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Influence of the addition of exogenous xylanase with or without pre-incubation on the *in vitro* ruminal fermentation of three fibrous feeds

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ABSTRACT: The effects of the exogenous fibrolytic enzyme (ENZ) commercial preparation Dyadic® xylanase PLUS (Dyadic International, Inc., Jupiter, USA), containing endo-1,4-β-D-xylanase, on ruminal fermentation of maize stover, oat straw, and sugarcane bagasse were examined using the *in vitro* gas production (GP) technique. The ENZ commercial preparation was added at 0 (control), 60 (low), 120 (medium), and 240 (high) μg/g dry matter of substrate, and at two times of application (direct addition just before fermentation or with a 72-h pre-incubation before fermentation). Ruminal GP volumes were recorded at 2, 4, 6, 8, 10, 12, 14, 24, and 48 h of incubation, and substrate degradability and concentration of fermentation end-products (volatile fatty acids, ammonia, methane) in the cultures were determined at 48 h of incubation. Increased ($P < 0.05$) GP volume was observed in fibrous feeds treated with ENZ compared with untreated substrates. The pre-incubation vs the direct addition of ENZ resulted in greater GP volume ($P < 0.05$) with the three tested feeds. Enzyme addition decreased ($P < 0.05$) rumen pH compared to control when maize stover and sugarcane bagasse were incubated. Ammonia-N and total volatile fatty acids (VFA) were not affected ($P > 0.05$) by ENZ application in maize stover and oat straw. However, total and individual VFA concentrations, and CH_4 and CO_2 volumes were greater ($P < 0.05$) when sugarcane bagasse was incubated with 240 μg ENZ/g ($P < 0.05$). It can be concluded that the application of endo-1,4-β-D-xylanase enhances rumen fermentation of roughages, although the magnitude of the effects depends on the fibrous substrate fermented, the time of application, and the amount of enzyme added.

Keywords: roughage; gas production; fibre degradation; rumen; fibrolytic enzyme

INTRODUCTION

The high cost of cereals and quality forages has raised the interest in using fibrous roughages in the diet of ruminants (Rojo et al. 2015; Salem et al. 2015). Crop production is associated with the production of a number of harvesting residues and by-products, which may have important eco-

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nomic and environmental impacts. Most often these residues constitute a waste with limited potential use within the farm, and their disposal becomes a costly affair. Stover and straw are some of the crop residues produced in large quantities and left in the field after harvest. Bagasse is another important by-product remaining after sugarcane is crushed to extract the juice. In 2013, the annual production of maize stover, sugarcane bagasse, and oat straw in Mexico accounted for 12.7, 0.524, and 11.2 million of tonnes, respectively (http://www.siap.gob.mx/cierre-de-la-produccion-agricola-por-cultivo/).

These by-products could be used as animal feedstuffs, but their use is limited by their high fibre content, which invariably lowers the efficiency of digestive utilization (Khattab et al. 2013; Kholif et al. 2014). Fibrous feeds are characterized by high lignocellulose content, low crude protein (CP) content, poor palatability, and low nutrient digestibility (Kholif et al. 2014; Togtokhbayar et al. 2015). The structural carbohydrates of the fibre are less digestible than other nutrients, and the cell wall (mainly the lignin) may be a physical barrier for the bacterial attachment and the access of ruminal enzymes resulting in limited ruminal degradability (Karunanandaa et al. 1995). Maize stover, oat straw, and sugarcane bagasse have poor nutritional value because of their low N and high fibre contents (Diaz et al. 2013; Lara-Bueno et al. 2013).

For an effective utilization of such fibrous feeds, exogenous fibrolytic enzymes have been used to improve carbohydrate and cell wall degradation (Morsy et al. 2016), thus upgrading their nutritive value. Supplementing ruminant diets with fibrolytic enzymes has been shown to improve feed utilization and animal performance (Rojo et al. 2015; Morsy et al. 2016). However, the effectiveness of enzymes depends upon substrate, enzyme specificity, and enzyme dose (Lara-Bueno et al. 2013; Elghandour et al. 2015), resulting in variable responses.

The experiment reported herein was designed to investigate the effect of a xylanase preparation, with or without (direct addition) pre-incubation, on *in vitro* fermentation of maize stover, oat straw, and sugarcane bagasse. The hypothesis is that an exogenous xylanase can enhance digestibility of poor quality feeds, and this effect may be affected by the time of application of the enzyme (direct addition of enzyme to the feed or with pre-incubation for 72 h before feeding).

MATERIAL AND METHODS

Fibrous feed substrates and the enzyme product. Three roughages, namely maize stover, oat straw, and sugarcane bagasse were used. Whole maize and oat plants were collected at harvest maturity stage (after completing their physiological development). Bagasse was collected from sugar factories as the fibrous waste remaining after juice extraction. Sugarcane had been harvested at peak maturity stage (ripening and maturation phase). Three samples of each feed were collected from different sites in the State of Mexico. Each plant material collected was chopped into particles of 0.5–1.0 cm size and dried at 65°C for 48 h in a forced air oven until constant weight. The three samples of each roughage were thoroughly mixed and the composite sample was ground in a Wiley mill to pass a 1 mm sieve and stored in plastic bags for subsequent determination of chemical composition and *in vitro* incubations.

The fibrous feeds were treated by the addition of the commercial enzymatic product Dyadic® Xylanase PLUS (Dyadic International, Inc., Jupiter, USA), a concentrated liquid acid-neutral endo-1,4-β-D-xylanase (E.C. 3.2.1.8) produced by a non-genetically modified strain of *Trichoderma longibrachiatum*. The declared enzymatic activities in Dyadic® Xylanase PLUS are 34 000–41 000 units of xylanase/g, 12 000–15 000 units of β-glucanase/g, and 45 000–55 000 units of cellulase/g, able to break down some non-starch polysaccharides including arabinoxylans and β-glucans in cereal grains and fibrous feeds. The activity of endoglucanase was assayed as the release of glucose from carboxymethyl cellulose, which was determined colorimetrically using an alkaline copper reagent (Robyt and Whelan 1972). One unit of endoglucanase catalyzes the release of 1 mmol of glucose per min from sodium carboxymethyl cellulose at 40°C and pH 4.5. α-Amylase was assayed as the release of reducing sugars from starch, which were measured by the reduction of 3,5-dinitrosalicylic acid as described by Bernfeld (1955). One unit of α-amylase catalyses the release of 1 mmol of reducing sugars per min from soluble starch at 25°C and pH 6.0, and was calculated as maltose equivalents. Protease activity was determined by the hydrolysis of dimethyl casein (DMC) and the liberated amino acids were determined using 2,4,6-trinitrobenzene sulfonic acid (Lin et al.

