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Influence of individual or mixed cellulase and xylanase mixture on in vitro rumen gas production kinetics of total mixed rations with different maize silage and concentrate ratios

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Abstract: This study assessed the effects of cellulase, xylanase, and their mixture (1:1, v/v) on in vitro rumen fermentation of five mixed rations with different maize silage (F) to concentrate (C) ratios (0F:100C, 25F:75C, 50F:50C, 75F:25C, 100F:0C). Samples were incubated using rumen inoculum from Brown Swiss cows fed ad libitum a total mixed ration of concentrate and alfalfa hay (1:1). Gas production (GP) was recorded after 2, 4, 6, 8, 10, 12, 24, 36, 48, and 72 h of incubation. Interaction effects ($P < 0.0001$) were observed between type of ration and enzyme for discrete lag time prior to GP. Ration type affected asymptotic GP (linear and quadratic, $P < 0.0001$) and in vitro GP at 24 h onward. Moreover, rations affected pH (linear and quadratic, $P < 0.0001$), dry matter degradability (quadratic, $P = 0.05$), metabolizable energy (linear, $P = 0.038$), short-chain fatty acids (linear, $P = 0.0005$), gas yield from truly digested dry matter at 24 h (linear, $P = 0.0026$), and microbial crude protein yield (linear, $P = 0.0264$). The most effective rations for in vitro GP during different times differed between different measured parameters. Enzyme administration was more effective when the ratio of maize silage was increased.

Key words: Cellulase, fibrolytic enzymes, maize silage:concentrate ratio, xylanase

1. Introduction

Ruminants can digest plant cell walls in fibrous feeds due to the unique enzymes produced by the rumen microflora. Stimulating the rumen microorganism activities using cell wall-degrading enzymes or by using exogenous fiber-degrading enzymes can improve the degradation of fibrous feeds (1–3). Fibrous feeds are characterized by their high cellulose and hemicellulose content that can create an insoluble complex network of cellulose, hemicellulose, and lignin, thereby causing reduced digestibility and inefficient utilization of forages. Moreover, the increased content of lignin in the fibrous feeds should reduce nutrient digestibility. Exogenous fibrolytic enzymes can be used to alter insoluble complexes of forage cell wall constituents (4) and also to create stable enzyme feed complexes (5). Exogenous enzymes treatments affect the forage fiber structure, which could stimulate microbial colonization (3). Exogenous fibrolytic enzymes also accelerate the rate of digestion by enhancing attachment and/or improve access of rumen microorganisms to the cell wall matrix (6). They can also work synergistically with rumen microbial enzymes to increase the digestion and nutritive value of fibrous rations (7).

Cellulase, xylanase, and β-D glucanase enzymes are specific for the breaking of internal β-1,4 linkages of cellulose, hemicellulose (xylan), and glucans to release soluble sugars and facilitate the growth of microbes, thereby increasing the in vitro dry matter (DM) digestibility (8). Morgavi et al. (7) demonstrated the synergism between exogenous and endogenous rumen enzymes such that the net combined hydrolytic effect in the rumen was much higher than that estimated from individual enzyme activities. Exogenous enzymes may be applied during ensiling or directly fed to animals during feeding (1). Enzyme application during ensiling gives an economical benefit to farmers by increasing the feed intake (i.e. palatability) and digestion rate (1,9). Direct addition of enzymes to animal feeds just before feeding is much easier and more applicable in terms of agronomic practices. A synergism between exogenous enzymes and endogenous

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enzymes in the ruminal fluid results in increased numbers of nonfibrolytic and fibrolytic bacteria causing increased feed digestibility and utilization (10).

Roughage to concentrate ratio is regarded as one of the most important limiting factors for efficient utilization of dietary nutrients (11). Balancing the roughage to concentrate ratio should improve the activity of the microbial population of the host animal more than high concentrate or high roughage rations (12).

It was hypothesized that the use of exogenous enzymes would affect and improve in vitro rumen fermentation kinetics of rations with different roughage to concentrate ratios. Therefore, the aim of this study was to assess the effects of maize silage:concentrate ratio and cellulase and/or xylanase supplementation on in vitro rumen fermentation.

