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Environmental impact of yeast and exogenous xylanase on mitigating carbon dioxide and enteric methane production in ruminants

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ABSTRACT

This study was carried out to establish *Saccharomyces cerevisiae*, xylanase, and their mixture as environmentally friendly feed additives that can reduce enteric biogas production. Rumen liquor was obtained from two rumen cannulated Holstein steers, two rumen cannulated Creole goats and two rumen cannulated Rambouillet sheep. The basal ration was supplemented (per g dry matter (DM)) with 2 mL xylanase, 4 mg *S. cerevisiae* or a mixture of both additives. There was no interaction ($P > 0.05$) between inoculum source and additive type. Inclusion of additives resulted in higher ($P = 0.045$) asymptotic gas production (GP) with sheep inoculum. Furthermore, higher ($P < 0.05$) GP rates with goat and sheep inocula were noted with the additives. *S. cerevisiae* or/and xylanase decreased ($P < 0.05$) proportional methane (CH_4) irrespective of the inoculum source with an increase ($P < 0.05$) in carbon dioxide production. Higher ($P < 0.05$) bacterial counts were observed with the inclusion of the additives. Metabolizable energy and short fatty acid concentrations were higher ($P < 0.05$) when the additives were supplemented to goat and sheep inocula. Additionally, inclusion of the additives resulted in higher ($P < 0.05$) DM degradability in sheep and steer inocula and higher ($P < 0.05$) organic matter degradability in goat and sheep inocula. *S. cerevisiae*, xylanase and their mixture did not affect total GP but altered the proportion of the gases generated. Furthermore, CH_4 production was reduced in the presence of the additives. Thus, the additives can be utilized in an environmentally friendly and sustainable way to reduce biogas emissions from livestock; thereby improving environmental conditions.

1. Introduction

In recent years, the earth has become warmer due to the increase in greenhouse gases (Moss et al., 2010). Livestock production was reported to be responsible for about 18% of methane (CH_4) emission and 9% of carbon dioxide (CO_2) production (FAO, 2006). Methane and CO_2 are a consequence of enteric fermentation of feeds, resulting in about 2–12% loss in gross energy (Hristov et al., 2013). The use of additives like *S. cerevisiae* (Elghandour et al., 2017a), salts of organic acids (Elghandour et al., 2016), enzymes (Kholif et al., 2017), and essential oils (Hernandez et al., 2017) in ruminants have been shown to mitigate ruminal CH_4 and CO_2 emissions. A reduction in CH_4 emission was assumed to result in an increase in body weight gain in cattle of 75 g/d as well as an increase of 1 L/d in milk production in dairy cows (Nkrumah et al., 2006).

Exploring alternative natural additives capable of modifying ruminal fermentation in order to enhance feed utilization (Salem et al., 2014) and reduce biogas emission (Elghandour et al., 2017a; Kholif et al., 2017) is gaining traction due to the ban of antibiotics and ionophores in animal feed within the European Union. It has been shown that inclusion of exogenous enzymes in the diets of

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ruminants resulted in a better performance of the animals by improved nutrient digestion and utilization (Valdes et al., 2015). Several modes of action of improving feed utilization have been described including solubilization of dietary fiber, supplementation of ruminal microorganisms with readily fermentable substrate, increased activity of microorganism-derived enzymes in the rumen (McAllister et al., 2001), and a better colonization of plant cell walls by microorganisms present in the rumen (Chung et al., 2012). However, Lewis et al. (1999) only observed a slight improvement in feed utilization with exogenous enzymes inclusion in ruminants. The observed differences might be due to the use of enzymes from different sources (Gado et al., 2017), different enzyme activities (Morsy et al., 2016), different physical properties of the diets (Elghandour et al., 2015), different application methods for exogenous enzymes (Elghandour et al., 2016) and differences in animal productivity (Beauchemin et al., 2003).

