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Effectiveness of xylanase and *Saccharomyces cerevisiae* as feed additives on gas emissions from agricultural calf farms

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ABSTRACT

The aim of the present study was to evaluate the potential of supplementing calves' diets with exogenous enzymes (xylanase; XYL) and yeast (*Saccharomyces cerevisiae* [SC]) on the sustainable control of methane (CH_4) and carbon dioxide (CO_2) productions in agricultural calves farming. Three different levels of supplemented diets of XYL (0, 3 and 6 mg/g of dry matter (DM)), SC (0, 2 and 4 mg/g of DM) and mixture of XYL and SC (0, 2 μL XYL + 2 mg SC, 6 μL XYL + 4 mg SC/g of DM) were tested. Asymptotic gas production (GP) consistently decreased by each of the additives with the lowest value at the high dose of XYL + SC mixture ($P < 0.05$) compared with the control and the low dose of XYL + SC mixture. Methane production was reduced by additives inclusion ($P < 0.05$) when compared with the control treatment with no additive. Xylanase + SC at all doses increased CO_2 production ($P < 0.05$) whereas the high dose had the most statistically significant ($P < 0.05$) reduction in GP and CH_4 production compared with control, XYL and SC additives at different doses. Interaction between additive and rumen liquor was observed for rate of GP ($P = 0.027$) and initial delay before GP ($P < 0.001$). Inclusion of XYL, SC, and XYL + SC mixture had less asymptotic GP while XYL + SC mixture had the lowest initial delay (39%) before GP began. The XYL + SC had the lowest rate of CH_4 production (9%) and highest asymptotic CO_2 production (81%). The findings of this study indicate that inclusion of XYL or SC additives can improve rumen fermentation and reduce greenhouse gases production. The study also established that the mixture of XYL and SC is more efficient in reducing gas and CH_4 emissions for cleaner environmental production conditions in calf farming.

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1. Introduction

Worldwide, agricultural farming systems, such as livestock production, face the increasing challenge of maintaining future global demand for meat and dairy products because of an expected increase in population (Wiedemann et al., 2017). The Food and Agriculture Organization (FAO, 2006) expects that an increase in purchasing power for food from animal sources raises the yearly demand to 465 and 1.043 million t for meat and milk products. Besides, the FAO estimates the growth of global population to reach 9.6 billion by the y 2050 (FAO, 2016), with a doubled purchasing power for meat and dairy products. To meet this rise in demand, agricultural systems need to devise a means to adapt to the probability of dangerous climate change and become more resilient, productive and sustainable (FAO, 2016). This will not only reduce greenhouse gas (GHG) emissions but also ensure the wellbeing of

Abbreviations: ADF, acid detergent fiber; *b*, the asymptotic gas, methane, or carbon dioxide production; *c*, the fractional rate of fermentation; CFU, colony-forming unit; CH_4, methane; CO_2, carbon dioxide; DM, dry matter; DMD, dry matter disappearance; EE, ether extract; GHG, greenhouse gas; GP, gas production; *Lag*, the discrete lag time prior to any gas, CO_2 or CH_4 formation; NDF, neutral detergent fiber; SC, *Saccharomyces cerevisiae*; XYL, xylanase.

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the ecosystem and rural populations.

Today, agricultural waste products are one of the largest contributors of anthropogenic sources of three major GHGs: methane (CH_4), carbon dioxide (CO_2), and nitrous oxide, with livestock production accounting for approximately two-thirds of the direct emissions (Slade et al., 2016), largely from digestion by livestock. Methane is the major GHG emitted from enteric fermentation through the typical digestive process of ruminants (Hristov et al., 2015). Methane accounts for approximately 12 to 17% GHG emission (Beauchemin et al., 2009). However, the major constraints in ruminant farming include: excessive excretion of nutrients, inefficient digestibility and high CH_4 emission which represent a net loss of 2 to 12% of gross dietary energy (Hristov et al., 2015). The efficient reduction of such energy losses may be potentially used for the production of more meat and milk, rather than contributing to GHG production which impacts negatively on climate change (Eckard et al., 2010). Recently, the use of exogenous enzymes (Rojo et al., 2015) and yeast (*Saccharomyces cerevisiae*; SC) additives in ruminant diets has attracted considerable interest (Hassan et al., 2016). Many research studies have shown that supplementing exogenous enzymes in livestock diets improved forage quality (Kholif et al., 2017), increase digestibility, rumen fermentation, and ruminant production (Valdes et al., 2015). However, Lewis et al. (1999) reported that exogenous enzymes did not consistently enhance forage quality and utilization by ruminants. This inconsistency may be attributed to several factors such as the source of the enzyme (Khattab et al., 2011), doses and activities of the enzyme (Jalilvand et al., 2008), physical properties of the substrate (Elghandour et al., 2015), treatment duration, enzyme application method (Elghandour et al., 2016a), composition of the diet to which enzyme is added (Elghandour et al., 2016a) and level of animal productivity (Beauchemin et al., 2003).