2. Materials and methods

2.1. Substrate and treatments

Three samples of each total mixed ration of different maize silage (F) to concentrate (C) ratios (0F:100C, 25F:75C, 50F:50C, 75F:25C, 100F:0C) were prepared using ingredients (Table 1) prepared in the State of Mexico in Mexico. Samples of rations 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 composition and in vitro gas production (GP) profile. Five different types of rations were used in the absence (control) or presence of 1 µL/g DM of cellulase (C, 0.033 unit/g DM), xylanase (X, 0.038 unit/g DM), or a mixture of C and X (1:1, v/v) (XC) as exogenous fibrolytic commercial enzymes (Dyadic PLUS, Dyadic International, Inc., Jupiter, FL, USA) in liquid form.

2.2. In vitro incubations

Effects of enzymes on rumen fermentation of forages are widely determined using the in vitro GP technique as described by Salem et al. (13). Briefly, rumen inoculum was collected from Brown Swiss cows (450 kg body weight) fitted with permanent rumen cannula and fed ad libitum a total mixed ration of 1:1 commercial concentrate and alfalfa hay formulated to meet all of their nutrient requirements (14) with free access to water.

Rumen contents was obtained before the morning feeding, mixed and strained through four layers of cheesecloth into a flask with O_2-free headspace. Samples of different rations (1 g) were weighed into 120-mL serum bottles followed with the addition of 10 mL of particle-free rumen fluid and 40 mL of the buffer solution according to Goering and Van Soest (15), with no trypticase added. Exogenous fibrolytic enzymes of C, X, or CX were added to bottle contents (i.e. substrate and buffered rumen fluid) immediately before closing. Once all the bottles were filled, they were immediately closed with rubber stoppers, shaken, and placed in an incubator at 39 °C.

A total of 180 bottles (three bottles for each ration in addition to three bottles for each enzyme in three different runs with three bottles as blanks (rumen fluid only)) were incubated for 72 h. The technique of Theodorou et al. (16) was used for measuring the pressure of gas produced at 2, 4, 6, 8, 10, 12, 24, 36, 48, and 72 h of incubation employing a pressure transducer to measure gas from incubations in 120-mL gas-tight culture bottles. Gas accumulated in the headspace of the bottle as the fermentation proceeded was measured with a digital manometer (Extech Instruments, Waltham, MA, USA). After each gas pressure reading, and with a syringe needle, the produced gases were discarded; then bottles were gently shaken and returned to the water bath before losing temperature. The amounts of produced gases were calculated using gas pressure readings using some equations. At the end of incubation at 72 h bottles were uncapped, pH was measured using a pH meter (Conductronic pH15, Puebla, Mexico), and the contents of each bottle were filtered under vacuum through glass crucibles with a sintered filter to obtain the nonfermented residue for gravimetrical determination of degraded substrate. Fermentation residues were dried at 105 °C overnight to determine in vitro DM degradability (DMD), with loss in weight after drying being the measure of undegradable DM.

2.3. Chemical analyses

Samples of the each ration were analyzed for DM, ash, N, ether extract (EE), and acid detergent lignin (ADL) according to the AOAC (17). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to Van Soest et al. (18). Analyses of NDF, ADF, and ADL were carried out using an ANKOM 200 Fiber Analyzer Unit (ANKOM Technology Corp., Macedon, NY, USA). The NDF was assayed with the use of alpha amylase and sodium sulfite in the NDF. Both NDF and ADF are expressed without residual ash.

2.4. Calculations

As previously mentioned by Salem et al. (3,13), the kinetic parameters of GP were calculated by fitting results of GP (mL/g DM) in the NLIN option of SAS (19) according to France et al. (20) 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 (mL/h), and L (h) is the discrete lag time prior to initiation of GP.

Metabolizable energy (ME; MJ/kg DM) and in vitro organic matter (OM) digestibility (OMD, g/kg OM) were estimated according to Menke et al. (21) as:

ME = 2.20 + 0.136 GP (mL/0.5 g DM) + 0.057 crude protein (CP) (g/kg DM),

OMD = 148.8 + 8.89 GP + 4.5 CP (g/kg DM) + 0.651 ash (g/kg DM),
where GP is net GP in mL from 200 mg of dry sample after 24 h of incubation.

Gas yield (GP_{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:

Gas yield (GY_{24}) = (mL GP/g DM)/g DMD.

Short-chain fatty acid (SCFA) concentrations were calculated according to Getachew et al. (22) as:

SCFA (mmol/200 mg DM) = 0.0222 GP – 0.00425
where GP is the 24-h net GP (mL/200 mg DM).

Microbial biomass production (MCP) was calculated (22) as:

MCP (mg/g DM) = mg DMD – (mL gas × 2.2 mg/mL), where 2.2 mg/mL is a stoichiometric factor that expresses mg of C, H, and O required for the SCFA gas associated with production of 1 mL of gas (22).