The capability of *S. cerevisiae* to modulate ruminal *in vitro* and *in vivo* fermentation was recently reported by Elghandour et al. (2014) and Ahmed et al. (2015). Inclusion of baker's yeast in animal diets was also shown to enhance the nutritional value of poor quality forages (Ahmed et al., 2015), the digestibility of nutrients in such forages (Hassan et al., 2016), and the carcass characteristics (Velázquez-Garduño et al., 2015). Furthermore, Rodriguez et al. (2015) observed higher *in vitro* rumen degradability and a higher gas production from feed when using *S. cerevisiae* as a feed additive. However, information on the effects of natural feed additives such as baker's yeast and xylanase on biogas productions is very limited. This study therefore aims to elucidate the effect of baker's yeast, xylanase and their mixture on the abatement of CH_4 and CO_2 emissions, ruminal microbial population, and rumen fermentation of a high-concentrate ration using rumen inoculum from goats, sheep and steers. The hypothesis was that different feed additives will have different effects on rumen microflora and that these changes are dependent on the ruminant species. Differences in the microflora could result in higher nutritive value of feeds and lower ruminal biogas production.

2. Materials and methods

2.1. Substrate for in vitro fermentation

The substrate (basal ration) was formulated in triplicates. The basal ration consisted of 520 g ground sorghum grain, 300 g corn stover, 60 g soybean meal, 80 g molasses and 40 g urea per kg DM. It contained 963 g organic matter, 183 g crude protein (CP), 304 g neutral detergent fiber (NDF) and 261 g acid detergent fiber (ADF) per kg DM. The substrate was supplemented with 2 mL xylanase (Dyadic® Xylanase PLUS, Dyadic International, Inc., Jupiter, USA), 4 mg *S. cerevisiae* (Procreatin 7, Safmix, Toluca, Mexico), or both (Please include actual amount of both additives used). The control treatment comprised of the substrate without an additive.

2.2. In vitro fermentation

Animal studies were carried out according to the official Mexican standards (NOM-062-ZOO-1999). Two Holstein steers (450 ± 20 kg body weight), two Rambouillet sheep (60 ± 2 kg body weight), and two Creole goats (50 ± 2 kg body weight) were ruminally cannulated and used for inoculum collection. All animals were housed in individual pens. Animal feed consisted of 60% oat hay and 40% of a concentrate (PURINA®, Toluca, Mexico). Feed was given at 8 a.m. and 4 p.m. and the animals had free access to water. For inoculum collection, rumen contents were placed in a plastic thermos preheated to 39 °C. Immediately after arriving in the laboratory, the containers were flushed with carbon dioxide. After mixing, rumen contents were pressed through 4 layers of cheesecloth into an oxygen-free flask. The flasks were kept at 39 °C under a continuous flow of carbon dioxide.

For *in vitro* fermentation, 0.5 g of substrate were incubated at 39 °C for 48 h in 50 mL rumen liquor (a 1:4 v/v mixture of ruminal inoculum and buffer as described by Goering and Van Soest (1970)) in the presence of either xylanase, *S. cerevisiae* or their mixture. Furthermore, blanks were run in the absence of additives. Three incubation runs were performed in three weeks. Bottles containing the samples (three bottles for each ration × four additives used × three different runs) plus three bottles as blanks (rumen fluid only) were incubated for 48 h.

At specific time point of incubation (2, 4, 6, 8, 10, 12, 24, 48 h), biogas production readings were recorded at 2, 4, 6, 8, 10, 12, 24 and 48 h of incubation using a water displacement apparatus according to Fedorak and Hrudey (1983) which previously detailed in Elghandour et al. (2017b). However, the apparatus designed with a conical funnel and the vials were punctured with a 16-gauge needle placed at the end of the hose. Gas production (mL) was measured by the displacement of water in the burette.