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1969). One DMC unit catalyzes the cleavage of 1 mmol of peptide bond per min from DMC at 25°C and pH 7.0, and was expressed in terms of newly formed terminal amino groups. Xylanase catalyzes the hydrolysis of xylan from oat spelts, and the reducing sugars released were determined using an alkaline copper reagent (Robyt and Whelan 1972). One unit catalyzes the release of 1 mmol of reducing sugars per h from xylan at 37°C and pH 5.5, and was expressed as xylose equivalents.

Experimental design. The experiment was designed to ascertain the effects of the addition of the enzyme (ENZ) preparation on *in vitro* rumen fermentation of the three selected fibrous feeds, as well as the effects of conditions of application (dose and time of addition). Accordingly, three doses (referred to as low, medium and high) and two times of application (direct addition just before fermentation or with a pre-incubation) of the ENZ preparation were tested. The addition of ENZ was tested against a control with no ENZ added. Feed samples (1 g dry matter (DM)) were weighed into 120 ml serum bottles with appropriate addition of ENZ. Rates of addition of the enzymatic preparation (ENZ) tested were 60 (low, L_ENZ), 120 (medium, M_ENZ), and 240 (high, H_ENZ) μg/g DM. A stock solution of the ENZ was prepared for each treatment (at concentrations of 60, 120, and 240 mg/l, respectively) by diluting the product in distilled water, so that the intended dose in the fermentation cultures was achieved by dispensing 1 ml of each stock solution on top of the sample of feed weighed into each serum bottle. The addition of ENZ solutions to the test feeds was either immediately before fermentation (no pre-incubation or direct addition) or 72 h before (with pre-incubation) *in vitro* rumen fermentation.

In vitro incubations. Rumen inoculum was collected before morning feeding from four sheep (35–45 kg body weight) fitted with a permanent rumen cannula and fed *ad libitum* a total mixed ration with (per kg) 500 g of a commercial concentrate (PURINA®, Toluca, Mexico) and 500 g of alfalfa hay. Sheep had free access to fresh water at all times during the experiment. Straightway after collection, the rumen contents obtained from the donor sheep were flushed with CO_2, mixed and strained through four layers of cheesecloth into a flask with O_2 free headspace. Filtered rumen fluid was immediately transported to the laboratory where it was mixed with a buffer solution at a ratio 1 : 4 (v/v). The buffer solution used was that described by Goering and Van Soest (1970) containing macro- and micro-minerals, with no trypticase added. Diluted rumen fluid (50 ml containing 10 ml of rumen liquor) was dispensed to each incubation bottle, where substrates had been previously weighed out and ENZ solutions dosed.

Three incubation runs were performed in three different weeks. A total of 66 bottles were inoculated within each incubation run, three bottles as blanks (i.e. rumen fluid only, with no substrate) and then 21 bottles for each feedstuff (corresponding to 7 experimental treatments (control plus six ENZ treatments) × 3 repetitions). After filling all bottles, they were immediately closed with rubber stoppers, shaken, and placed in a water bath at 39°C. The volume of gas produced was recorded at 2, 4, 6, 8, 10, 12, 14, 24, and 48 h of incubation using a pressure transducer (Extech Instruments, Waltham, USA) following the technique of Theodorou et al. (1994). At the same incubation times, methane and CO_2 concentrations in the headspace of the bottles were measured using a diffusion based gas detector Tetra 3 (Crowcon Detection Instruments Ltd., Abingdon, UK).

After 48 h of incubation, bottles were opened, pH was measured using a pH meter PH15 (Conductronic, Puebla, Mexico), and samples of the supernatant (5 ml) from each bottle were collected in glass tubes for volatile fatty acids (VFA) and ammonia-N determinations. A subsample of 3 ml was preserved in 3 ml of 0.2M HCl for ammonia-N analysis, whereas another subsample (0.8 ml) was mixed with 0.2 ml of a solution of metaphosphoric acid (250 g/l) for VFA analyses. Subsamples were stored at –20°C until laboratory analyses.

After sampling the supernatant, the contents of each bottle were filtered under vacuum through sintered glass crucibles (coarse porosity No. 1, pore size 100–160 μm) (Pyrex, Stone, UK). The incubation residues were dried then at 105°C overnight to estimate apparent DM disappearance. Neutral (NDF) and acid detergent fibre (ADF) were determined in the dried residues. Blanks were used to correct for gas production from ruminal fluid.

Chemical analyses. AOAC (1997) official methods were used to analyze the feed samples for DM (method No. 934.01), ash (method No. 942.05), and N (method No. 954.01). The NDF and ADF contents in both feeds and incubation residues were determined using an ANKOM 200 Fiber

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Analyzer (ANKOM Technology Corp., Macedon, USA). The NDF analysis was done with sodium sulphite, but without α-amylase. Both NDF and ADF were expressed without residual ash.

Ruminal fluid samples for VFA analysis were thawed and centrifuged at 9000 × *g* for 10 min at 10°C, and then analyzed using a gas chromatograph (Agilent Technologies 6890 N (G153ON) Network GC System; Agilent Technologies, Inc., Santa Clara, USA), equipped with a flame ionization detector and a capillary column Elite-FFAP (PerkinElmer, Waltham, USA) of 30 m, 0.32 mm i.d., 0.25 mm film thickness. The ammonia-N concentration was determined using the method of McCullough (1967).