2.5. Statistical analyses

Data of each of the three runs within the same sample of each of the three individual samples of each ration were averaged prior to statistical analysis. Mean values of each individual sample were used as the experimental unit. Results of in vitro GP and rumen fermentation parameters were analyzed as a factorial experiment using the PROC GLM option of SAS (19) as:

$Y_{ijk} = \mu + D_i + EZ_j + (D \times EZ)_{ij} + \varepsilon_{ijk}$,
where Y_{ijk} is every observation of the *i*th ration (D_i) when incubated with the *j*th EZ types (EZ_j: type of enzyme preparation), μ is the general mean, D_i (i = 1–5) is the total mixed rations of different maize silage to concentrate ratios, EZ_j is the enzyme type effect (j = 1–4), $(D \times EZ)_{ij}$ is the interaction between rations and enzyme type, and ε_{ijk} is the random error.

3. Results

The content of OM, NDF, ADF, and ADL increased (P < 0.05) with increasing maize silage ratio of the ration. However, the ration of 0F:100C had the highest content of CP (Table 1).

An interaction effect (P < 0.0001) was observed between ration type and enzyme type for the *L* value. However, no interactions were observed for the other measured parameters for in vitro rumen gas kinetics. Ration type affected the asymptotic GP (linear and quadratic effects, P < 0.0001) and the *L* value (linear effect, P = 0.003). No significant effects (P > 0.05) were seen on *b* (mL/g DM), *c* (fraction per h), and in vitro GP during the different incubation times due to addition of cellulose, xylanase, or their mixture. However, increasing the ratios of maize silage to concentrate caused a lowered in vitro GP volume when no enzymes were added (Table 2). In vitro GP values after 36, 48, and 72 h of incubation were affected linearly (P < 0.0001) with ration types. Different enzyme types also affected GP_{24} (linear effect, P = 0.0005) and GP_{36}, GP_{48}, and GP_{72} (linear and quadratic effects, P < 0.0001) (Table 2). The effect of individual or mixed enzymes varied between different F:C ratios for different measured parameters. However, xylanase had numerically higher values of GP with higher rates of production than cellulase for all different F:C ratios with the exception of 25F:75C (Table 2).

No interactions (P > 0.05) were observed for any measured parameters of the in vitro rumen fermentation profile. However, ration type affected pH (linear and quadratic effects, P < 0.0001), DMD (quadratic effect, P = 0.05), ME (linear effect, P = 0.0381), SCFA (linear effect, P = 0.0005), P = GY_{24} (linear effect, P = 0.003), and MCP (linear effect, P = 0.026; quadratic effect, P = 0.0409). Enzyme type tended to affect ME (P = 0.073), OMD (P =

Table 1. Chemical composition[1] (g/kg DM) of the five mixed rations of different maize silage and concentrate ratios (adapted from Elghandour et al. (11)).

Ration	OM	CP	NDF	ADF	ADL
0F:100C	927.4[b]	172.0[a]	145.1[e]	70.3[e]	8.1[e]
25F:75C	932.6[ab]	133.2[b]	217.7[d]	88.2[d]	10.3[d]
50F:50C	939.6[a]	138.7[b]	302.2[c]	127.0[c]	12.6[c]
75F:25C	943.7[a]	92.0[c]	371.7[b]	149.0[b]	15.0[b]
100F:0C	944.2[a]	85.0[c]	499.4[a]	229.3[a]	20.4[a]
SEM	14.82	9.34	12.56	10.98	1.42

[1]OM: Organic matter, CP: crude protein, ADF: acid detergent fiber, NDF: neutral detergent fiber, ADL: acid detergent lignin, SEM: standard error of the mean.
[a,b,c,d,e]: Different superscripts following means within a column indicate differences at P < 0.05.

Table 2. In vitro rumen gas kinetics of five different mixed rations of maize silage and concentrate ratios as affected by cellulase (C, 0.033 unit/g DM) and xylanase (X, 0.038 unit/g DM) or their mixture (CX, 1:1, v/v).