After 48 h of incubation, 5 mL of gas were taken and stored in the vials with saturated saline solution prepared with 400 g of NaCl in 1 L of distilled water and the pH adjusted accordingly, as also described in Elghandour et al. (2017b). Briefly, five mL of 20% methyl orange was added as indicator for CH_4 and CO_2 determination. A sample of 10 μL of the gas phase was taken from the vials with saturated saline and injected into a PerkinElmer, Claurus 500 gas chromatograph (Mexico City, Mexico) and the retention times at 0.73 min and 1.05 min was used for CH_4 and CO_2, respectively.

Fermentation was terminated after 48 h by putting the bottles in ice for 5 min and thereafter pH was determined. The entire contents of the bottles were filtered and dried at 55 °C to constant weight (48 h).

2.3. Total bacteria and protozoa counts

Quantification of total bacteria and protozoal counts were according to the method described in Elghandour et al. (2017b). Briefly, the concentration of total bacteria was determined after 48 h of incubation using a count chamber bacterium at a magnification of 100 × . About 0.5 mL of the 10% formaldehyde fixed medium sample was taken and diluted in 4.5 mL of distilled water. The bacterial concentration was determined as the average of bacteria observed in each grid, multiplied by the dilution factor and the chamber factor (2×10^7).

For the protozoal count, 1 mL of the 10% formaldehyde fixed sample was obtained and diluted in 1 mL of distilled water, then 0.5 mL of the mixture was taken and deposited into a Neubauer chamber, subsequently observed on a contrast microscope at 400 × magnification. The protozoa count was made in eight quadrants (4 of each grid) and viable protozoa are those that maintained their morphological integrity. The concentration of protozoa per mL of culture medium was estimated as the average of protozoa observed in each grid, multiplied by the dilution factor and the chamber factor (1×10^4).

2.4. Chemical analysis

Methods described by AOAC (1997) were used to quantify DM (#934.01), ash (#942.05), N (#954.01), EE (#920.39), ADF (#973.18), and lignin (#973.18). Neutral detergent fiber was quantified according to Van Soest et al. (1991).

Ruminal ammonia-N was quantified as described by Broderick and Kang (1980). Aliquots of the incubation medium were centrifuged at 3000 g for 10 min. Thereafter, 20 μl of the supernatant

were mixed with 1 mL of hypochlorite and 1 mL of phenol. The mixture was incubated at 39 °C for 30 min and thereafter diluted with 5 mL of distilled water. Samples were read on a visible ultraviolet light spectrophotometer (Varian, model Cary 1E, California, USA) at 630 nm. The resulting mg/dL concentration was divided by the factor 0.8, which is the 25% metaphosphoric acid dilution factor.

2.5. Calculations and statistical analyses

Fitting of gas production (GP) kinetics were performed as described by France et al. (2000). Metabolizable energy (MJ/kg DM) and OMD (g/kg DM) were determined as described by Menke et al. (1979). The partitioning factor of incubation at 24 h (PF_{24}) was defined as DMD (mg) per GP (mL) (Blümmel et al., 1997). The GY_{24} was calculated as follows: GY_{24} = mL gas/g DM/g DMD. Short-chain fatty acid concentration was calculated as described by Getachew et al. (2002) and MCP according to Blümmel et al. (1997).

The experimental design was a factorial design with 3 replicates in a randomized complete block design. However, data of each of the three runs within the same sample of each of the three individual samples of the substrate (ration) were averaged prior to statistical analysis, then mean values of each individual sample were used as the experimental unit. The GLM procedure of SAS (SAS, 2002) was used to analyze the data using the following model:

$$Y_{ijk} = \mu + R_i + A_j + (R \times A)_{ij} + \varepsilon_{ijk} \quad (1)$$

where Y_{ijk} represents the observation, μ the population mean, R_i the inoculum source effect, A_j the type of feed additive, $(R \times A)_{ij}$ the interaction of the feed additive with the inoculum, and ε_{ijk} the residual error. Tukey test was used to compare means.

3. Results

3.1. Gas production

There was no interaction between inoculum source and additive type for all gas parameters (Table 1). Total gas, CH_4, and CO_2 production were affected ($P < 0.05$) by inoculum source but only GP and CH_4 were affected ($P < 0.05$) by the additives.

