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Mitigation of ruminal biogases production from goats using *Moringa oleifera* extract and live yeast culture for a cleaner agriculture environment

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ABSTRACT

The present investigation was assessed to explore the sustainable mitigation of methane and carbon dioxide production from goats using *Moringa oleifera* extract and live yeast culture (*Saccharomyces cerevisiae*) as feed supplements. Treatments include supplementation of 0 (control), 0.6, and 1.8 mL/g dry matter of *M. oleifera* extract and 0 (control), 2, and 4 mg/g dry matter of commercially available *S. cerevisiae* into the feeding diet. Higher doses of *M. oleifera* extract and *S. cerevisiae* increased the asymptotic gas production from 88.8 to 147.5 mL/g dry matter. The fractional rate of gas production was increased ($P < 0.05$) due to the supplementation of *M. oleifera* extract and *S. cerevisiae*. Lag time increased linearly from 1.32 to 3.99 h but only *M. oleifera* extract affected it quadratically ($P = 0.041$). The asymptotic methane production, rate of methane emission, and lag time decreased ($P > 0.05$) with the varied doses of additives. *M. oleifera* extract × *S. cerevisiae* interaction had non-significant ($P > 0.05$) influence on asymptotic carbon dioxide emission, fractional rate of carbon dioxide emission, and lag time. Furthermore, the inclusion of *S. cerevisiae* exhibited increased gas production in a time dependent manner. The proportional methane production was estimated to be decreased ($P > 0.05$) at high doses of *M. oleifera* extract and *S. cerevisiae* at 72 h of incubation with the lowest emission of 11.7%. In contrary to this, the proportional carbon dioxide production was reduced (quadratic effect, $P = 0.031$) at 72 h of incubation with the lowest emission of 50.3%. In conclusion, the addition of *M. oleifera* extract and *S. cerevisiae* in diets would be an invaluable approach for mitigating methane and carbon dioxide emission from goats. These additives at diversified concentrations may be utilized as pronounced cleaner product and additive agents for the ecosystem as well as livestock.

1. Introduction

The perpetual production of greenhouse gases (GHG), particularly methane (CH_4) and carbon dioxide (CO_2) from livestock due to ruminal fermentation is the huge burden for ruminant nutritionists globally. These GHG are considered not only environmental pollutants but also hazardous to human health. The emission of CH_4 and CO_2 from livestock is an energetically extravagant mechanism, contributing about 18% and 9% of all GHG emissions, respectively (FAO, 2006). The production of GHG into the ecosystem is the preeminent cause of global warming (Elghandour et al., 2017a,b). Researchers have focused on the manipulation of ruminal microbiota as well as fermentation system through diversified means in order to improve feed utilization, and mitigate the production of detrimental gases. Conventional antibiotics have exhibited promising influence on the utilization of feed, but the European Union has banned their further exploitation in view of the public concern. The quest for auspicious natural alternative resources to mitigate

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the emission of GHG for cleaner society and sustainable environment has gained immense interest among worldwide veterinarians.

Researchers concentrated on the safer utilization of distinct natural feed additives for estimating the emission of biogases. Elghandour et al. (2016) estimated increased GHG productions due to the supplementation of organic acid salt into prickly pear cactus flour. The addition of fibrolytic enzymes increased *in vitro* biogas production and implied its effectiveness in enhancement of rumen fermentation (Vallejo et al., 2016). The supplementation of garlic oil into high concentrate diet revealed reduction *in vitro* CH_4 and CO_2 emission from dairy calves (Hernandez et al., 2017). Various strains of *Saccharomyces cerevisiae* improved fermentation kinetics and enhanced the emission of gases from sheep (Elghandour et al., 2017a,b). The supplementation of diverse doses of *Lactobacillus farciminis* into oat straw exhibited the increment in asymptotic gas emission from horses (Elghandour et al., 2018). Diversified feed supplements may improve animal performances and may alter the production of gas pollutants into the ecosystem (Johnson and Johnson, 1995). Few supplements metabolize hydrogen for other mechanism than its utilization with methanogenic microbes which causes reduction in CH_4 emission (Reddish and Kung, 2007).