Ration	Enzyme[1]	Gas production parameters[2]			In vitro gas production (mL/g DM)									
		b	*c*	*L*	Gas2	Gas4	Gas6	Gas8	Gas10	Gas12	Gas24	Gas36	Gas48	Gas72
0F:100C	0	146.7	0.083	1.75	22.57	41.66	57.79	71.44	82.98	92.74	126.72	139.25	143.89	146.27
	C	140.7	0.077	1.92	20.17	37.43	52.20	64.85	75.68	84.96	118.49	131.82	137.14	140.14
	X	145.6	0.073	1.93	19.78	36.88	51.65	64.42	75.45	84.98	120.36	135.09	141.22	144.84
	XC	152.1	0.072	1.40	20.28	37.83	53.02	66.17	77.56	87.42	124.35	140.09	146.85	151.07
	P-value	0.4114	0.3238	0.0650	0.4039	0.4186	0.4342	0.4502	0.4664	0.4824	0.5459	0.5276	0.4824	0.4315
25F:75C	0	145.0	0.079	1.98	21.45	39.74	55.33	68.62	79.96	89.62	124.04	137.28	142.37	145.09
	C	150.3	0.069	1.96	19.48	36.41	51.14	63.95	75.09	84.78	121.58	137.65	144.70	149.19
	X	161.7	0.080	1.76	25.51	46.85	64.73	79.73	92.32	102.92	139.47	153.02	158.22	161.09
	XC	144.9	0.075	2.04	20.09	37.36	52.23	65.02	76.03	85.52	120.37	134.69	140.62	144.14
	P-value	0.2818	0.5381	0.2845	0.4516	0.4457	0.4396	0.4331	0.4263	0.4192	0.3734	0.3324	0.3058	0.2859
50F:50C	0	271.3	0.031	2.32	16.52	32.03	46.59	60.26	73.10	85.15	143.50	183.50	210.90	242.70
	C	268.8	0.032	2.57	16.62	32.21	46.84	60.56	73.43	85.50	143.80	183.50	210.60	241.60
	X	264.4	0.031	2.26	15.98	30.99	45.10	58.35	70.10	82.40	139.20	178.20	205.00	236.20
	XC	270.4	0.034	2.47	18.23	35.21	51.01	65.74	79.45	92.22	152.50	192.10	218.20	247.00
	P-value	0.9026	0.6408	0.6149	0.6365	0.6391	0.6418	0.6444	0.6472	0.6502	0.6702	0.6951	0.7251	0.7914
75F:25C	0	193.5	0.038	1.69	14.10	27.26	39.40	50.66	61.10	70.77	115.62	144.05	162.09	180.80
	C	183.8	0.036	2.62	12.93	24.95	36.12	46.50	56.15	65.12	107.12	134.23	151.74	170.37
	X	185.9	0.369	2.43	60.85	75.34	83.56	90.55	96.99	102.99	131.35	150.01	162.28	175.66
	XC	186.0	0.036	2.29	12.87	24.84	35.99	46.36	56.02	65.01	107.29	134.79	152.68	171.89
	P-value	0.3742	0.4453	0.1404	0.4503	0.4571	0.4643	0.4713	0.4779	0.4841	0.5041	0.4749	0.3796	0.2192
100F:0C	0	316.5	0.026	6.69	16.00	31.18	45.60	59.29	72.29	84.63	146.62	192.02	225.29	267.51
	C	284.9	0.024	3.71	13.47	26.31	38.53	50.17	61.27	71.80	125.49	165.59	195.57	234.76
	X	299.4	0.032	3.46	18.27	35.39	51.43	66.47	80.58	93.80	157.57	201.12	230.97	265.78
	XC	320.5	0.025	3.32	15.49	30.22	44.25	57.60	70.30	82.39	143.59	189.06	222.85	266.60
	P-value	0.4924	0.0574	0.0007	0.0366	0.0381	0.0399	0.0419	0.0442	0.0468	0.0705	0.1112	0.1683	0.2946
Pooled LSD[3]		18.797	0.4679	21.939	23.537	22.784	21.864	21.009	20.227	16.801	15.23	14.892	15.686	28.858
Interactions														
Ration														
Linear		<0.0001	0.4082	0.0003	0.6177	0.4827	0.4356	0.4772	0.6155	0.8682	0.0005	<0.0001	<0.0001	<0.0001
Quadratic		<0.0001	0.6218	0.3197	0.6696	0.5265	0.4450	0.4239	0.4558	0.5414	0.1629	0.0001	<0.0001	<0.0001
Enzyme		0.3820	0.3797	0.0013	0.3093	0.2561	0.2112	0.1765	0.1500	0.1296	0.0736	0.0693	0.0902	0.1692
Ration × enzyme		0.6183	0.4890	<0.0001	0.5388	0.5845	0.6229	0.6490	0.6625	0.6648	0.5254	0.3554	0.3003	0.3722

[1]Activities of the exogenous fibrolytic enzymes were as follows:
For cellulase product it contained 30,000 to 36,000 units of cellulase/g and 7500 to 10,000 units of β-glucanase/g.
For xylanase product it contained 34,000 to 41,000 units of xylanase/g, from 12,000 to 15,000 units of β-glucanase/g, and 45,000 to 55,000 units of cellulose/g.
[2]*b* is the asymptotic gas production (mL/g DM); *c* is the rate of gas production (/h); *L* is the initial delay before gas production begins (h).
[3]LSD: Least significant difference.