Animal nutritionists and microbiologists have recently developed keen interest in the use of dietary feed additives to modify ruminal fermentation and reduce rumen GHG production (Hernandez et al., 2017). Dick et al. (2015) reported that the use of legumes as a replacement for nitrogen fertilizer enhanced reduction of GHG emission. Nguyen et al. (2010) suggested that increasing the proportion of concentrate-based diet would reduce the CH_4 production. Supplementation of SC improved digestibility of low quality forages and altered microbial environment by increasing the number of ruminal microflora which could enhance fiber fraction digestion in ruminants (Ahmed et al., 2015) and horses (Salem et al., 2016). Salem et al. (2015a) observed that inclusion of exogenous fibrolytic enzymes (e.g., cellulase and xylanase (XYL)) improved feed utilization in ruminants. Exogenous XYL was selected to be studied in the present study because emerging evidences show better results with XYL than cellulase (Vallejo et al., 2016).

Few studies investigated the effect of natural feed additives on CH_4 and GHG productions. The present study evaluated the use of exogenous enzyme (xylanase), yeast (*S. cerevisiae)* and their mixture, as dietary feed additives, to enhance the nutritive value of feeds and to mitigate the production of CO_2 and CH_4 from the agriculture calf farms using the *in vitro* gas production (GP) technique.

2. Materials and methods

2.1. In vitro incubations and treatments

Rumen inoculum was collected by stomach tube from 40 weaned Holstein calves (40–55 kg body weight) before morning feeding. They were divided into 4 groups (n = 10) which were fed a basal diet with no additive (Control rumen liquor), or daily supplemented with 5 mL of XYL (Dyadic PLUS; Dyadic international, Inc, Jupiter, FL, USA) [XYL rumen liquor], or 4 g of SC, with a minimum guaranteed concentration of live yeast cells of 1.5×10^{10} CFU of SC/g of product (Procreatin 7, Safmix, Toluca, Mexico) [SC rumen liquor] or their mixture (2.5 mL XYL + 2 g SC) (XYL + SC rumen liquor) for 60 d of age. All the 40 calves were stabled and reared under the same condition. The calves were fed *ad libitum* a total mixed ration of a commercial concentrate (Ultra Malta Clayton®, Toluca, Mexico) formulated to meet their nutrient requirements (NRC, 1985) with free access to fresh water. The diet contained per kg dry matter (DM) of 200 g crude protein, 230 g neutral detergent fiber (NDF), 50.3 g acid detergent fiber (ADF) and 35.6 g ether extract (EE). The treatments which were tested against control treatment (no additives) were as follows: XYL treatment (at 3 and 6 μL/g DM), SC treatment (at 2 and 4 mg/g DM) and their mixture at 3 μL XYL + 2 mg SC, and 6 μL XYL + 4 mg SC (XYL + SC treatment). The diet fed to the calves was used as the substrate for the *in vitro* incubation. The product of XYL contained: 34,000 to 41,000 U of XYL/mL, 12,000 to 15,000 units of beta-glucanase/mL, and 45,000 to 55,000 U of cellulase/mL.

Immediately after collection, the rumen contents obtained from the donor calves were flushed with CO_2, mixed and strained through four layers of cheesecloth into a flask with oxygen-free headspace. Filtered rumen fluid was immediately transported to the laboratory where it was mixed in a 1:4 (v/v) proportion with the buffer solution (containing micro- and macro-elements, a reducing agent and a reduction indicator of resazurin) as described by Goering and Van Soest (1970), with no trypticase added. Diluted rumen fluid (50 mL containing 10 mL of rumen liquor) was added to each incubation bottle of 120 mL containing 0.5 g of substrate, which had been previously weighed out and additive solutions dispensed.

Three incubation runs were performed in three different weeks. Bottles were inoculated within each incubation run, with three bottles as blanks (i.e., rumen fluid only with no substrate or additive). After filling all bottles, they were flushed with CO_2 and 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, 16, 18, 24, 36, 48 and 70 h of incubation using a pressure transducer (Extech Instruments, Waltham, USA) following the technique of Theodorou et al. (1994). At the same incubation times, CH_4 and CO_2 concentrations in the headspace of the bottles were measured using a diffusion based gas detector (Gas Analyzer CROWCON Model Tetra3, Abingdon, UK). The *in vitro* incubation process can be summarized in Fig. 1.