Phytogenic metabolites are non-toxic and are generally known to modify ruminal fermentative mechanism (Salem et al., 2014a). Presence of potent bioactive secondary metabolites and their sources indicates the efficiency of those supplements in livestock industries (Kholif et al., 2015). *Moringa oleifera* (Moringaceae), commonly called as 'drumstick tree' is a multipurpose drought-tolerant tropical tree that has numerous ethno-pharmacological and agricultural uses. Leaves of this plant are valuable sources of protein for ruminants which have a moderate palatability. According to the US Food and Drug Administration, the utilization of *Saccharomyces cerevisiae* as feed supplements for livestock is considered as safe. Yeast exhibits potentiality to maintain rumen fermentative process by improving the viable counts of microbiota (Jouany, 2001). The probiotic yeasts are known to stimulate bacterial activity within the rumen of cattle and avoid any kind of disorder in rumen (Pinloche et al., 2013). Yeasts are also responsible for enhancing the utilization of ammonia by ruminal microbiota (Chaucheyras-Durand et al., 2008).

While incorporation of natural feed additives as technological management system for reducing GHG emissions in livestock, it is often recommended to choose energy efficient and climate friendly supplements. Among the bioenergy additives, previous studies focused on the utilization of distinct plant sources and few potent probiotics. This is mainly due to the fact that those additives are not only less energy intensive but also do not compete with feed property (Djomo et al., 2015). Most of the plant sources used in livestock industries are seasonal and their impact on the slight reduction of detrimental gases production from ruminants/non-ruminants emphasized the worldwide researchers for the exploitation of prominent natural sources as new feed supplement. To the best of our knowledge, the combination of *M. oleifera* with *S. cerevisiae* as alternative feed sources in goat nutrition for mitigating the emission of GHG is not evidenced yet. In order to reduce the GHG emission from livestock, the synergistic role of *M. oleifera* and *S. cerevisiae* as alternative resources could be a promising approach in the current scenario. Considering the prominent role of plants as wells as yeasts in livestock industries, a significant attempt was undertaken in this context to fill the gap of research by determining the fermentation kinetics and GHG production mitigation attributes of *M. oleifera* as well as *S. cerevisiae* in goats not only as a process for a cleaner and eco-friendly products but also unique approaches for understanding livestock feed fermentation property.

2. Materials and methods

2.1. Location of the study

Experiments were carried out in the Animal Nutrition Laboratory, Faculty of Veterinary Medicine and Animal Science, Autonomous University of the State of Mexico. Ruminal liquid donors were treated and managed in accordance with Mexican official standards for animal care.

2.2. Plant extract preparation

Fresh leaves of *M. oleifera* were collected from Veracruz, Mexico and were ground into powder form using mixer. Ten grams of powder were immersed into 90 mL of distilled water. Extraction was performed in closed jars for 72 h at 28 °C, followed by a second extraction at 39 °C for 1 h. The extract was filtered through gauze and preserved at 4 °C.

2.3. Substrates and treatments

In vitro fermentation was carried out using a balanced diet based on 75% forage and 25% concentrate. Treatments include supplementation of 0, 0.6, and 1.8 mL/g dry matter (DM) of *M. oleifera* extract and 0, 2, and 4 mg/g DM of commercially available *S. cerevisiae* (2×10^{10} cfu) into the feeding diet. The composition of the basal diet used as substrate during *in vitro* fermentation and feed for goats (Nubia × Criollo; approximately 18 ± 3 kg live weight) consisted of 40% ground oat straw and 60% of a mixture of ground corn (36%), soybean paste (12%), urea (1%), molasses (7%), sunflower oil (3%), and vitamin and mineral premix (1%). A control substrate (without supplementation of *M. oleifera* extract and *S. cerevisiae*) was also used in the study.

2.4. In vitro incubations

Rumen inoculum was collected in the morning before feeding 10 male goats, housed in individual cages of approximately 1.1 m × 1 m. Initial feeding was formulated for goats as per the National Research Council (NRC, 2001). Goats were provided fresh water during the inoculum collection phase. Rumen content of goats was rinsed with CO_2 and filtered using cheese cloth in a flask with oxygen-free space. The collected rumen fluid was brought to the laboratory, and then mixed with buffer solution (1:4 v/v), avoiding the addition of trypticase (Goering and Van Soest, 1970). Rumen fluid was diluted and then added to incubation bottles containing pre-weighed substrates (0.5 g of DM) and additive solutions. Three incubation runs were performed in three weeks. Bottles containing the samples [three doses of *M. oleifera* extract (0, 0.6, and 1.8 mL/g of diet DM) × three doses of commercially *S. cerevisiae* (0, 2, and 4 mg/g DM) × three different runs] plus three bottles as blanks (rumen fluid only) were incubated for 72 h.