Calculations and statistical analyses. To estimate ruminal fermentation kinetic parameters, gas volumes recorded (ml/g DM) were fitted to France et al. (2000) model using the NLIN procedure of SAS (Statistical Analysis System, Version 9.0, 2002):

$$y = A \times [1 - e^{-c\,(t-L)}]$$

where:

y = volume of gas production at time t (h)

A = asymptotic gas production (ml/g DM)

c = fractional rate of fermentation per h

L = discrete lag time prior to any gas is released (h)

Separate analyses of variance were performed for each feedstuff (maize stover, oat straw, and sugarcane). For each substrate, treatments were arranged in a 2 (ENZ application with or without pre-incubation) × 3 (rates of application at 60, 120 or 240 μg ENZ/g DM) factorial plus one control (no enzyme additive) design, for a total of seven experimental treatments. For each end-point studied, values recorded from the three repetitions within each incubation run were averaged. Thus, within each feed there were three replicates per treatment (each corresponding to the average value recorded at each incubation run), and each replicate was considered as an experimental unit. The statistical model included the fixed effects of enzyme addition (control vs ENZ), time of application (with or without pre-incubation), rate of application, and the interaction of the last two factors. The analysis was performed using the PROC MIXED of SAS (Statistical Analysis System, Version 9.0, 2002) for factorial arrangements of treatments plus one control as described by Marini (2003). Treatment vs control contrasts were done comparing all the treatments with enzyme additive vs the control (no enzyme additive). Multiple comparisons of means were performed using the Tukey's test.

RESULTS

The three roughages differed in their chemical composition but all of them had moderate fibre contents (459–538 g NDF and 274–380 g ADF/kg DM). Maize stover contained 956 g organic matter (OM), 63 g crude protein (CP), 477 g NDF, and 281 g ADF per kg DM. The chemical composition of the oat straw used in the study was 924 g OM, 39 g CP, 538 g NDF, and 380 g ADF per kg DM. The sugarcane bagasse contained 982 g OM, 27 g CP, 459 g NDF, and 324 g ADF per kg DM.

For maize, elevated pH ($P = 0.028$) was observed in control (6.82) compared to ENZ (on average 6.71) treatments (Table 1). An interaction was observed ($P < 0.05$) between time of addition and application rates for GP volumes at different incubation times (Table 1). Greater ($P \leq 0.007$) GP volumes were observed with ENZ treatments vs control at all incubation times (average GP at 24 h of incubation was 117 and 166 ml/g DM, and average asymptotic GP was 171 and 236 ml/g DM for control and ENZ treatments, respectively). Pre-incubation of maize stover with ENZ gave rise to greater GP volumes and asymptotic GP ($P < 0.05$) compared to the direct ENZ addition before incubation (average asymptotic GP was 219 and 253 ml/g DM for direct application and pre-incubation, respectively). When ENZ was added directly with no pre-incubation, the increasing ENZ application rate had nonsignificant effect on GP volume throughout incubation. However, with a pre-incubation in ENZ, GP volume was increased with the H_ENZ compared to L_ENZ application rates ($P < 0.05$) at all incubation times (216 vs 150 ml/g at 24 h of incubation for H_ENZ and L_ENZ, respectively).

For oat straw (Table 2), interactions were significant only for molar proportion of propionate ($P = 0.015$) and for acetate to propionate ratio ($P = 0.029$). Pre-incubation of oat straw with ENZ at 240 μg/g DM showed greater ($P \leq 0.004$) GP volumes at all incubation times and thus an increased asymptotic GP ($P = 0.001$) vis-a-vis the untreated control. The pre-incubation with 120 μg ENZ/g DM resulted in a longer lag time ($P = 0.007$) if compared to the direct addition of ENZ. After a 72-h pre-incubation, the ENZ application rate

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Table 1. *In vitro* rumen gas production kinetics, degradability, and rumen fermentation end-products from maize stover treated with increasing addition rates of ENZ (μg ENZ/g DM) applied at two different times (direct addition just before fermentation or 72 h pre-incubation)

	Control	Direct application			Pre-incubation			SEM	*P*-value			
	0	60	120	240	60	120	240		control vs ENZ[1]	time (T)[2]	rate (R)[3]	T × R
Gas production and kinetics (ml/g DM incubated)												
Gas2[4]	16.1^{c}	21.5^{b}	21.8^{b}	21.1^{b}	22.6^{b}	23.4^{b}	27.4^{a}	2.9	< 0.001	< 0.001	0.049	0.016
Gas4	34.5^{c}	39.4^{b}	39.6^{b}	39.0^{b}	41.2^{b}	46.0^{a}	48.5^{a}	5.0	< 0.001	< 0.001	0.009	0.005
Gas6	52.3^{c}	61.0^{bc}	61.3^{bc}	59.9^{bc}	61.0^{bc}	70.0^{ab}	76.6^{a}	6.2	< 0.001	< 0.001	0.017	0.008
Gas8	71.8^{b}	85.4^{ab}	81.7^{b}	79.7^{b}	77.4^{b}	88.6^{ab}	103.9^{a}	9.2	0.007	0.041	0.075	0.006
Gas10	89.1^{b}	106.7^{b}	101.5^{b}	101.1^{b}	95.8^{b}	110.9^{ab}	136.6^{a}	12.4	0.005	0.022	0.016	0.003
Gas24	117.4^{c}	148.6^{bc}	151.8^{bc}	151.1^{bc}	150.1^{bc}	178.1^{ab}	215.5^{a}	15.1	< 0.001	0.001	0.008	0.013
Gas48	167.8^{c}	204.2^{bc}	208.9^{bc}	207.4^{bc}	199.3^{bc}	238.6^{ab}	276.8^{a}	14.1	< 0.001	0.007	0.016	0.027
A (ml/g DM)	170.5^{b}	212.2^{ab}	226.1^{ab}	219.3^{ab}	211.3^{ab}	254.4^{ab}	294.1^{b}	18.1	0.005	0.037	0.074	0.146
c (per h)	0.062	0.064	0.060	0.056	0.058	0.056	0.060	0.008	0.691	0.747	0.901	0.748
L (h)	0.04	0.22	0.13	0.16	0.10	0.34	0.56	0.02	0.230	0.209	0.428	0.270
Degradability (%)												
DM	43.9	56.4	60.9	66.3	46.1	50.0	50.1	5.5	0.084	0.015	0.470	0.843
NDF	25.5	33.0	32.2	36.1	24.9	28.4	28.1	3.2	0.178	0.022	0.625	0.750
ADF	14.3	18.8	18.3	20.6	14.6	16.2	17.1	2.0	0.150	0.068	0.539	0.878
Fermentation parameters												
pH	6.82	6.74	6.71	6.71	6.81	6.63	6.65	0.04	0.028	0.474	0.048	0.148
NH_3-N (mg/dl)	10.4	15.3	9.6	10.8	12.7	22.8	17.7	4.6	0.386	0.142	0.874	0.253
Total VFA (mmol/l)	46.1	70.1	50.3	52.1	58.1	70.9	59.0	12.5	0.317	0.621	0.792	0.444
A (mmol/l)	28.5	44.0	30.7	31.2	37.9	47.3	38.3	7.6	0.255	0.356	0.713	0.348
% of VFA	62.6	63.6	61.1	60.3	65.8	67.5	64.9	2.0	0.589	0.018	0.563	0.601
P (mmol/l)	10.8	16.1	11.9	12.7	11.5	13.4	11.8	2.9	0.511	0.579	0.859	0.594
% of VFA	23.1^{ab}	22.5^{ab}	23.7^{ab}	24.1^{a}	19.6^{ab}	18.6^{b}	20.1^{ab}	1.1	0.185	< 0.001	0.542	0.578
Butyrate (mmol/l)	6.8	10.1	7.7	8.2	8.7	10.3	8.9	2.2	0.369	0.737	0.928	0.673
% of VFA	14.3	14.0	15.2	15.6	14.6	14.0	14.9	1.1	0.745	0.663	0.675	0.709
A : P ratio	2.76	2.85	2.57	2.50	3.42	3.66	3.24	0.26	0.330	0.002	0.537	0.614
CO_2 (ml/100 ml gas)	27.1	41.1	29.9	31.1	34.8	42.4	35.4	7.7	0.314	0.583	0.827	0.491
CH_4 (ml/100 ml gas)	14.9	23.0	16.2	16.5	20.4	25.4	20.7	4.2	0.246	0.307	0.745	0.384