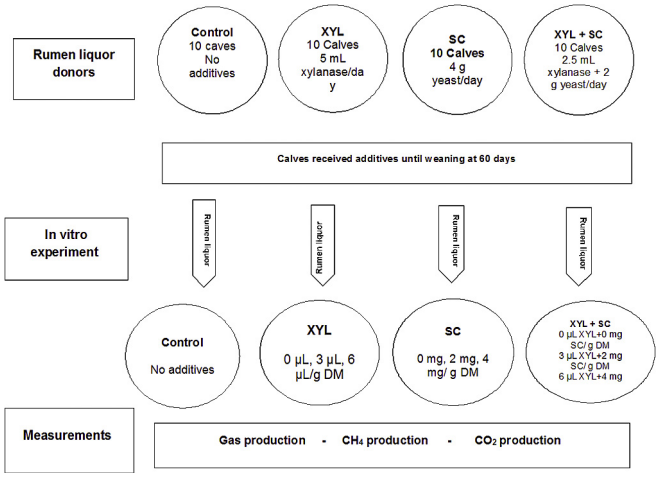

Fig. 1. Flowchart of the *in vitro* incubation process.

After sampling the supernatant for pH determination, 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 then dried at 70 °C overnight to estimate apparent DM disappearance (DMD).

2.2. Chemical analyses

Samples of the incubated substrate were analyzed for DM (method ID 934.01), ash (method ID 942.05), nitrogen (method ID 954.01) and EE (method ID 920.39) using Association of Official Analytical Chemists (AOAC, 1997) official methods. The NDF (Van Soest et al., 1991) and ADF (AOAC, method ID 973.18) contents were determined using an ANKOM^{200} Fiber Analyzer Unit (ANKOM Technology Corp., Macedon, NY, USA). The NDF analysis was done with sodium sulfite, and with α-amylase. Both NDF and ADF were expressed without residual ash.

2.3. Calculations and statistical analyses

Volumes (mL/g DM) of gas, CO_2, and CH_4 were used to estimate the fermentation kinetic parameters using the NLIN procedure of Statistical Analysis System (SAS, 2002) according to France et al. (2000) model as:

$$y = b \times \left[1 - e^{-c(t-Lag)}\right]$$

where y is the volume of gas, CO_2 or CH_4 at time t (h); b is the asymptotic GP, CO_2 or CH_4 production (mL/g DM); c is the fractional rate of fermentation (per h), and *Lag* (h) is the discrete lag time prior to any gas, CO_2 or CH_4 formation.

The experimental design for the *in vitro* ruminal GP and fermentation parameters analysis was a completely randomized design, considering as fixed factors, additive type and additive doses in the linear model (Steel and Torrie, 1980). Data of each of the three runs within the same sample were averaged prior to statistical analysis. Mean values of each individual extract within each species (three samples of each) were used as the experimental unit. Multiple comparisons of means were performed using the Tukey's test. Significance was declared at a level of $P < 0.05$.

3. Results

Fig. 2 shows the *in vitro* rumen GP (mL/g incubated DM) of a calf's diet, supplemented with XYL, SC, and XYL + SC mixture. Interaction between additive and rumen liquor was observed for rate of GP ($P = 0.027$) and initial delay before GP ($P < 0.001$; Table 1). No effect ($P > 0.05$) was noted between additive × dose for asymptotic GP, rate of GP and initial delay before GP. Inclusion of XYL, SC, and XYL + SC had a higher asymptotic GP while XYL + SC mixture had the lowest initial delay (39%) before GP began. There was a decrease ($P < 0.05$) in the average asymptotic GP (at all doses) of the treatment XYL + SC mixture compared with the control treatment (no additive). The supplementation of XYL and SC to the diets of the calves had no statistically significant effects ($P > 0.05$) on the asymptotic GP at all tested doses, while the supplementation of a mixture of XYL + SC at a high dose affected it ($P < 0.05$) compared with the control. The rate of GP showed a positive effect ($P < 0.05$) on all the doses of XYL addition while no statistically significant effect ($P > 0.05$) was observed with the addition of SC and XYL + SC mixture additives, when compared with the control. In addition, there was an increase in the lag time of GP at each dose of XYL, SC and XYL + SC mixture (only at the high dose) additives, and a statistically significant effect ($P < 0.05$) on all the doses of XYL and SC additives compared with the control.

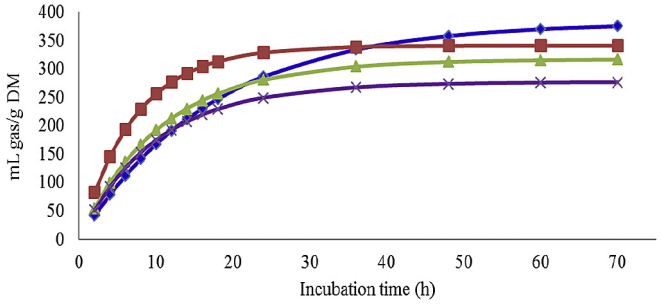

Fig. 2. *In vitro* rumen gas production (mL/g incubated DM) of calf's diet supplemented with: no additive (control) (-◆-), xylanase (-■-), *Saccharomyces cerevisiae* (-▲-), and their mixture (-×-) incubated with rumen inoculum from calves fed on diet supplemented with the same feed additives for 60 d of age.