2.5. Estimation of CH_4 and CO_2 emission

Three replications were made in substrates containing bottles. Bottles were filled, closed using rubber stoppers, mixed, and incubated at 39 °C in water bath. The gas production (GP) was estimated up to 72 h using a pressure transducer (Extech Instruments, Waltham, U.S.) as per the methodology of Theodorou et al. (1994). The CH_4 and CO_2 levels in the upper space of bottles were estimated up to 72 h using a diffusion-based gas detector (CROWCON Gas Analyzer Model Tetra3, Abingdon, United Kingdom). pH was measured after 72 h using a digital pH meter (Conductronic pH15.0, Puebla, Mexico). Residues collected after

vacuum filtration was dried at 65 °C for calculating DM degradability (DMD) (Orskov and McDonald, 1979).

2.6. Calculations and statistical analyses

The kinetic parameters of GP, CH_4, and CO_2 were calculated by NLIN option of SAS (2002) as per the below mentioned equation of France et al. (2000).

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

where: A is the volume of GP, CH_4, or CO_2 at time t; *b* is the asymptotic GP, CH_4, or CO_2 (mL/g DM); *c* is the rate of GP, CH_4, or CO_2 (/h), and *Lag* (h) is the discrete lag time prior to GP, CH_4, or CO_2.

Experiments were completely randomized with repeated measures in time. However, data of each of the three runs within the same treatment of each of the three individual treatments doses (*M. oleifera* extract and/or *S. cerevisiae*) were averaged prior to statistical analysis, then mean values of each individual sample were used as the experimental unit. Analysis was done using statistical model (Elghandour et al., 2017a,b) as follows:

$$y_{ijk} = \mu + d_i + a(d)_{j(i)} + p_k + (dp)_{ik} + e_{ijk} \quad (2)$$

where, y_{ijk} is the value measured at period k (day of rumen collection) on the j^{th} goats assigned to the i^{th} plant, μ the overall mean effect, d_i is the i^{th} fixed plant effect, $a(d)_{j(i)}$ is the random effect of the j^{th} goats within the *i*th extract, p_k is the fixed k^{th} period (age time) effect when the measurement was taken, $(dp)_{ik}$ is the fixed interaction effect between plant and period, and ε_{ijk} is the random error associated with the j^{th} goats assigned to the i^{th} diet at period k. Data were estimated using MIXED procedure of SAS (2002) for repeated measures. Results shown in tables were least square means of fixed effects with their corresponding standard errors.

3. Results

3.1. In vitro fermentation kinetics

Despite the increment in asymptotic GP at higher doses of *M. oleifera* extract and *S. cerevisiae*, the effect was observed to be non-significant ($P > 0.05$). The fractional rate of GP was significantly increased due to the supplementation of *M. oleifera* extract (linear = 0.004; quadratic = 0.024) and *S. cerevisiae* (linear = 0.006; quadratic = 0.005). Lag time increased linearly (1.32–3.99 h) but only *M. oleifera* extract affected it quadratically ($P = 0.041$). *M. oleifera* extract × *S. cerevisiae* interaction had non-significant ($P > 0.05$) effect on asymptotic total GP, rate of GP, and lag time. The asymptotic CH_4 emission (22.4 mL CH_4/0.5 g DM), rate of CH_4 emission (0.01/h), and lag time (8.19 h) reduced non-significantly ($P > 0.05$) due to varied doses of *M. oleifera* extract, *S. cerevisiae*, and *M. oleifera* extract × *S. cerevisiae* interaction. The supplementation of *M. oleifera* extract and *S. cerevisiae* showed non-significant ($P > 0.05$) impact on the asymptotic and fractional rate of CO_2 production. *S. cerevisiae* decreased (linear = 0.029) the lag time (2.43 h). *M. oleifera* extract × *S. cerevisiae* interaction had no significant ($P > 0.05$) influence on asymptotic CO_2 emission, fractional rate of CO_2 emission, and lag time (Table 1).

