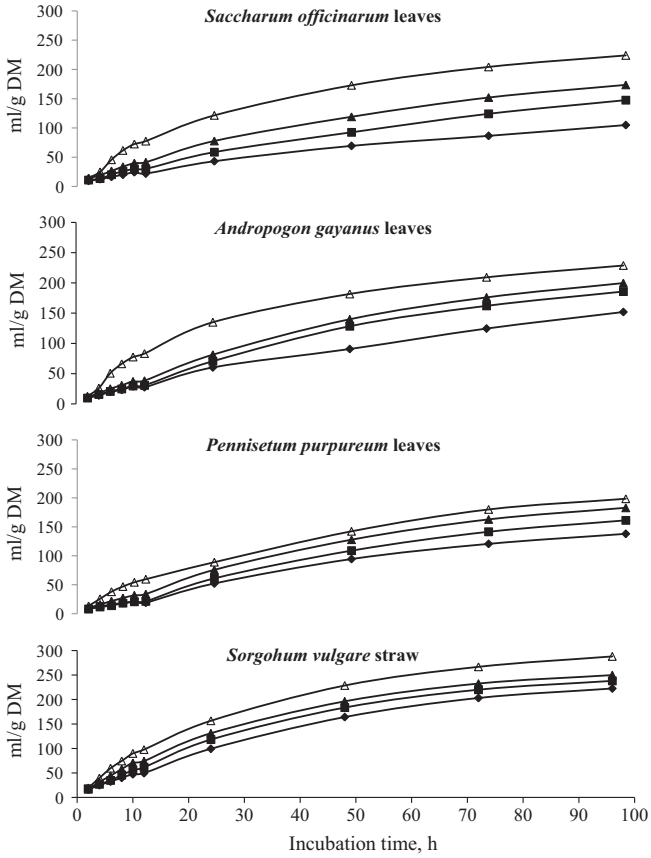

Fig. 1. Cumulative gas production profiles (ml gas/g DM) from *in vitro* fermentation of four fibrous feed species at four doses of exogenous enzyme preparation (ENZ, mg/g DM) (-♦-, 0; -■-, 6; -▲-, 12; -△-, 24 mg ENZ/g DM of substrate; SEM is for the overall fit and *P* is for the effect of enzyme dose).

Addition of ENZ, in general, increased (P<0.05, quadratic effect) parameters of ruminal fermentation, while final pH and MCP decreased (P<0.05, quadratic effect). However, OMD, ME, GY_{24} and SCFA linearly increased (P<0.05), and MCP decreased (P<0.05), with the addition of ENZ *versus* control (Table 3).

4. Discussion

Differences in CP contents between the feed were probably due to stage of maturity, the N profile of the soils where they were grown and differences in efficiency of protein accumulation in them during growth. However, the nutrient levels are comparable to those reported by Rubanza et al. (2003). Differences in nutrient composition of the feeds are also likely due to differences in the stage of growth and plant part (*i.e.*, twigs, leaves, soft stem) sampled. Inconsistencies could also be due to sampling site and climatic influences on species growth and plant nutrient accumulation.

In general, GP appeared to be related to the chemical composition of the feeds, in particular to the fibre content (Table 2 and Fig. 1). Several studies have shown that adding this enzyme preparation to ruminant diets increased digestion of DM and fibre measured *in situ*, *in vitro* or *in vivo* (Gado, 1997; Gado et al., 2009, 2011; Salem et al., 2011). Increased *in vitro* GP with ENZ may allow higher voluntary feed intake (Gado et al., 2009) by decreasing physical rumen fill, increasing the net energy density of the diets and stimulating MCP production (Oba and Allen, 2000).

The ENZ addition was effective in improving *in vitro* GP (Table 2) and ruminal fermentation parameters such as OMD, GY_{24} and SCFA when applied to *S. vulgare*, which compared with smaller effects when it was added to *S. officinarum*, *A. gayanus* and *P. purpureum*. This effect could be due to differences in the internal plant cell wall structures of the feeds, but results are consistent with Yu et al. (2005) who found that responses of alfalfa hay, wheat straw and oat hulls to a multi-enzyme cocktail differed, and concluded that a multi-enzyme cocktail is most effective for oat hulls, followed by alfalfa hay lastly wheat straw.

The linear increase in GP of the feeds with increasing ENZ addition in *S. officinarum* and *S. vulgare* and *A. gayanus* at all the incubation times could support the hypothesis that a suitable enzyme dose could improve fermentation efficiency, and/or that ENZ addition stimulated fermentation (Nsereko et al., 2002). That the ENZ affected GP to the fermentation end point of 96 h (Fig. 1) could indicate that ENZ increased fermentable material (Colombatto et al., 2003b), which is inconsistent with Wang et al. (2004) who reported that 1.5 g/kg of a fibrolytic multi-enzyme added to wheat straw only increased GP up to 8 h of incubation. The rapid and linear increase in GP with the addition of ENZ to these feeds, although it had no effect on lag time, could be due to addition of the polysaccharidase enzymes in ZADO® which provided fermentable carbohydrate

Table 3
In vitro rumen fermentation profile[1] as affected by exogenous enzyme dose (ENZ, mg/g DM).