Fig. 3 shows the *in vitro* rumen CH_4 production (mL/g incubated DM) of a calf's diet supplemented with XYL, SC and XYL + SC mixture. Interactions between additive and rumen liquor were observed ($P < 0.05$) for asymptotic and rate of CH_4 productions (Table 1). Moreover, interactions were observed ($P < 0.05$) between additive and dose for asymptotic CH_4, initial delay before CH_4 production and at 48 h incubation for mL/g incubated DM. Xylanase, SC, and XYL + SC mixture in all doses except for 0 mg dose of XYL + SC mixture additive affected ($P < 0.05$) CH_4 production when compared with the control treatment. Also the mean productions of CH_4 from the XYL, SC and XYL + SC mixture were decreased ($P < 0.05$) compared with the control. The lowest asymptotic CH_4 production was observed at the high dose of XYL + SC mixture which was lower ($P < 0.05$) than that of the control treatment. No effect ($P > 0.05$) was observed in all the doses on the rate of CH_4 production except at 3 μL XYL/g DM that was increased ($P < 0.05$), compared with the control, with no observable effect ($P > 0.05$) being noticed with the addition of SC and XYL + SC mixture additives. Similarly, there was no statistically significant difference ($P > 0.05$) in the lag time of CH_4 production at all doses when compared with the control treatment. The asymptotic CH_4 production reduced for all the additives, while the rate of CH_4 production was lowest for XYL + SC (9%).

Fig. 4 shows the *in vitro* rumen CO_2 production (mL/g incubated DM) of a calf's diet supplemented with XYL, SC and XYL + SC mixture. Interaction was observed ($P < 0.05$) between additive and dose for mL/g incubated DM but there were no effects for mL/g degraded DM and proportional CO_2 production at 6, 24 and 48 h incubation. The XYL + SC had the highest asymptotic CO_2 (81%) followed by SC (37%) and XYL (20%). The mean asymptotic CO_2 production was higher ($P < 0.05$) for SC and XYL + SC mixture additives addition than for the control (without additive). The highest asymptotic CO_2 production was recorded for the treatment containing 2 mg SC/g DM and high dose of XYL + SC mixture; it was greater in the two treatments ($P < 0.05$) than in the control but a decrease below the control was observed at 6 μL XYL/g DM and 0 mg SC/g DM. The mean rate of GP differed ($P < 0.05$) only with the addition of XYL and not with SC and XYL + SC mixture additives when compared with the control. All the values of the lag time of CO_2 production ranged from the lowest scale of 8.2 mL/g DM in 2 mg SC to the highest gauge of 10.9 mL/g DM in the high dose of XYL + SC mixture. There was no statistically significant effect ($P > 0.05$) on mean lag time of CO_2 production for XYL, SC additives and the control, but a statistically significant effect was observed for XYL + SC mixture compared with the control treatment.

From previous studies, negligible amounts of CH_4 were released during the first 6 h of fermentation. Because the production peak

Table 1
In vitro gas, methane (CH_4) and carbon dioxide (CO_2) kinetics[1] as affected by addition of xylanase (XYL), yeast (SC) and mixture of both in rumen liquor of calves fed on diet supplemented with xylanase and/or yeast for 60 d of age.