3.2. Ruminal gas production

The fermentation pH and DMD were found to be non-significant ($P > 0.05$) because of the inclusion of *M. oleifera* extract. The supplementation of *S. cerevisiae* affected significantly (linear = 0.004) the DMD. *M. oleifera* extract × *S. cerevisiae* interaction revealed significant ($P = 0.031$) impact on DMD (Table 2).

Fig. 1 illustrates *in vitro* ruminal GP (mL/0.5 g incubated DM) from goats as influenced due to the dietary inclusion of varied levels of *M. oleifera* extract and *S. cerevisiae*. The supplementation of *S. cerevisiae* with respect to *M. oleifera* extract estimated the enhanced amount of GP in a time dependent manner. The supplementation of *M. oleifera* extract and *S. cerevisiae* exhibited no significant ($P > 0.05$) increment in GP up to 72 h with respect to the control. The GP (mL/0.5 g degraded DM) was increased at all incubation periods, showing maximum production of 206 (mL/0.5 g degraded DM) at 72 h due to inclusion of *M. oleifera* extract with *S. cerevisiae* but the production was not significant (linear and quadratic, $P > 0.05$) (Table 2).

Table 1
Effect of *M. oleifera* and *S. cerevisiae* at different concentrations as feed additives on *in vitro* rumen total gas production, CH_4 and CO_2 kinetics.[1].

M. oleifera extract (mL/g DM)	*S. cerevisiae* (mg/g DM)	Total gas production			CH_4			CO_2		
		b	*c*	*Lag*	*b*	*c*	*Lag*	*b*	*c*	*Lag*
0	0	95.4	0.046	1.32	31.3	0.011	9.05	64.7	0.02	4.83
0	2	128.4	0.027	3.33	41.6	0.012	8.90	84.0	0.017	7.36
0	4	88.8	0.069	1.69	77.8	0.01	13.64	45.0	0.019	2.43
0.6	0	128.8	0.023	3.69	51.4	0.013	14.18	87.7	0.018	8.75
0.6	2	107.9	0.019	5.60	58.2	0.013	14.56	81.8	0.018	8.18
0.6	4	101.1	0.041	3.99	33.9	0.015	13.87	62.3	0.019	8.06
1.8	0	121.6	0.027	1.95	77.6	0.014	16.68	61.9	0.021	12.01
1.8	2	147.5	0.029	0.57	46.8	0.013	12.04	100.8	0.019	6.62
1.8	4	129.1	0.033	2.46	22.4	0.013	8.19	86.8	0.02	4.98
Pooled SEM[2]		16.8	0.004	1.25	11.4	0.001	3.18	10.0	0.0009	1.10
Additive effect:										
Extract										
Linear		0.22	0.004	0.231	0.934	0.117	0.774	0.242	0.448	0.057
Quadratic		0.753	0.024	0.041	0.89	0.214	0.233	0.789	0.347	0.123
S. cerevisiae										
Linear		0.683	0.006	0.896	0.558	0.872	0.782	0.653	0.77	0.029
Quadratic		0.383	0.005	0.083	0.987	1.0	0.354	0.131	0.241	0.67
Extract × *S. cerevisiae*		0.834	0.105	0.836	0.106	0.817	0.511	0.609	0.874	0.207

[1] *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).
SEM[2], Standard error of mean.

Table 2
Effect of *M. oleifera* and *S. cerevisiae* at different concentrations as feed additives on *in vitro* rumen fermentation parameters as well as total gas production at different incubation period (h).