ENZ = xylanase enzyme, DM = dry mater, *A* = asymptotic gas production, *c* = rate of gas production, *L* = initial delay before gas production beginning, VFA = volatile fatty acids, A = acetate, P = propionate, CO_2 = carbon dioxide, CH_4 = methane, NDF = neutral detergent fibre, ADF = acid detergent fibre, SEM = standard error of the mean

[1]treatment vs control contrasts comparing all treatments with enzyme additive vs control (no enzyme additive)

[2]direct application vs pre-incubation

[3]60, 120 or 240 μg ENZ/g DM

[4]gas production at *t* hours of incubation (e.g. Gas2 = gas at 2 h of incubation etc.)

[a–c]means in the same row with different superscripts are significantly different ($P < 0.05$)

increasing from 60 to 240 μg/g resulted in increased GP volumes at 24 and 48 h of fermentation, greater asymptotic GP (from 176 to 245 ml/g for 60 and 240 μg/g doses, respectively), and slower fermentation rate (from 0.089 to 0.061 per h for 60 and 240 μg/g doses, respectively).

For sugarcane bagasse (Table 3), control had higher pH values ($P < 0.001$) than ENZ treatments

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Table 2. *In vitro* rumen gas production kinetics, degradability, and rumen fermentation end-products from oat straw treated with increasing addition rates of ENZ (µg ENZ/g DM) applied at two different times (direct addition just before fermentation or 72 h pre-incubation)

	Control	Direct application			Pre-incubation			SEM	*P*-value			
	0	60	120	240	60	120	240		control vs ENZ[1]	time (T)[2]	rate (R)[3]	T × R
Gas production and kinetics (ml/g DM)												
Gas2[4]	20.8^{b}	23.4^{ab}	22.8^{ab}	24.3^{a}	23.6^{ab}	23.9^{ab}	24.3^{a}	2.4	0.003	0.512	0.389	0.716
Gas4	38.2^{b}	44.4^{b}	47.8^{ab}	47.5^{ab}	48.0^{ab}	49.3^{ab}	56.9^{a}	4.3	< 0.001	0.019	0.055	0.225
Gas6	57.6^{b}	68.4^{ab}	68.2^{ab}	75.1^{ab}	71.0^{ab}	73.9^{ab}	77.8^{a}	7.0	0.004	0.274	0.231	0.905
Gas8	73.6^{b}	89.0^{ab}	91.5^{ab}	90.0^{ab}	90.8^{ab}	94.8^{ab}	99.4^{a}	9.0	0.004	0.261	0.629	0.731
Gas10	88.2^{b}	106.3^{ab}	112.7^{ab}	104.2^{ab}	112.5^{ab}	116.5^{ab}	117.4^{a}	11.0	0.003	0.136	0.677	0.724
Gas24	109.5^{c}	132.7^{bc}	143.9^{ab}	150.0^{ab}	135.4^{bc}	156.9^{ab}	173.9^{a}	13.7	< 0.001	0.030	0.003	0.317
Gas48	162.0^{c}	186.6^{bc}	196.6^{abc}	213.3^{ab}	182.7^{bc}	200.6^{abc}	238.2^{a}	15.7	0.002	0.310	0.003	0.333
A	158.0^{c}	182.0^{bc}	194.2^{bc}	216.2^{ab}	175.8^{bc}	198.6^{abc}	244.8^{a}	14.3	0.001	0.262	< 0.001	0.198
c (per h)	0.071^{bc}	0.076^{abc}	0.075^{abc}	0.062^{c}	0.089^{a}	0.081^{ab}	0.061^{c}	0.003	0.426	0.042	< 0.001	0.135
L (h)	0.00^{b}	0.00^{b}	0.07^{ab}	0.00^{b}	0.17^{ab}	0.28^{a}	0.00^{b}	0.05	0.127	0.007	0.012	0.107
Degradability (%)												
DM	46.3^{ab}	55.0^{ab}	56.4^{ab}	61.4^{a}	44.7^{b}	43.4^{b}	45.0^{b}	3.1	0.184	< 0.001	0.498	0.632
NDF	36.9	33.6	35.2	38.9	28.0	26.5	28.7	3.3	0.172	0.009	0.590	0.782
ADF	26.8	24.7	26.3	30.0	21.2	20.1	22.1	2.4	0.305	0.008	0.364	0.639
Fermentation parameters												
pH	6.84^{ab}	6.85^{ab}	6.80^{abc}	6.67^{c}	6.90^{a}	6.84^{ab}	6.71^{bc}	0.03	0.187	0.088	< 0.001	0.970
NH_3-N (mg/dl)	10.3	15.3	20.3	13.1	14.0	19.8	13.6	4.1	0.221	0.904	0.260	0.978
Total VFA (mmol/l)	44.9	58.1	67.9	51.5	39.1	61.2	50.2	9.6	0.367	0.290	0.285	0.653
A (mmol/l)	30.9	37.8	44.7	34.5	27.7	32.0	32.6	6.4	0.573	0.137	0.650	0.687
% of VFA	68.8	66.0	66.5	67.7	71.6	69.1	65.6	2.1	0.631	0.246	0.585	0.203
P (mmol/l)	9.5	12.9	13.9	9.9	6.8	9.2	11.0	2.6	0.693	0.144	0.800	0.354
% of VFA	21.1	21.8	20.2	19.0	17.0	19.2	21.4	1.1	0.249	0.211	0.735	0.015
Butyrate (mmol/l)	4.5	7.3	9.3	7.1	4.7	5.8	6.6	1.9	0.278	0.160	0.716	0.713
% of VFA	10.0	12.2	13.3	13.3	11.4	11.8	13.0	1.2	0.072	0.361	0.529	0.884
A : P ratio	3.26	3.07	3.31	3.56	4.24	3.63	3.11	0.27	0.444	0.135	0.508	0.029
CO_2 (ml/100 ml gas)	24.6	33.1	39.8	30.4	22.5	26.9	28.9	6.6	0.436	0.142	0.698	0.665
CH_4 (ml/100 ml gas)	15.3	19.4	23.5	18.3	14.5	16.6	16.8	3.5	0.461	0.141	0.647	0.740