Rumen liquor from calves fed on:	Additive:	Dose (/g DM)	Gas production (mL/g DM)[2]			CH_4 production (mL/g DM)[3]			CO_2 production (mL/g DM)[4]		
			b	*c*	*Lag*	*b*	*c*	*Lag*	*b*	*c*	*Lag*
Control	No additive	0	383^{Aa}	0.058^{Cc}	2.69^{Ce}	108^{Aa}	0.044^{Bbc}	7.15^{ab}	38^{Cde}	$0.041B^{c}$	$8.48B^{c}$
XYL	XYL	0 μL	375^{a}	0.141^{a}	5.28^{abc}	67^{bc}	0.093^{ab}	8.93^{a}	58^{c}	0.147^{ab}	10.17^{ab}
		3 μL	348^{a}	0.148^{a}	5.28^{abc}	60^{c}	0.102^{a}	8.30^{ab}	50^{cd}	0.162^{a}	9.78^{abc}
		6 μL	303^{ab}	0.128^{ab}	5.43^{abc}	64^{c}	0.070^{abc}	6.87^{ab}	35^{de}	0.060^{c}	8.39^{c}
		Mean	352^{AB}	0.119^{A}	4.67^{A}	75^{B}	0.077^{A}	7.81	45^{BC}	0.103^{A}	9.21^{AB}
SC	SC	0 mg	331^{ab}	0.000^{bc}	6.59^{a}	63^{c}	0.049^{bc}	8.53^{ab}	31^{e}	0.074^{c}	8.74^{bc}
		2 mg	329^{ab}	0.105^{abc}	5.58^{ab}	69^{bc}	0.062^{abc}	8.01^{ab}	90^{ab}	0.087^{bc}	8.18^{c}
		4 mg	295^{ab}	0.108^{abc}	6.02^{a}	67^{bc}	0.050^{bc}	9.03^{a}	47^{cde}	0.043^{c}	8.50^{bc}
		Mean	334^{AB}	0.086^{BC}	5.22^{A}	77^{B}	0.051^{B}	8.18	51^{B}	0.061^{B}	8.48^{B}
XYL + SC	XYL + SC	0	295^{ab}	0.100^{abc}	3.78^{de}	105^{ab}	0.042^{c}	7.30^{ab}	97^{a}	0.041^{c}	10.49^{a}
		3 μL XYL+2 mg SC	297^{ab}	0.105^{abc}	4.07^{cde}	74^{abc}	0.050^{bc}	6.40^{b}	58^{c}	0.064^{c}	9.54^{abc}
		6 μL XYL + 4 mg SC	240^{b}	0.111^{abc}	4.37^{bcd}	55^{c}	0.055^{abc}	7.73^{ab}	80^{b}	0.049^{c}	10.88^{a}
		Mean	304^{B}	0.094^{AB}	3.73^{B}	86^{B}	0.048^{B}	7.15	68^{A}	0.049^{B}	9.85^{A}
Additive effectiveness (as % of no additive treatment)[5]:											
		XYL	- 8	105	74	- 31	75	9	20	151	9
		SC	- 13	49	94	- 29	16	14	37	49	0
		XYL + SC	- 21	62	39	- 21	9	0	81	20	16
SEM[6]			20.6	0.0119	0.291	7.5	0.0091	0.434	3.2	0.0140	0.327
P value											
Additive type			0.138	0.033	<0.001	0.306	0.022	0.579	<0.001	0.008	0.008
Rumen liquor type			0.007	<0.001	<0.001	<0.001	0.038	0.004	<0.001	0.001	<0.001
Additive dose			0.031	0.255	0.133	0.024	0.551	0.159	<0.001	0.002	0.154
Additive type × Rumen liquor type			0.175	0.027	<0.001	0.017	0.025	0.168	<0.001	0.003	0.030
Additive type × additive dose			0.798	0.468	0.754	0.015	0.109	0.007	<0.001	0.004	0.001

[1]Determined as: $y = b \times [1 - e^{-c\,(t-Lag)}]$, where *y* is the volume of gas production at time *t* (h); *b* is the asymptotic gas, CO_2 or CH_4 production (mL/g DM); *c* is the fractional rate of fermentation (per h), and *Lag* (h) is the discrete lag time prior to any gas, CO_2 or CH_4 formation.
[2]*b* is the asymptotic gas production (mL/g DM); *c* is the rate of gas production (per h); *Lag* is the initial delay before gas formation (h).
[3]*b* is the asymptotic methane production (mL/g DM); *c* is the rate of methane production (per h); *Lag* is the initial delay before methane formation (h).
[4]*b* is the asymptotic carbon dioxide production (mL/g DM); *c* is the rate of carbon dioxide production (per h); *Lag* is the initial delay before carbon dioxide formation (h).
[5]Based on the mean value of each feed additive at different doses.
[6]SEM, standard error of the mean.
(A,B,C) arithmetic mean in the same column with different letters differ ($P < 0.05$) among additives (the mean of all doses for each additive).
(a,b,c,d,e) arithmetic mean in the same row with different letters differ ($P < 0.05$) among doses of different feed additives.

occurred approximately at 24 h of fermentation, and then started to decrease until 48 h of incubation, after which negligible amounts were still released, only productions at 6, 24, and 48 h of incubation were tabulated, while productions at other times of incubation were presented as figures. Table 2 shows the *in vitro* CH_4 and CO_2 productions at 6, 24 and 48 h after incubation as affected by the addition of XYL, SC and a mixture of both in rumen liquor of calves fed on diet supplemented with the same three additives for 60 d of age. At 6, 24 and 48 h of incubation, XYL, SC and XYL + SC mixture did affect CH_4 production (mL/g incubated DM) compared with the control treatment. However, the mixture of XYL and SC had no effect on the CH_4 production at 24 and 48 h compared with their respective controls. Moreover, CH_4 production (mL/g degraded DM) was decreased at the high dose of XYL + SC mixture at 24 and 48 h of incubation. There was an observable reduction in the CH_4 production (mL/g degraded DM) in XYL, SC and mixture of XYL + SC mixture compared with the control. The proportional CH_4 production at 6, 24 and 48 h of incubation was reduced slightly but the reduction was marginal ($P > 0.05$) compared with the control treatment, while addition of additives resulted in a decreased proportional CH_4 production. On the other hand, addition of XYL, SC and XYL + SC mixture increased ($P < 0.05$) the production of CO_2 (mL/g incubated DM) and mL/g degraded DM but had no effect ($P > 0.05$) on the proportional CO_2 production when compared with the control treatment.