M. oleifera extract (mL/g DM)	*S. cerevisiae* (mg/g DM)	Fermentation parameters			Gas production (mL/0.5 g dry matter incubated)			Gas production (mL/0.5 g dry matter degraded)			
		pH	DMD[1]	8	24	48	72	8	24	48	72
0	0	6.7	52.4	32.1	66.5	82.8	96.2	35.3	72.7	90.9	105.3
0	2	6.7	72.2	44.2	84.2	114.5	132.8	64.0	121.4	164.6	191.2
0	4	6.8	70.5	41.3	74.4	81.6	91.6	56.4	104.3	114.1	128.0
0.6	0	6.7	68.0	47.9	85.0	118.9	135.9	66.1	116.4	162.4	185.5
0.6	2	6.7	71.5	38.0	65.1	96.3	110.6	54.0	92.5	136.8	157.1
0.6	4	6.6	68.8	39.7	72.0	94.8	107.4	54.3	98.6	129.4	146.6
1.8	0	6.8	65.5	42.3	72.4	108.2	122.6	56.6	96.5	143.1	162.1
1.8	2	6.8	66.1	69.1	107.3	146.2	156.4	91.1	141.5	192.6	206.0
1.8	4	6.7	72.8	57.6	94.9	125.6	138.3	83.5	138.3	183.2	202.0
Pooled SEM[2]		0.04	2.03	9.11	12.62	15.57	17.18	12.57	17.54	21.75	13.98
Additive effect:											
Extract											
Linear		0.687	0.26	0.175	0.337	0.121	0.175	0.15	0.279	0.103	0.145
Quadratic		0.097	0.219	0.56	0.511	0.706	0.792	0.649	0.611	0.828	0.918
S. cerevisiae											
Linear		0.611	0.004	0.649	0.723	0.897	0.795	0.462	0.419	0.721	0.799
Quadratic		0.945	0.136	0.51	0.582	0.35	0.37	0.451	0.491	0.281	0.288
Extract × *S. cerevisiae*		0.714	0.031	0.804	0.744	0.736	0.742	0.755	0.64	0.592	0.56

[1]DMD is dry matter degradability.
SEM[2], Standard error of mean.

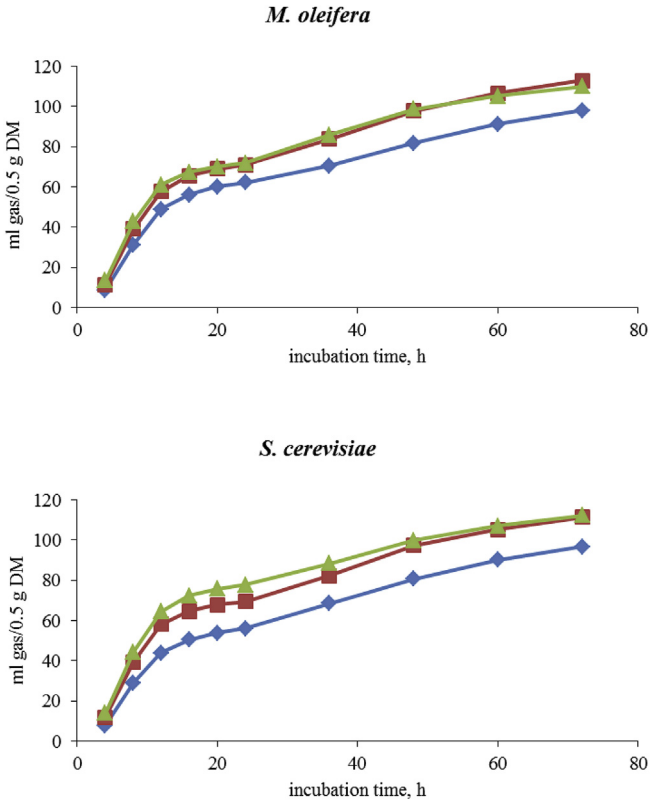

Fig. 1. Ruminal GP (mL/0.5 g incubated DM) in goats as affected by the dietary inclusion of *M. oleifera* extract [0 (-◆-), 0.6 (-■-), and 1.8 (-▲-) mL/g DM] and *S. cerevisiae* [0 (-◆-), 2.0 (-■-), and 4.0 (-▲-) mg/g DM].