Fig. 1 shows GP at different incubation hours and the effect of different inoculum sources and feed additives. The inclusion of *S. cerevisiae*, xylanase or their mixture did not affect ($P > 0.05$) asymptotic GP with goat and steer inocula; however, a higher ($P = 0.045$) asymptotic GP with sheep inoculum was observed (Table 1). Goat ($P = 0.48$) and sheep ($P = 0.046$) inocula increased the rate of GP when *S. cerevisiae*, xylanase or their mixture were added to the substrate. A decreased ($P = 0.029$) in lag time of GP was observed with steer inoculum but no effect was noted with goat and sheep inocula.

The additives had no effect on CH_4 production (mL/g DM). However, the additives decreased proportional CH_4 with goat ($P = 0.046$), sheep ($P = 0.04$) and steer ($P = 0.041$) inoculum and lowered CH_4 production (mL/g DM) at 48 h of incubation with goat ($P = 0.041$) and sheep ($P = 0.024$) inoculum (Table 1). Inclusion of *S. cerevisiae* and xylanase resulted in a higher production ($P < 0.05$) of CO_2 (mL/g DM) and proportional CO_2 at 48 h of incubation.

3.2. Microbial population

An interaction between inoculum source and additive type was observed ($P < 0.001$) for total protozoal counts, but no interaction ($P > 0.05$) was noted for total bacterial counts (Table 2). Additive type did not affect ($P > 0.05$) total bacterial and protozoal counts. However, protozoal counts differed ($P = 0.002$) among inoculum sources. For all inoculum sources, total bacterial counts were higher ($P < 0.05$) in the presence of all additives, whereas total protozoal counts were not affected ($P > 0.05$).

3.3. Fermentation kinetics

Interactions between inoculum source and additive type for all measured parameters of fermentation patterns were not affected ($P > 0.05$; Table 2). Most of the fermentation parameters

Table 1
Biogas production (mL/g DM) of a total mixed ration as affected by the addition of xylanase, *S. cerevisiae* yeast and their mixture using rumen liquor from goat, sheep and steer.

Inoculum	Additive	Gas production parameters				CO_2 production at 48 h of incubation			CH_4 production at 48 h of incubation		
		b	*c*	*Lag*	mL gas/g degraded DM	mL CO_2/g incubated DM	Proportional CO_2 production	mL CO_2/g degraded DM	CH_4 production (mL/g DM)[a]	Proportional CH_4 production	mL CH_4/g degraded DM
Goat	Control	270	0.062	4.91	357	226.7	80.0	329.2	36.4	12.9	51.5
	Xylanase	278	0.069	4.76	369	212.6	73.5	307.0	46.5	16.1	64.6
	Yeast	288	0.066	4.22	366	221.0	76.1	319.1	49.5	17.0	69.6
	Xylanase + yeast	267	0.069	4.83	352	222.9	76.3	315.7	48.9	16.8	67.6
	SEM	5.1	0.0021	0.187	6.0	1.3	0.40	2.7	0.86	0.31	0.62
	P-value	0.379	0.048	0.107	0.236	0.051	0.046	0.041	0.012	0.048	0.037
Sheep	Control	274	0.059	4.40	365	202.9	79.3	291.2	33.1	12.9	46.2
	Xylanase	281	0.064	4.32	367	199.9	74.8	283.7	47.3	17.7	65.4
	Yeast	283	0.062	4.04	374	198.9	75.6	282.6	49.3	18.7	68.5
	Xylanase + yeast	290	0.059	3.59	373	197.2	76.5	275.5	43.4	16.9	59.3
	SEM	6.5	0.0031	0.473	8.3	1.4	0.52	1.9	0.33	0.62	1.19
	P-value	0.045	0.046	0.634	0.854	0.760	0.040	0.024	0.048	0.047	0.019
Steer	Control	292	0.073	5.15	401	211.6	82.1	303.8	36.2	14.0	51.3
	Xylanase	297	0.075	4.54	402	202.5	75.7	285.7	44.9	16.8	61.7
	Yeast	300	0.073	4.41	409	204.4	76.1	292.4	44.0	16.4	61.3
	Xylanase + yeast	303	0.070	4.38	403	207.1	76.2	291.7	44.6	16.4	61.2
	SEM	6.5	0.0029	0.299	8.3	1.6	0.49	1.80	1.19	1.49	1.60
	P-value	0.677	0.595	0.029	0.907	0.404	0.041	0.605	0.069	0.055	0.007
Pooled SEM		6.1	0.0027	0.341	7.622	2.50	0.82	2.6	1.52	0.82	1.57
P value											
Inoculum		<0.001	<0.001	0.041	<0.001	<0.001	0.049	<0.001	0.027	0.527	0.018
Additive		0.016	0.024	0.014	0.547	0.012	0.045	0.621	0.887	0.959	0.872
Inoculum × additive		0.694	0.434	0.750	0.843	0.215	0.143	0.229	0.403	0.138	0.449