4. Discussion

Agricultural wastes are important sources of global GHG emissions which are estimated to rise to about 8.2 billion t of CO_2 equivalents by 2030, if adequate mitigation technique is not properly implemented (Slade et al., 2016). Apart from the impacts of GHG, enteric CH_4 emission contributes to a loss of net feed energy that cannot be used in ruminant animals for production purposes (Johnson and Johnson, 1995). Because of these challenges, intensive research efforts are recently directed towards ruminant animals CH_4 mitigation (Elghandour et al., 2016b). The use of *in vitro* GP technique is a powerful, simple and sensitive screening method for evaluating substrate fermentation or degradation and for monitoring the efficacy of feed additives (Elghandour et al., 2015) and GHG production (Elghandour et al., 2016c). The interactions between additive type and rumen liquor, as well as additive and additive dose, for some measured parameters, suggest that both fermentation kinetics and gas production are rumen liquor and additive-dose dependent, underpinning the importance of identifying optimal supplemental levels of each additive for each rumen liquor type. The addition of enzyme at all doses had no effects on the asymptotic GP. This finding is in agreement with the results of Jalilvand et al. (2008) who observed that the addition of enzyme additives to forage had negligible effects on GP kinetics, and opined that the effects of enzyme addition depend on the fiber content, structural polysaccharide compositions of the substrate and difference in enzyme composition. There were interactions between additive and rumen liquor for rate of GP and initial delay before GP. Enzyme addition significantly affected the rate of GP, which contradicts previous reports on other enzyme preparations and types (Jalilvand et al., 2008). Recent studies including *in vivo* (Morsy et al., 2016) and *in vitro* (Elghandour et al., 2016a) experiments showed that supplementation of ruminant diets with exogenous enzymes could improve feed utilization, digestion of

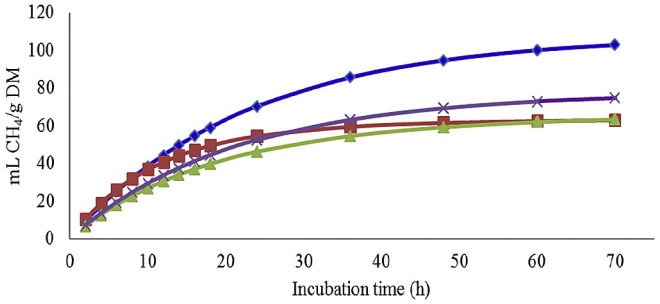

Fig. 3. *In vitro* rumen methane (CH_4) production (mL/g incubated DM) of calf's diet supplemented with: no additive (control) (-◆-), xylanase (-■-), *Saccharomyces cerevisiae* (-▲-), and their mixture (-×-) incubated with rumen inoculum from calves fed on diet supplemented with the same feed additives for 60 d of age.

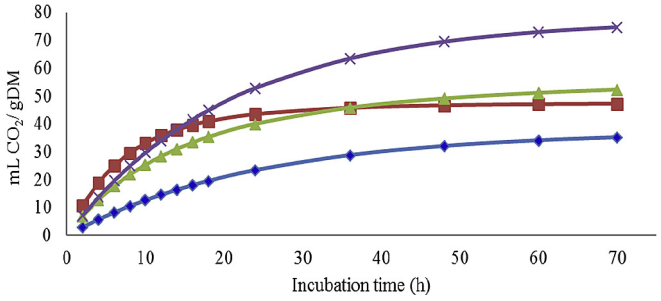

Fig. 4. *In vitro* rumen carbon dioxide (CO_2) production (mL/g incubated DM) of a calf's diet supplemented with: no additive (control) (-◆-), xylanase (-■-), *Saccharomyces cerevisiae* (-▲-), and their mixture (-×-) incubated with rumen inoculum from calves fed on diet supplemented with the same feed additives for 60 d of age.

DM, and animal performance by improving DM degradation (Alsersy et al., 2015).

Ahmed et al. (2015) showed that the supplementation of SC to diets of ruminants improved feed utilization. In contrast, Corona et al. (1999) reported that supplementation of SC to cow diets did not affect digestibility of DM, hemicellulose and starch. Yeast additives had no effect on rate of GP but there was a slight increase in rate of GP compared with the control. This result is in contrast to the work of Rodriguez et al. (2015) who reported a decreased rate of GP in response to SC additives. The differences in results may be due to the composition and incubation of the substrates (Elghandour et al., 2014).

Xylanase, SC and their mixture at all doses, except that of 0 mg dose of XYL + SC mixture, decreased CH_4 production when compared to the control treatment. This pronounced decrease in CH_4 production suggests that the use of XYL, SC or its mixture as additives in ruminant diets may serve as efficient methods to reduce CH_4 emission from ruminant production. Several researches have reported a reduction of CH_4 production with SC supplementation. For instance 58% reduction in CH_4 production have been reported by Newbold and Rode (2006) with the addition of SC in ruminant diets. Besides, Polyorach et al. (2014) noted a decrease in *in vitro* CH_4 production with supplementation of SC, which supports our findings.