ENZ = xylanase enzyme, DM = dry mater, *A* = asymptotic gas production, *c* = rate of gas production, *L* = initial delay before gas production beginning, VFA = volatile fatty acids, A = acetate, P = propionate, CO_2 = carbon dioxide, CH_4 = methane, NDF = neutral detergent fibre, ADF = acid detergent fibre, SEM = standard error of the mean

[1]treatment vs control contrasts comparing all treatments with enzyme additive vs control (no enzyme additive)

[2]direct application vs pre-incubation

[3]60, 120 or 240 µg ENZ/g DM

[4]gas production at *t* hours of incubation (e.g. Gas2 = gas at 2 h of incubation etc.)

[a–c]means in the same row with different superscripts are significantly different ($P < 0.05$)

at medium and high doses. The addition of ENZ resulted in increased ($P < 0.001$) GP volumes at all incubation times as well as asymptotic GP (average asymptotic GP was 165 and 259 ml/g DM for control and ENZ treatments, respectively). After pre-incubation for 72 h, increasing doses of ENZ (from 60 to 240 µg/g) decreased pH ($P < 0.001$), increased GP volume after 24 ($P = 0.003$) and 48 h

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Table 3. *In vitro* rumen gas production kinetics, degradability, and rumen fermentation end-products from sugarcane bagasse treated with increasing addition rates of ENZ (μg ENZ/g DM) applied at two different times (direct addition just before fermentation or 72 h pre-incubation)

	Control	Direct application			Pre-incubation			SEM	*P*-value			
	0	60	120	240	60	120	240		control vs. ENZ[1]	time (T)[2]	rate (R)[3]	T × R
Gas production and kinetics (ml/g DM)												
Gas2[4]	17.5^{c}	30.1^{b}	30.1^{b}	28.8^{b}	33.9^{ab}	37.2^{a}	37.4^{a}	3.2	< 0.001	< 0.001	0.406	0. 164
Gas4	38.8^{c}	66.8^{ab}	62.9^{b}	64.7^{ab}	69.1^{ab}	72.8^{a}	73.0^{a}	6.2	< 0.001	0.001	0.876	0.189
Gas6	57.5^{b}	102.0^{a}	97.2^{a}	100.8^{a}	105.5^{a}	106.3^{a}	107.8^{a}	8.8	< 0.001	0.013	0.642	0.618
Gas8	77.0^{b}	134.1^{a}	128.9^{a}	131.5^{a}	139.7^{a}	135.8^{a}	140.3^{a}	10.0	< 0.001	0.012	0.313	0.870
Gas10	93.3^{b}	155.0^{a}	154.4^{a}	161.4^{a}	169.5^{a}	156.6^{a}	163.2^{a}	11.8	< 0.001	0.064	0.153	0.201
Gas24	126.6^{c}	201.2^{b}	208.9^{b}	216.7^{ab}	210.9^{b}	213.8^{ab}	234.6^{a}	14.8	< 0.001	0.015	0.003	0.419
Gas48	165.3^{c}	249.3^{b}	261.0^{ab}	261.3^{ab}	253.9^{b}	263.1^{ab}	289.9^{a}	16.7	< 0.001	0.049	0.010	0.125
A	164.8^{c}	240.9^{b}	257.1^{ab}	257.6^{ab}	246.1^{b}	259.3^{ab}	290.8^{a}	17.1	< 0.001	0.034	0.003	0.086
c (per h)	0.078^{b}	0.103^{ab}	0.088^{ab}	0.098^{ab}	0.112^{a}	0.089^{ab}	0.080^{b}	0.006	0.013	0.550	0.007	0.072
L (h)	0.33^{ab}	0.63a^{b}	0.54^{ab}	0.79^{a}	0.74^{a}	0.14^{b}	0.17^{b}	0.01	0.167	0.004	0.018	0.012
Degradability (%)												
DM	51.4	57.2	58.5	59.5	52.7	54.2	54.9	2.1	0.050	0.019	0.570	0.997
NDF	28.1	30.3	31.3	31.1	28.7	27.9	28.5	1.2	0.267	0.024	0.973	0.753
ADF	20.4	21.8	22.6	22.8	20.9	20.5	20.9	0.8	0.231	0.034	0.843	0.774
Fermentation parameters												
pH	6.77^{a}	6.72^{ab}	6.65^{bcd}	6.59^{cd}	6.69^{abc}	6.64^{bcd}	6.55^{d}	0.03	< 0.001	0.199	< 0.001	0.825
NH_3-N (mg/dl)	18.5	11.4	12.0	20.4	14.0	19.1	20.9	3.8	0.590	0.286	0.138	0.680
Total VFA (mmol/l)	60.5	31.1	51.8	71.5	44.5	61.8	67.8	8.5	0.540	0.364	0.007	0.579
A (mmol/l)	39.8	20.5	34.6	44.4	30.9	40.1	44.1	5.6	0.510	0.274	0.016	0.633
% of VFA	65.8	67.6	67.2	61.9	70.0	65.0	64.4	1.7	0.913	0.530	0.019	0.319
P (mmol/l)	13.7	6.8	11.2	16.3	9.2	13.6	14.5	1.8	0.380	0.512	0.004	0.437
% of VFA	22.7	21.7	21.4	23.0	20.4	22.0	22.0	1.2	0.475	0.566	0.489	0.489
Butyrate (mmol/l)	6.9	3.8	6.0	10.8	4.3	8.1	9.2	1.3	0.952	0.761	0.002	0.414
% of VFA	11.5^{ab}	10.7^{b}	11.3^{ab}	15.1^{a}	9.6^{b}	13.0^{ab}	13.6^{ab}	0.8	0.409	0.626	< 0.001	0.150
A : P ratio	2.90	3.13	3.15	2.71	3.50	2.95	3.01	0.24	0.512	0.434	0.206	0.451
CO_2 (ml/100 ml gas)	33.8	17.6	29.1	42.5	24.3	35.5	39.5	5.1	0.678	0.439	0.006	0.577
CH_4 (ml/100 ml gas)	20.0	10.4	17.5	23.5	15.3	20.7	23.0	3.0	0.644	0.328	0.013	0.666