0.074), SCFA (P = 0.074), and GY_{24} (P = 0.0729). No effects (P > 0.05) were observed on the measured parameters of the in vitro rumen fermentation profile with the exception of DMD (P = 0.042) with the ration 75F:25C, and GY_{24} (P = 0.002) and MCP (P = 0.001) for the ration 100F:0C (Table 3). Almost all measured parameters were higher with xylanase than with cellulase with the different F:C ratios.

Table 3. In vitro rumen fermentation profile[1] of five different mixed rations of maize silage and concentrate ratios as affected by cellulase (C, 0.033 unit/g DM) and xylanase (X, 0.038 unit/g DM) or their mixture (CX, 1:1, v/v).

Ration	Enzyme[2]	pH	DMD	ME	OMD	SCFA	GY_{24}	MCP
0F:100C	0	6.67	793.3	6.68	460.4	2.79	160.5	514.5
	C	6.70	823.4	6.46	445.8	2.61	144.0	562.7
	X	6.69	836.1	6.51	449.1	2.65	144.0	571.3
	XC	6.68	845.1	6.62	456.2	2.74	147.1	571.6
	P-value	0.3623	0.1977	0.5463	0.5459	0.5453	0.3724	0.2593
25F:75C	0	6.66	853.4	6.52	448.3	2.73	145.4	580.4
	C	6.65	845.5	6.45	443.9	2.68	143.8	578.0
	X	6.65	871.7	6.94	475.7	3.08	161.9	564.8
	XC	6.65	811.4	6.42	441.8	2.65	148.4	546.6
	P-value	0.6288	0.4158	0.3730	0.3735	0.3730	0.9098	0.7150
50F:50C	0	6.34	836.7	6.88	468.8	3.16	171.6	521.0
	C	6.35	829.4	6.89	469.3	3.17	173.3	513.2
	X	6.38	826.5	6.76	461.2	3.07	168.4	520.3
	XC	6.36	831.8	7.12	484.9	3.36	183.5	496.2
	P-value	0.2744	0.5673	0.6701	0.6702	0.6706	0.7248	0.7949
75F:25C	0	6.75	800.3	6.08	415.7	2.55	144.4	545.9
	C	6.71	758.8	5.84	400.6	2.36	141.4	523.1
	X	6.73	796.1	6.50	443.7	2.90	165.7	507.1
	XC	6.69	779.7	5.85	400.9	2.36	137.6	543.6
	P-value	0.0740	0.0423	0.5042	0.5041	0.5039	0.6510	0.8143
100F:0C	0	6.68	732.5	6.71	453.4	3.23	200.18	409.9
	C	6.48	733.0	6.13	415.8	2.76	155.17	478.8
	X	6.67	743.9	7.00	472.9	3.48	211.84	397.2
	XC	6.65	758.6	6.62	448.0	3.17	189.32	442.7
	P-value	0.5397	0.1232	0.0705	0.0705	0.0704	0.0020	0.0013
Pooled LSD[3]		0.098	28.858	0.457	29.873	0.373	22.461	52.456
Interactions								
Ration								
Linear		<0.0001	0.5159	0.0381	0.0888	0.0005	0.0026	0.0264
Quadratic		<0.0001	0.0500	0.2676	0.2990	0.1629	0.0941	0.0409
Enzyme		0.4661	0.3183	0.0734	0.0736	0.0735	0.0729	0.6602
Ration × enzyme		0.6274	0.0932	0.5253	0.5255	0.5249	0.2857	0.5545

[1]DMD is the DM degraded substrate (mg/g DM); ME is the metabolizable energy (MJ/kg DM); OMD is the in vitro organic matter digestibility (mg/g DM); SCFA is the short-chain fatty acids (mmol/g DM); GY_{24} is the gas yield at 24 h (mL gas/g DMD); MCP is the microbial biomass production (mg/g DM).