Of the several studies which have evaluated the effects of exogenous enzymes on CH_4 emission in the rumen, few reported an absolute increase in production of CH_4 with addition of exogenous enzymes supplementation to the ruminant diets (Beauchemin et al., 2009). Dong et al. (1999) reported 43% increase in CH_4 production when cellulase and XYL were used as supplements with hay in RUSITEC system. Colombatto et al. (2003) reported that there was no effect of enzymes supplement on CH_4 production in continuous culture system. McGinn et al. (2004) observed no effect on CH_4 production in steers fed with barley silage-based diets supplemented with different feed additives including exogenous enzymes. In contrast, Kholif et al. (2016) reported that the addition of enzymes at certain doses reduced CH_4 production in equine diets. Salem et al. (2015b) observed the same results in horses fed diet supplemented with exogenous enzymes. In the present study, addition of enzymes at all doses decreased CH_4 production. This may be due to the possible stimulation of reductive acetogens in the rumen that alters hydrogen (H_2) metabolism and its utilization by methanogens in a manner that reduces CH_4 formation and emissions (Stewart et al., 1997). The reduction in CH_4 production by the addition of a combination of XYL and SC at high dose depicts a positive impact on rumen fermentation, although the observed pronounced decrease in CH_4 production at high dose of the added additive was accompanied by a slight decrease in asymptotic GP, indicating a direct inhibitory effect of rumen fermentation kinetics.

Many gases consisting of mainly CH_4, CO_2 and H_2 are produced during ruminal fermentation process within the rumen. In this study, addition of additives at all doses slightly increased CO_2 production at 6, 24 and 48 h of incubation. At 6 h of incubation, there was no CO_2 production in all the additives as well as in the control. Decreased CO_2 production below the control treatment was observed at 6 μL XYL/g DM and 0 mg SC/g DM. This reduction in CO_2 production and decrease in rate of CO_2 may be due to increased cell wall content that can reduce the microbial activities. Elghandour et al. (2016c) reported a decreased CO_2 production when corn grain was replaced with soybean hulls. Elghandour et al. (2016d) observed that replacement of corn grain with prickly pear cactus increased CO_2 production. However, both experiments used the same organic acid addition, indicating that the observed different effect may be ration dependent. To the best of our knowledge, there is little information on the effects of supplementing diets of ruminants with enzymes and SC additives on CO_2 production which makes it difficult to compare the present results with previous results. The asymptotic CO_2 production recorded the highest values for 2 mg SC/g DM (90 mL/g DM) and (97 and 80 mL/g DM) for 0 and high dose of XYL + SC mixture; the three treatments had greater productions than the control treatment (38 mL/g DM). In this study, inclusion of additives had an increasing effect on asymptotic CO_2 production.

5. Conclusions

Methane and CO_2 from enteric fermentation in the digestive system of ruminants are two major contributors of greenhouse gas emissions in the world. Mitigating the loss of these gases from ruminant production will not only reduce greenhouse gas production from agricultural wastes but also will decrease loss of net feed energy to the animal. This study demonstrated that supplementing ruminant's diets with xylanase, *S. cerevisiae* and their mixture at different doses for 60 d of age changed the pattern of ruminal production of gas, CH_4 and CO_2. Addition of a mixture of xylanase and *S. cerevisiae* at a high dose significantly reduced asymptotic gas production compared with other treatments with dose-dependent results. Inclusion of xylanase, *S. cerevisiae* and their mixture reduced asymptotic gas production to <1%. Again, addition of additives in the diets of ruminants at all doses had statistically significant reduction effect on CH_4 production. The pronounced decrease in CH_4 production shows that the use of xylanase, *S. cerevisiae* or their mixture as additives in ruminant diets may serve as efficient method to reduce CH_4 emission from ruminant production. This study also established that the mixture of xylanase and *S. cerevisiae* was more efficient and promising in

Table 2
In vitro dry matter disappearance (DMD), and production of methane (CH_4) and carbon dioxide (CO_2) at 6, 24 and 48 h after incubation as affected by addition of xylanase (XYL), yeast (SC) and mixture of both in rumen liquor of calves fed on diet supplemented with xylanase and/or yeast for 60 d of age.