[a] b is the asymptotic gas production (mL/g DM); *c* is the rate of gas production (/h); *Lag* is the initial delay before gas production begins (h).

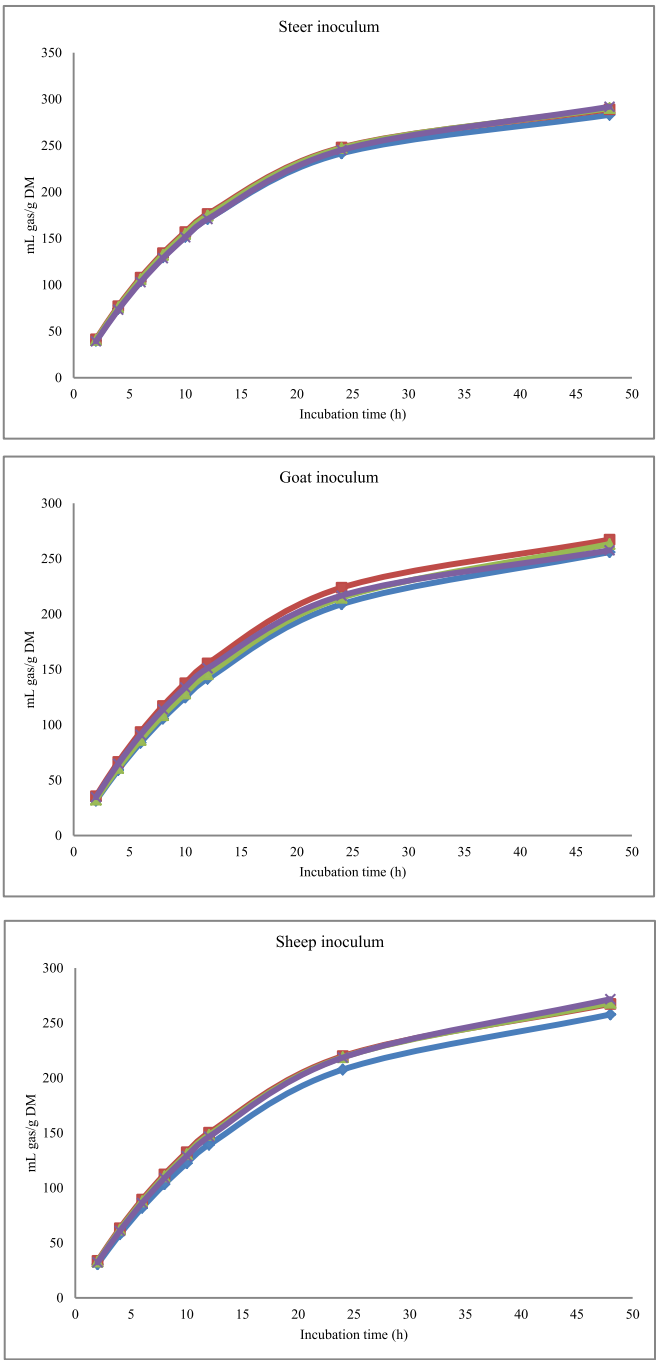

Fig. 1. Gas production (mL/g DM) as affected by the inclusion of *S. cerevisiae*, xylanase and their mixture using rumen liquor from goat, sheep and steer. Control (0 additive; -♦-), xylanase (2 mL; -■-), *S. cerevisiae* (4 mg; -▲-) and mixture of both additives (-X-) per g DM of the basal ration.

determined differed ($P < 0.05$) among goat, sheep and steer inocula as well as among feed additive types. Inclusion of *S. cerevisiae* or xylanase had no effects ($P > 0.05$) on rumen pH, NH_3-N concentration, PF_{24}, and GY_{24}. Higher SCFA concentrations were observed with the inclusion of the additives in goat ($P = 0.041$) and sheep ($P = 0.45$) inocula. The additives resulted in higher DMD with sheep ($P = 0.009$) and steer ($P = 0.037$) inocula and higher ($P = 0.048$) OMD with goat inoculum. *S. cerevisiae* and xylanase resulted in higher ME concentration with goat ($P = 0.019$) and sheep ($P = 0.046$) inocula and higher MCP ($P = 0.042$) with goat inoculum.

4. Discussion

4.1. Gas production

The absence of inoculum source and feed additive type interaction reveals that the effects of additive are not inoculum dependent. Besides, GP parameters differed among goat, sheep and steer inocula and therefore results from a specific ruminant species cannot be easily extrapolated to another. Aderinboye et al. (2016) observed different fermentation parameters among cows, sheep and goat inocula, which might be due to differences in bacterial and protozoal populations among the different ruminant species and therefore differences in the microbial activities. Because feed type is mainly responsible for the composition of ruminal microbial population (Mould et al., 2005), differences in the microbial populations in the rumen of the different animal species used in the study were not expected. However, variations in microbial species were also reported to be due to rumination, gut physiology, and residence time or sample preparation and inoculation (Mould et al., 2005).