[2]Activities of the exogenous fibrolytic enzymes were as follows:

For cellulase product it contained 30,000 to 36,000 units of cellulase/g and 7500 to 10,000 units of β-glucanase/g.

For xylanase product it contained 34,000 to 41,000 units of xylanase/g, from 12,000 to 15,000 units of β-glucanase/g, and 45,000 to 55,000 units of cellulose/g.

[3]LSD: Least significant difference.

4. Discussion

Feeding well-balanced concentrate:roughage rations has been described to improve ruminant productivity and decrease methanogenesis (23). Higher fiber rations do not encourage microbial growth and fermentation enough, causing decreased ration digestibility (24) and a decrease in readily available energy and protein contents, with increase in the structural carbohydrate content of those rations (11). Increasing rations' concentrate content may alter the rumen fermentation towards propionogenesis as carbon dioxide is produced when propionate is made by rumen bacteria via the succinate-propionate pathway (11), whereas fibrous rations result in the preferential production of acetate, butyrate, and methane compared to a concentrate ration (24).

In general, GP appeared to be related to the chemical composition of the feeds, and in particular to the fiber content (25). Significant effects of ration type on asymptotic GP parameter (*b*) and lag time (*L*) were observed. Increased cell wall content as a result of increased maize silage ratio was considered to reduce the microbial activities, causing a lowered GP. Baah et al. (26) indicated positive effects on rumen bacterial growth rate, volatile fatty acid, GP, DM intake, and milk production in cattle. Comparison of in vitro GP without enzyme addition at different incubation times showed lowered GP (value at each incubation hour, i.e. GP_2 to GP_{72}) with increasing ratios of maize silage to concentrate. This may be due to decreased microbial fermentation in the higher roughage proportions in the rations due to the suppressing effect that resulted from decreased attachment of rumen microbes to feed particles. Dutta et al. (27) reported a decrease in gas volume as the red gram straw level was increased in the complete ration by replacing the concentrate proportion. However, Kumar et al. (24) reported that total GP was not affected by forage:concentrate ratios.

Enzymes may be applied during feed ensiling or directly during animal feeding. Both of these methods have a different mode of action. Administration of exogenous fibrolytic enzymes improved in vitro GP and improved the nutritive value of fibrous feeds. This may be due to enhanced attachment by rumen microorganisms (6), creation of stable enzyme feed complexes (5), and/or the possibility of alteration in the fiber structure, which could stimulate microbial colonization. The effect of enzymes, however, seems to be dependent on many factors such as source, type and dose of enzyme, type of rations fed to the animals and enzyme applications, and method of administration (28), causing inconsistent results. Some commercial fibrolytic enzymes increase total GP and rates of in vitro fermentation of feed (24). The ability of cellulases and xylanases to increase the extent of fiber digestion may be limited by the lack of enzymes that degrade the core structure of lignin-cellulose complexes in low-quality forages (28). Khattab et al. (1) and Valdes et al. (29) showed that an enzymatic complex containing cellulase and xylanase enhanced the digestion of low-quality feeds and maize silage. The unaffected DMD and OMD with the mixture of cellulase and xylanase indicated that neither of them were able to degrade the crystalline complex of cellulose and hemicellulose with other cell wall complexes.

A significant interaction effect between type of ration and enzyme on discrete lag time prior to GP (i.e. *L*) was observed, which suggests that it is important to identify appropriate enzyme type and the ration's maize silage:concentrate ratio.

It was expected that enzyme administration could improve rumen fermentation (6) and enhance attachment and colonization to the plant cell wall material by rumen microorganisms (6) and/or by synergism between rumen enzymes and the enzymes of the exogenous enzyme preparations (7). Nsereko et al. (6) suggested that exogenous enzymes could increase fiber degradation through a hydrolytic action resulting in more effective rumen fermentation. Tang et al. (30) suggested that in vitro GP variables usually reflect the characteristics of the fermentation process. In the present study, the interactive effects for in vitro GP parameters suggest that it is important to identify appropriate fibrolytic enzyme administration and rations with ratio of maize silage to concentrate. The interactive effects for in vitro GP suggest that cellulase and xylanase supplementation with rations could affect and improve GP.

In conclusion, the administration of cellulase or/and xylanase at the rate of 1 µL/g DM of substrate improved the in vitro rumen gas kinetics and cumulative GP, which may enhance the productive performance of ruminants in some further in vivo experiments. The responsiveness varied among different rations. However, enzyme administration was more affective when the ratio of maize silage was increased in the ration.

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