ENZ = xylanase enzyme, DM = dry mater, *A* = asymptotic gas production, *c* = rate of gas production, *L* = initial delay before gas production beginning, VFA = volatile fatty acids, A = acetate, P = propionate, CO_2 = carbon dioxide, CH_4 = methane, NDF = neutral detergent fibre, ADF = acid detergent fibre, SEM = standard error of the mean

[1]treatment vs control contrasts comparing all treatments with enzyme additive vs control (no enzyme additive)

[2]direct application vs pre-incubation

[3]60, 120 or 240 μg ENZ/g DM

[4]gas production at *t* hours of incubation (e.g. Gas2 = gas at 2 h of incubation etc.)

[a–d]means in the same row with different superscripts are significantly different ($P < 0.05$)

($P = 0.010$) of incubation as well as asymptotic GP (from 246 to 291 ml/g DM, $P = 0.003$), and decreased the rate of fermentation (from 0.112 to 0.080 per h, $P = 0.007$). Increasing ENZ application rate increased the concentration of fermentation end-products (total VFA ($P = 0.007$), acetic acid ($P = 0.016$), propionic acid ($P = 0.004$), butyric acid ($P = 0.002$), CO_2 ($P = 0.006$), and CH_4 ($P = 0.013$)).

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DISCUSSION

The GP technique is a simple, powerful, and sensitive screening tool to evaluate substrate fermentation and test efficacy of feed additives, and hence applied for the current study (Diaz et al. 2013).

The chemical composition of tested plants differed to some extent from that reported in other studies. Individual feed ingredients may vary widely in composition because of variation in cultivars and genotype of the crops, growing environments including climate, the soil and agronomic practice, harvest conditions, post harvesting treatments, and morphological (stalk, stem, leaf, husk, chaff) fractions of the samples (Welch 1995).

Gas production. Differences in fibre content, composition, and structure are likely to be related to the variability specificity and activity of ENZ on different roughage sources (Elghandour et al. 2015). Elghandour et al. (2015) reported that effects of an exogenous enzyme on ruminal fermentation appear to be substrate-dependent.

Compared to untreated roughages, the ENZ treatments showed greater GP volume, suggesting a stimulation of ruminal fermentation, as GP is closely correlated with the amount of organic matter fermented (Diaz et al. 2013). Our findings suggest that the ENZ could degrade some cell wall constituents or facilitate the access of ruminal microorganisms. The application of ENZ may stimulate fibrolytic and non-fibrolytic bacteria in the rumen due to the release of carbohydrates that are readily utilized by the bacteria (Nsereko et al. 2002). Mao et al. (2013) found that the addition of cellulase and xylanase increased the numbers of total bacteria and *Fibrobacter succinogenes* in the incubation medium *in vitro* and thereby improved the substrate fermentation.

Giraldo et al. (2007) showed that treatment of poor quality forage or forage-based diets with exogenous enzymes stimulated the initial phases of microbial colonization in the rumen, and this is recognized as one mode of action of enzymatic products (Elghandour et al. 2015). The addition of fibrolytic enzymes facilitates the access of microorganisms to feed components enabling a faster microbial growth. Gas production during the first hours of ruminal fermentation was increased in the present study, supporting the hypothesis that a suitable ENZ level could improve the fermentation of feeds during the initial stages of forage digestion (Elghandour et al. 2013).

Diaz et al. (2013) observed that the addition of a fibrolytic enzyme increased the fermentation rate of maize stover, although asymptotic GP was unaffected. The application of ENZ may be a mean of overcoming the problem of low intakes and slow digestion rates of low quality forages (Salem et al. 2015). Elghandour et al. (2013) concluded that it is necessary to determine the optimal application rate of ENZ preparation for individual feeds.

The pre-incubation of substrates with ENZ for 72 h improved GP compared to the direct addition of enzyme just prior to fermentation. Mao et al. (2013) explained a possible mode of action. The pre-treatment of feed with fibrolytic enzymes could release reducing sugars (Nsereko et al. 2002) and other products of hydrolysis, which, in turn, can enhance the ruminal microbial colonization (Giraldo et al. 2007).

In a study by Wang et al. (2012), the application of enzymes to barley straw increased GP volume at short periods of fermentation both with an immediate application (prior to incubation) and with a previous hydrolysis by incubation with the enzyme. The addition of exogenous enzymes to feed directly before incubation with a ruminal inoculum has also been reported to improve ruminal fermentation by Khattab et al. (2011).