Gas production differed among the feed additives studied. This was expected because each of the additives has a different mode of action in respect to their effect on ruminal fermentation (Hernandez et al., 2017). Previous reports on the inclusion of *S. cerevisiae* (Elghandour et al., 2014) and enzymes (Vallejo et al., 2016) resulted in higher GP. In the present study, *S. cerevisiae* and xylanase had a weak effect on GP with goat and steer inocula, but a higher GP was observed with sheep inocula. Consistent with our results, Hernandez et al. (2017) reported negligible effects of exogenous xylanase and *S. cerevisiae* on GP.

The additives increased GP rate and decreased the lag time of GP revealing better nutrients utilization. It was shown recently that addition of exogenous enzymes to ruminant diets improved DM degradation and therefore feed utilization, DM digestion and animal performance (Morsy et al., 2016). *S. cerevisiae* addition was reported to decrease the rate of GP (Rodriguez et al., 2015). This inconsistency was explained by differences in substrate composition (Elghandour et al., 2014). The lower lag time may be due to higher degradation of feed nutrients especially fibers (Kholif et al., 2016; Elghandour et al., 2017a). Exogenous enzymes have the ability to stimulate microbial colonization in the rumen and facilitate bacterial attachment to feed particles (Giraldo et al., 2007). *S. cerevisiae* was reported to effectively consume O_2 molecules from the rumen thereby creating a favorable condition for the activity of various microorganisms (Newbold et al., 1996). In addition, Williams et al. (1991) reported a stimulating effect of *S. cerevisiae* on cellulose degradation, which was associated with a lower lag time and higher initial rates of digestion without affecting the extent of ruminal digestion. Lowering the lag time of GP with xylanase and *S. cerevisiae* reveals the ability of these additives to provide a solution when poor quality forages are fed to ruminants (Salem et al., 2015).