Substrate (S)	Enzyme dosage (ENZ)	pH	DMD (mg/g DM)	OMD (g/g DM incubated)	ME (MJ/kg DM)	PF_{96} (mg DMD:ml gas)	GY_{24} (ml gas/g DMD)	SCFA (mmol/g DM)	MCP (mg/g DM)
Saccharum officinarum (leaves)	0	6.9	446	0.235	3.4	12.8	80	0.76	369
	6	6.9	471	0.249	3.6	14.3	95	0.93	377
	12	6.8	479	0.293	4.3	7.2	142	1.48	331
	24	6.6	452	0.377	5.6	3.9	255	2.53	200
	P								
	Linear	0.012	0.636	0.056	0.058	0.932	0.091	0.055	0.492
	Quadratic	<0.001	0.748	<.0.001	<0.001	0.032	<0.001	<0.001	0.012
Andropogon gayanus (leaves)	0	6.7	550	0.249	3.7	13.4	90	1.05	444
	6	6.6	538	0.293	4.3	7.34	136	1.61	377
	12	6.6	551	0.302	4.5	7.0	143	1.72	378
	24	6.4	554	0.386	5.8	4.4	228	2.77	277
	P								
	Linear	0.257	0.678	0.002	0.002	0.074	0.003	0.002	0.028
	Quadratic	0.004	0.659	<0.001	<0.001	0.071	<0.001	<0.001	0.011
Pennisetum purpureum (leaves)	0	6.7	547	0.277	4.0	9.7	104	1.24	422
	6	6.7	555	0.295	4.3	8.3	120	1.46	408
	12	6.6	584	0.311	4.5	7.8	130	1.66	417
	24	6.6	563	0.320	4.7	8.4	145	1.78	385
	P								
	Linear	0.904	0.915	0.483	0.484	0.612	0.564	0.486	0.485
	Quadratic	0.057	0.054	0.170	0.173	0.565	0.625	0.169	0.242
Sorghum vulgare (straw)	0	6.6	641	0.336	5.0	6.9	146	2.05	435
	6	6.7	627	0.365	5.4	5.7	174	2.40	386
	12	6.6	584	0.387	5.7	4.8	208	2.69	316
	24	6.5	558	0.426	6.3	3.9	258	3.16	242
	P								
	Linear	0.072	0.364	0.086	0.087	0.003	0.026	0.086	0.010
	Quadratic	<0.001	0.004	0.004	0.004	<0.001	<0.001	0.004	<0.001
Pooled SEM		0.035	16.8	0.0147	0.22	2.04	15.8	0.183	26.1
P value									
S		<.0001	<.0001	<.0001	<.0001	0.04	<.0001	<.0001	0.01
ENZ		<.0001	0.45	<.0001	<.0001	0.003	<.0001	<.0001	<.0001
S × ENZ		0.26	0.07	0.04	0.04	0.27	0.01	0.04	0.08

[1] DMD is dry matter degradability; OMD is *in vitro* organic matter digestibility; ME is metabolizable energy; PF_{96} is the partitioning factor at 96 h of incubation; GY_{24} is gas yield at 24 h; SCFA is short chain fatty acids; MCP is microbial CP production.

to stimulate microbial growth (Forsberg et al., 2000). It could also be due to the increased numbers of fibrolytic and non-fibrolytic bacteria in the rumen due to release of polysaccharides which are readily utilized by the bacteria (Nsereko et al., 2002).

We hypothesize that curvilinear increase in GP and fermentation kinetics of these feeds with increasing levels of ENZ was due to excessive levels of ENZ that may have prevented binding of all enzymes to substrate receptors, which reduced proportional attachment by ruminal microorganisms to fibre (Treacher and Hunt, 1996). Colombatto et al. (2003a) concluded that increasing the level of enzyme from 1× to 5× increased the rate and extent of GP, but that addition at the 10× levels was not effective.

The linear increase of DMD and OMD, ME, GY_{24} and SCFA of these feeds with the addition of ENZ may have been due to increased fibre digestion and altered ruminal fermentation (Nsereko et al., 2002), enhanced attachment and colonization to the plant cell wall material by rumen microorganisms (Nsereko et al., 2002; Wang et al., 2001) and/or by synergism between ruminal enzymes and the enzymes of the ENZ as the most likely mode of action of the enzyme (Morgavi et al., 2001). Indeed Morgavi et al. (2001) demonstrated synergism between exogenous and ruminal enzymes such that the net combined hydrolytic effect in the rumen was much higher than that estimated from individual enzyme activities. Increased SCFA with increased ENZ dose was opposite of the pH response, but is consistent with the increase OMD and ME. Our results also agree with Omar et al. (2009), who concluded that supplementation of enzymes to steer rations improved digestibility and rumen SCFA concentrations.

Addition of ENZ linearly decreased MCP compared with the control in all feeds. This may have been due to a poor synchronization between energy and N availability and a deficiency in N due to the generally low CP content of the feeds.

5. Conclusions

The effectiveness of the enzyme preparation differed among the feeds being highest for *S. vulgare*, intermediate *A. gayanus* and *P. purpureum*, and lowest for *S. officinarum*. Increasing the enzyme dose, linearly increased ruminal gas production including fermentation rate and asymptotic gas production, but decreased microbial CP production.

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