Substrate degradability. Elghandour et al. (2013) observed that the amount of enzyme added to different fibrous feeds has a positive effect on *in vitro* nutrient digestibility. Lara-Bueno et al. (2013) reported a greater disappearance *in situ* for DM and NDF after 24 and 48 h of incubation of oat straw treated with a fibrolytic enzyme. Enzyme treated roughages had greater nutrient digestibility compared to untreated feeds. The greater DM digestion may be related to enhanced attachment and colonization to the plant cell wall material by rumen microorganisms (Nsereko et al. 2002). A synergism interaction between ruminal enzymes and the exogenous enzyme applied has been considered as the most likely mode of action. Diaz et al. (2013) stated that the addition of xylanase (the same preparation used in the current study) improved *in vitro* DM degradability (DMD) of roughage with increasing the amount of enzyme added.

The direct addition of ENZ just prior to incubation has resulted in improved nutrient digestibility to a greater extent than a 72-h pre-incubation.

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With the direct application of ENZ, the products of cell-wall hydrolysis can be instantly fermented by ruminal bacteria. Wang et al. (2012) stated that the application of an exogenous enzyme to barley straw immediately prior to incubation showed greater DMD at 4, 12, and 48 h.

Ruminal fermentation end-products. One of the main mechanisms of action of exogenous enzymes application is the shifting and altering of ruminal fermentation (Khattab et al. 2011). Togtokhbayar et al. (2015) showed that with the addition of an exogenous enzyme (xylanase; the same preparation used in the current study) the molar proportions of VFA were changed, and that this shift was dose-dependent.

Mean ruminal fluid pH was lowered with ENZ treatment vs the control diets, in the case of all the three feeds. This may be due to greater enzymatic hydrolysis of feeds into readily fermentable substrates that depress pH when fermented. Elghandour et al. (2013) recorded reduced ruminal pH values when four fibrous substrates were incubated with different levels of an exogenous enzyme. On the other hand, Diaz et al. (2013) stated that enzyme treatment of maize stover had no effects on final pH values *in vitro*.

The enzyme addition to the three substrates had no effects on VFA production. Mao et al. (2013) found that xylanase addition did not affect the molar proportions of VFA, except for butyrate. However, Tang et al. (2013) observed increased concentrations of VFA resulting from the enzymatic treatments of maize stover, rice straw, and wheat straw. The inconsistent results of enzyme use on the changes of VFA composition may be related to the activities of the selected enzyme product, the type of forage used as substrate, the diet of donor animal, and even the species of the donor animal used (Mao et al. 2013; Tang et al. 2013).

The increase in acetate and butyrate concentrations when fibrous feeds were incubated with exogenous enzymes could be associated with an improved digestion of structural carbohydrates. In the present case, increasing rates of enzyme application significantly increased VFA production with sugarcane bagasse. Likewise, Diaz et al. (2013) have also observed increased total VFA production, molar proportions of acetate, propionate and butyrate, and acetate : propionate ratio with increasing enzyme doses. Further, a shift in VFA profile was evident in the present study for some levels of enzyme, although the effects varied with the roughage incubated. Lara-Bueno et al. (2013) found that ammonia-N and VFA concentrations were not affected in sheep fed an oat straw based ration treated with an exogenous fibrolytic enzyme.

Only a few studies have investigated the effects of exogenous enzymes on ruminal CH_4 and CO_2 outputs, and the results are inconsistent (Mao et al. 2013). Methane production from ruminants fed fibrous diets is greater than when fed better quality forages (Tang et al. 2013). The methanogenic *Archaea* can utilize H_2 produced from the ruminal degradation of organic matter, and use it for CH_4 production. Goel and Makkar (2012) suggested that CH_4 production is associated with the increase in fermented and digested organic matter. The application of enzyme to maize stover and oat straw did not affect the CH_4 volume significantly, but a change was observed with sugarcane bagasse. A probable reason could be the residual soluble carbohydrates (sucrose) present in sugarcane bagasse that would provide energy for microbial growth. Mao et al. (2013) noted that with the addition of cellulase and xylanase the production of CH_4 was increased. Shojaeian and Thakur (2007) have shown that supplementing fibre degrading enzymes in livestock diets may improve feed utilization by enhancing fibre degradation and reducing CH_4 production per unit of animal product.

CONCLUSION

The current study showed that the supplemental exogenous enzyme preparation increased gas production when different roughages were incubated *in vitro*, suggesting that the enzyme (xylanase) can enhance rumen fermentation of fibrous substrates. This response, however, is influenced by both the rate and the time of application of the enzymatic additive. Hence, optimal conditions of application need to be established for an effective treatment of low quality roughage with exogenous enzymes to upgrade their ruminal degradation.

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REFERENCES

AOAC (1997): Official Methods of Analysis. 16th Ed. Arlington, USA.

Bernfeld P. (1955): Amylases, alpha and beta. Methods in Enzymology, 1, 149–158.

Diaz A., Carro M.D., Saro C., Mateos I., Odongo E., Ranilla M.J. (2013): In vitro evaluation of commercial fibrolytic enzymes for improving the nutritive value of low-quality forages. Animal Nutrition and Feed Technology, 13, 461–476.

Elghandour M.M.Y., Salem A.Z.M., Gonzalez-Ronquillo M., Brquez J.L., Gado H.M., Odongo N.E., Penuelas C.G. (2013): Effects of exogenous enzymes on in vitro gas production kinetics and ruminal fermentation of four fibrous feeds. Animal Feed Science and Technology, 179, 46–53.

Elghandour M.M.Y., Kholif A.E., Marquez-Molina O., Vazquez-Armijo J.F., Puniya A.K., Salem A.Z.M. (2015): Influence of individual or mixed cellulase and xylanase mixtures on in vitro rumen gas production kinetics of total mixed rations with different maize silage and concentrate ratios. Turkish Journal of Veterinary and Animal Sciences, 39, 435–442.

France J., Dijkstra J., Dhanoa M.S., Lopez S., Bannink A. (2000): Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed in vitro: derivation of models and other mathematical considerations. British Journal of Nutrition, 83, 143–150.

Giraldo L.A., Tejido M.L., Ranilla M.J., Carro M.D. (2007): Effects of exogenous cellulase supplementation on microbial growth and ruminal fermentation of a high-forage diet in Rusitec fermenters. Journal of Animal Science, 85, 1962–1970.