4.2. Biogas production

Production of CH_4 and CO_2 differed among goats, sheep, and steer inocula. This may be due to different ruminal microflora population. Hook et al. (2010) observed different CH_4 production from different ruminant species. Boeckaert et al. (2007) reported that ruminal protozoal population is animal dependent. Differences in CH_4 production among ruminal species indicate that a single species could not be used to predict CH_4 production (Bueno et al., 1999) for another species. As previously shown, the loss in net feed energy is partly due to enteric CH_4 emission (Hristov et al., 2013). Therefore, intensive research efforts have recently been

Table 2
Fermentation characteristics of a total mixed ration as affected by the addition of xylanase, *S. cerevisiae* yeast and their mixture using rumen liquor from goat, sheep and steer.

Inoculum	Additive	pH	SCFA	NH_3-N	DMD	OMD	ME	PF_{24}	GY_{24}	MCP	Total bacteria × 10^8	Total protozoa × 10^5
Goat	Control	6.50	4.62	66.2	716	605	8.92	5.39	185	667	7.0	2.03
	Xylanase	6.50	4.95	65.3	724	632	9.33	5.30	189	695	11.6	2.65
	Yeast	6.49	4.74	64.7	719	615	9.08	5.35	187	677	11.3	2.79
	Xylanase + yeast	6.51	4.79	66.7	733	619	9.14	5.35	187	681	11.5	2.83
	SEM	0.020	0.076	2.02	6.0	2.1	0.093	0.022	0.7	1.4	0.25	0.417
	P-value	0.821	0.041	0.895	0.289	0.048	0.019	0.102	0.091	0.042	0.028	0.510
Sheep	Control	6.50	4.59	55.0	706	603	8.89	5.40	185	664	8.4	2.77
	Xylanase	6.52	4.86	55.2	728	625	9.23	5.33	188	687	10.9	2.30
	Yeast	6.48	4.84	54.4	719	623	9.20	5.33	188	685	11.4	2.32
	Xylanase + yeast	6.49	4.83	59.7	729	622	9.19	5.34	188	685	11.9	2.29
	SEM	0.013	0.130	1.29	3.7	10.5	0.160	0.034	1.2	11.0	0.42	0.289
	P-value	0.279	0.045	0.068	0.009	0.043	0.046	0.457	0.459	0.457	0.045	0.446
Steers	Control	6.50	5.34	62.2	707	663	9.82	5.21	192	728	7.9	2.89
	Xylanase	6.48	5.49	62.5	720	674	9.99	5.18	193	740	11.1	2.23
	Yeast	6.48	5.47	57.3	711	673	9.97	5.19	193	738	10.8	2.28
	Xylanase + yeast	6.49	5.42	60.7	724	670	9.91	5.20	192	735	11.5	2.16
	SEM	0.003	0.079	1.33	3.7	6.3	0.096	0.016	0.6	6.6	0.45	0.435
	P-value	0.222	0.597	0.085	0.037	0.624	0.604	0.556	0.625	0.602	0.044	0.170
Pooled SEM		0.014	0.098	1.59	4.6	7.9	0.120	0.025	0.9	8.3	0.38	0.215
P value												
Inoculum		0.545	<0.001	<0.001	0.076	<0.001	<0.001	<0.001	<0.001	<0.001	0.167	0.002
Additive		0.289	0.035	0.075	0.002	0.034	0.035	0.033	0.038	0.035	0.368	0.096
Inoculum × additive		0.661	0.907	0.363	0.843	0.905	0.907	0.865	0.861	0.909	0.649	<0.001

[1]DMD is dry matter degradability (mg/g DM), GY_{24} is gas yield at 24 h (mL gas/g DMD), MCP is microbial protein production (mg/g DM), ME is metabolizable energy (MJ/kg DM), NH_3-N is ammonia-N, OMD is *in vitro* organic matter digestibility (g/kg DM), PF_{24} is partitioning factor at 24 h of incubation (mg DMD/mL gas), pH is ruminal pH, SCFA is short-chain fatty acids (mmol/g DM).