Rumen liquor from calves fed on:	Additive:	Dose (mg/g DM)	DMD	CH_4 production									CO_2 production								
				mL/g incubated DM			mL/g degraded DM			Proportional CH_4 production			mL/g incubated DM			mL/g degraded DM			Proportional CO_2 production		
				6 h	24 h	48 h	6 h	24 h	48 h	6 h	24 h	48 h	6 h	24 h	48 h	6 h	24 h	48 h	6 h	24 h	48 h
Control	No additive	0	693	25	70.2^{Aa}	94.7^{Aa}	2.47	85	128^{Aa}	1.53^{Aa}	21^{A}	25^{A}	8^{Bb}	24^{Bd}	32^{Ce}	0	34	65	0	8	13
XYL	XYL	0 μL	656	29	60^{ab}	66^{abc}	2.56	72	82^{ab}	0.79^{ab}	13	15	34^{a}	56^{ab}	58^{bc}	0	64	70	0	12	12
		3 μL	644	28	55^{ab}	60^{bc}	2.22	78	91^{ab}	0.70^{ab}	15	17	31^{a}	49^{abc}	50^{cd}	0	72	78	0	14	14
		6 μL	642	22	49^{ab}	59^{c}	2.59	63	81^{ab}	1.00^{ab}	14	17	11^{b}	26^{d}	33^{e}	0	53	68	0	12	14
		Mean	659	26	59^{AB}	70^{B}	2.46	75	96^{B}	1.00^{B}	16^{AB}	19^{B}	21^{A}	39^{A}	43^{B}	0	56	70	0	11	13
SC	SC	0 mg	665	16	43^{b}	56^{c}	0.85	53	79^{ab}	0.51^{b}	13	16	11^{b}	25^{d}	30^{e}	0	37	51	0	9	11
		2 mg	671	22	54^{ab}	66^{abc}	1.41	60	90^{ab}	0.62^{b}	14	19	$31.^{a}$	65^{a}	77^{a}	0	115	137	0	25	28
		4 mg	625	16	42^{b}	56^{c}	1.42	59	84^{ab}	0.58^{b}	13	17	11^{b}	30^{cd}	41^{de}	0	64	81	0	13	15
		Mean	664	20	52^{B}	68^{B}	1.54	64	95^{B}	0.81^{B}	15^{B}	19^{B}	15^{AB}	36^{A}	45^{B}	0	63	83	0	14	17
XYL + SC	XYL + SC	0	681	23	67^{ab}	91^{ab}	2.48	77	102^{ab}	1.28^{ab}	20	24	21^{ab}	60^{ab}	83^{a}	0	52	74	0	13	16
		3 μL XYL + 2 mg SC	674	19	50	66^{abc}	2.20	58	77^{ab}	1.09^{ab}	15	18	18^{ab}	44^{bcd}	54^{cd}	0	39	49	0	10	12
		6 μL XYL + 4 mg SC	678	16	40^{b}	51^{c}	1.80	53	68^{b}	1.13^{ab}	17	19	20^{ab}	55^{ab}	72^{ab}	0	50	68	0	15	19
		Mean	681	21	57^{AB}	76^{B}	2.24	68	94^{B}	1.26^{AB}	18^{AB}	21^{AB}	17^{AB}	46^{A}	60^{A}	0	44	64	0	12	15
Additive effectiveness (as % of no additive treatment)[1]																					
	XYL		- 5	2.8	−17	- 26	- 0.4	- 12	- 25	- 35	- 24	- 26	154	64	34.27	0	63	8.	0	36	6
	SC		- 4	- 21	−25	- 28	- 38	- 24	- 26	- 47	- 28	- 22	88	53	40	0	83	29	0	67	31
	XYL + SC		- 2	- 17	−19	- 20	- 9	- 20	- 27	- 18	- 13	- 14	105	94	88	0	28	- 0.8	0	39	17
SEM[2]			23.8	2.9	5.3	6.1	0.400	9.5	10.4	0.175	2.0	2.1	3.4	4.1	3.0	0	17.8	23.6	0	3.4	4.3
P value																					
Additive type			0.433	0.065	0.170	0.219	0.128	0.439	0.784	0.208	0.607	0.632	0.054	<0.001	<0.001	1.000	0.775	0.937	1.000	0.962	0.962
Rumen liquor type			0.040	0.347	0.004	<0.001	0.134	0.032	<0.001	<0.001	0.003	0.001	<0.001	<0.001	<0.001	1.000	0.257	0.983	1.000	0.330	0.926
Additive dose			0.422	0.083	0.013	0.008	0.712	0.496	0.476	0.872	0.936	0.840	<0.001	<0.001	<0.001	1.000	0.062	0.178	1.000	0.031	0.092
Additive type × Rumen liquor type			0.431	0.111	0.090	0.025	0.076	0.434	0.499	0.107	0.158	0.081	0.008	<0.001	<0.001	1.000	0.749	0.876	1.000	0.859	0.822
Additive × additive dose			0.228	0.835	0.361	0.048	0.575	0.443	0.202	0.566	0.245	0.121	0.003	0.001	<0.001	1.000	0.694	0.753	1.000	0.589	0.661

[1]Based on the mean value of each feed additive at different doses.
[2]SEM, standard error of the mean.
(A,B,C) arithmetic mean in the same column with different letters differ ($P < 0.05$) among additives (the mean of all doses for each additive).
(a,b,c,d,e) arithmetic mean in the same row with different letters differ ($P < 0.05$) among doses of different feed additives.

reducing gas and methane emissions arising from ruminant production. If this mitigation practice is adopted, it can serve as an environmental friendly way of feeding livestock leading to cleaner environmental production conditions in calf farming.

Conflict 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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