Goel G., Makkar H.P.S. (2012): Methane mitigation from ruminants using tannins and saponins, a status review. Tropical Animal Health and Production, 44, 729–739.

Goering M.K., Van Soest P.J. (1970): Forage Fibre Analysis (Apparatus, Reagents, Procedures and Some Applications). Agriculture Handbook No. 379. Agricultural Research Service, USDA, Washington, USA.

Karunanandaa K., Varga G.A., Akin D.E., Rigsby L.L., Royse D.J. (1995): Botanical fractions of rice straw colonized by white-rot fungi: changes in chemical composition and structure. Animal Feed Science and Technology, 55, 179–199.

Khattab H.M., Gado H.M., Kholif A.E., Mansour A.M., Kholif A.M. (2011): The potential of feeding goats sun dried rumen contents with or without bacterial inoculums as replacement for berseem clover and the effects on milk production and animal health. International Journal of Dairy Science, 6, 267–277.

Khattab H.M., Gado H.M., Salem A.Z.M., Camacho L.M., El-Sayed M.M., Kholif A.M., Elshewy A.A., Kholif A.E. (2013): Chemical composition and in vitro digestibility of Pleurotus ostreatus spent rice straw. Animal Nutrition and Feed Technology, 13, 507–516.

Kholif A.E., Khattab H.M., El-Shewy A.A., Salem A.Z.M., Kholif A.M., El-Sayed M.M., Gado H.M., Mariezcurrena M.D. (2014): Nutrient digestibility, ruminal fermentation activities, serum parameters and milk production and composition of lactating goats fed diets containing rice straw treated with Pleurotus ostreatus. Asian-Australasian Journal of Animal Sciences, 27, 357–364.

Lara-Bueno A., Mendoza Martinez G.D., Hernandez Garcia P.A., Martinez Garcia J.A., Plata Perez F.X. (2013): Evaluation of high doses of exogenous fibrolytic enzymes in lambs fed an oat straw based ration. Animal Nutrition and Feed Technology, 13, 355–362.

Lin Y., Gary E., Robert E. (1969): Action of proteolytic enzymes on N,N-dimethyl proteins. Basis for a microassay for proteolytic enzymes. Journal of Biological Chemistry, 244, 789–793.

Mao H.L., Wu C.H., Wang J.K., Liu J.X. (2013): Synergistic effect of cellulase and xylanase on in vitro rumen fermentation and microbial population with rice straw as substrate. Animal Nutrition and Feed Technology, 13, 477–487.

Marini R.P. (2003): Approaches to analyzing experiments with factorial arrangements of treatments plus other treatments. HortScience, 38, 117–120.

McCullough H. (1967): The determination of ammonia in whole blood by direct colorimetric method. Clinica Chimica Acta, 17, 297–304.

Morsy T.A., Kholif A.E., Kholif S.M., Kholif A.M., Sun X., Salem A.Z. (2016): Effects of two enzyme feed additives on digestion and milk production in lactating Egyptian buffaloes. Annals of Animal Science, 16, 209–222.

Nsereko V.L., Beauchemin K.A., Morgavi D.P., Rode L.M., Furtado A.F., McAllister T.A., Iwaasa A.D., Yang W.Z., Wang Y. (2002): Effect of a fibrolytic enzyme preparation from Trichoderma longibrachiatum on the rumen microbial population of dairy cows. Canadian Journal of Microbiology, 48, 14–20.

Robyt J.F., Whelan W.J. (1972): Reducing value methods for maltodextrins: 1. Chain-length dependence of alkaline 3,5-dinitrosalicylate and chain length independence of alkaline copper. Analytical Biochemistry, 45, 510–516.

Rojo R., Kholif A.E., Salem A.Z.M., Elghandour M.M.Y., Odongo N.E., Montes de Oca R., Rivero N., Alonso M.U. (2015): Influence of cellulase addition to dairy goat diets on digestion and fermentation, milk production and fatty acid content. Journal of Agricultural Science, Cambridge, 153, 1514–1523.

doi: 10.17221/52/2015-CJAS

Salem A.Z.M., Alsersy H., Camacho L.M., El-Adawy M.M., Elghandour M.M.Y., Kholif A.E., Rivero N., Alonso M.U., Zaragoza A. (2015): Feed intake, nutrient digestibility, nitrogen utilization, and ruminal fermentation activities in sheep fed Atriplex halimus ensiled with three developed enzyme cocktails. Czech Journal of Animal Science, 60, 185–194.

Shojaeian K., Thakur S.S. (2007): Effect of exogenous fibrolytic enzymes supplementation to substrates containing different roughage : concentrate ratios on in vitro rumen fermentation, DM and NDF degradability. Indian Journal of Dairy Science, 60, 94–101.

Tang S.X., Zou Y., Wang M., Salem A.Z.M., Odongo N.E., Zhou C.S., Han X.F., Tan Z.L., Zhang M., Fu Y.F., Huang S.Q., He Z.X., Kang J.H. (2013): Effects of exogenous cellulase source on in vitro fermentation characteristics and methane production of crop straws and grasses. Animal Nutrition and Feed Technology, 13, 489–505.

Theodorou M.K., Williams B.A., Dhanoa M.S., McAllan A.B., France J. (1994): A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Animal Feed Science and Technology, 48, 185–197.

Togtokhbayar N., Cerrillo S.M.A., Rodriguez G.B., Elghandour M.M.Y., Salem A.Z.M., Urankhaich C., Jigjidpurev S., Odongo N.E., Kholif A.E. (2015): Effect of exogenous xylanase on rumen in vitro gas production and degradability of wheat straw. Animal Science Journal, 86, 765–771.

Wang Y., Ramirez-Bribiesca J.E., Yanke L.J., Tsang A., McAllister T.A. (2012): Effect of exogenous fibrolytic enzyme application on the microbial attachment and digestion of barley straw in vitro. Asian-Australasian Journal of Animal Sciences, 25, 66–74.

Welch R.W. (1995): The chemical composition of oats. In: Welch R.W. (ed.): The Oat Crop: Production and Utilization. Chapman and Hall, London, UK, 279–320.

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