directed towards mitigation of CH_4 production in ruminants (Elghandour et al., 2016b). Methane, CO_2 and H_2 are the major gases generated during the fermentation process within the rumen. Therefore, lack of effect on GP and lower proportional CH_4 production are proofs that the additives were effective in reducing CH_4 production and consequently CH_4 emission in ruminants. Polyorach et al. (2014) observed that *S. cerevisiae* supplementation decreased *in vitro* CH_4 production, which is consistent with the current findings. In ruminant nutrition, xylanase is not expected to reduce CH_4 production because it increases fiber digestibility by increasing the availability and utilization of hemicellulose (Elghandour et al., 2016b). The reasons for the reduction in CH_4 production in the current study are unclear.

S. cerevisiae lowered CH_4 production because of its ability to stimulate the acetogens through competition and co-metabolization of H_2 with methanogens (Hristov et al., 2013). Moreover, inclusion of baker's yeast in ruminant feed has shown to enhance nutrient digestibility (Hassan et al., 2016) and alter SCFA production in the rumen by increasing cellulolytic and amylolytic bacteria population in the rumen (Kumar et al., 1997). The full mode of action for the reduction of CH_4 production is not clear, because some studies reported increased CH_4 production with *S. cerevisiae* supplementation (Elghandour et al., 2017a). Newbold and Rode (2006) reported a decrease in CH_4 production with feeding live *S. cerevisiae* products.

4.3. Microbial population

Additives did not affect total protozoal counts but resulted in higher total bacterial numbers. Chung et al. (2012) reported that protozoa populations were not affected by *S. cerevisiae* and fibrolytic enzyme supplementation in rams and cows. Newbold et al. (1996) observed that *S. cerevisiae* supplementation increased total anaerobic and cellulolytic bacteria counts. Higher bacterial number with *S. cerevisiae* was as a result of providing the incubation medium with vital nutrients for optimum microbial production (Callaway and Martin, 1997). In addition, *S. cerevisiae* provides conducive anaerobic conditions favorable for the growth of microorganisms (Mosoni et al., 2007), making the rumen environment more suitable for microbial growth. On the other hand, exogenous enzyme, like xylanase, may have positive effects on ruminal fibrolytic and non-fibrolytic bacteria by releasing readily usable carbohydrates (Nsereko et al., 2002).

4.4. Fermentation patterns

Greater SCFA concentrations were observed with the inclusion of the additives with goat and sheep inocula. Inclusion of *S. cerevisiae* in the diet of ruminants increased total SCFA and propionic acid production (Mao et al., 2013). Greater SCFA production and ME concentrations are as a result of enhanced microbial activities. As previously shown, higher ruminal bacterial population in the presence of the additives is the reason for enhanced ruminal fermentation. At the same time, improved DMD with sheep and steer inocula and higher OMD with goat inoculum might be a result of increased colonization of plant cell walls by fungi resulting in enhanced DM and fiber digestion (Windham and Akin, 1984). Enhanced degradability in the presence of *S. cerevisiae* may be a result of enhanced ruminal environment (Newbold et al., 1996; Callaway and Martin, 1997).

5. Conclusion

S. cerevisiae and xylanase did not affect gas production of the substrate; however, they made qualitative changes in the gases produced and they improved rumen conditions. *S. cerevisiae* and xylanase decreased CH_4 production, which is very important from an environmental standpoint, and therefore, these feed additives can be applied as a sustainable strategy to reduce biogases from livestock. Further research is needed to study dose response of these additives *in vitro* and *in vivo* in order to validate or disprove the present results and to determine whether these additives could be utilized as feed additives to enhance feed efficiency and abatement of CH_4 production in ruminants.

Conflicts of interest

All authors declare that there are no present or potential conflicts of interest among the authors and other people or organizations that could inappropriately bias their work.

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