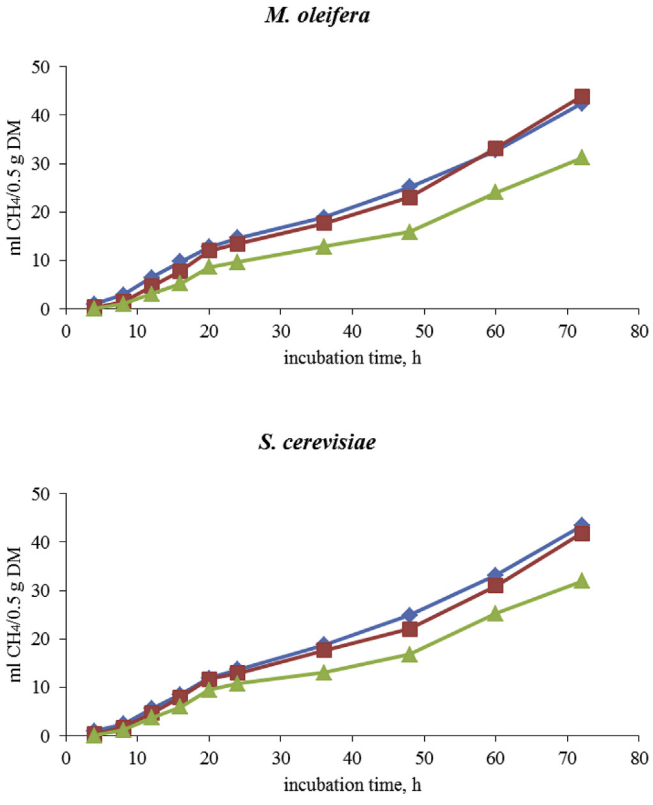

Fig. 2. Ruminal CH_4 production (mL/0.5 g incubated DM) in goats as affected by the dietary inclusion of *M. oleifera* extract [0 (-◆-), 0.6 (-■-), and 1.8 (-▲-) mL/g DM] and *S. cerevisiae* [0 (-◆-), 2.0 (-■-), and 4.0 (-▲-) mg/g DM].

3.3. *Ruminal CH_4 production*

In vitro ruminal CH_4 emission (mL/0.5 g incubated DM) as influenced due to the inclusion of varied levels of *M. oleifera* extract and *S. cerevisiae* in diet fed to goats is shown in Fig. 2. The supplementation of *M. oleifera* extract and *S. cerevisiae* revealed mitigation in CH_4 emission (mL/0.5 g incubated DM) with respect to the control diet. No significant differences were reported for *M. oleifera* extract × *S. cerevisiae* interaction. On the other hand, CH_4 emission (mL/0.5 g degraded DM) was improved ($P > 0.05$) at 8, 24, 48, and 72 h due to the inclusion of *M. oleifera* extract and

Table 3
Effect of *M. oleifera* and *S. cerevisiae* at different concentrations as feed additives on *in vitro* rumen CH_4 production at different incubation period (h).

M. oleifera extract (mL/g DM)	*S. cerevisiae* (mg/g DM)	mL CH_4/0.5 g dry matter incubated				mL CH_4/0.5 g dry matter degraded				Proportional CH_4 production			
		8	24	48	72	8	24	48	72	8	24	48	72
0	0	0.4	9.9	15.3	28.8	0.5	10.9	17.2	31.8	1.4	14.5	17.8	29.1
0	2	2.2	14.7	21.7	38.5	3.2	21.2	31.2	55.4	3.5	12.2	13.3	20.3
0	4	0.8	9.9	15.7	30.3	1.07	14.1	22.4	43.02	1.4	8.4	11.8	21.0
0.6	0	0.8	13.5	23.2	46.1	1.1	18.4	31.7	63.2	1.5	11.9	14.4	24.7
0.6	2	1.7	14.8	26.7	54.3	2.5	20.8	37.6	76.8	2.8	14.9	18.4	33.6
0.6	4	0.3	10.7	16.8	32.4	0.5	14.9	23.1	44.5	0.7	10.9	13.1	22.3
1.8	0	0.7	10.5	18.9	39.5	0.9	13.8	24.7	51.8	2.3	14.3	15.5	26.6
1.8	2	0.6	12.8	22.4	46.08	0.9	16.8	29.2	60.3	0.7	9.1	11.8	22.5
1.8	4	1.7	9.4	12.5	23.07	2.5	13.8	18.3	33.7	2.08	6.9	6.9	11.7
Pooled SEM[1]		0.4	2.92	5.11	7.82	0.56	4.1	7.11	10.87	0.94	1.8	2.45	2.78
Additive effect:													
Extract													
Linear		0.825	0.88	0.956	0.728	0.845	0.913	0.96	0.72	0.516	0.512	0.387	0.396
Quadratic		0.779	0.586	0.433	0.260	0.807	0.521	0.381	0.209	0.634	0.421	0.368	0.119
S. cerevisiae													
Linear		0.596	0.736	0.536	0.354	0.52	0.98	0.724	0.548	0.577	0.051	0.11	0.03
Quadratic		0.126	0.312	0.277	0.163	0.109	0.269	0.242	0.132	0.161	0.659	0.656	0.376
Extract × *S. cerevisiae*		0.302	0.995	0.992	0.94	0.244	0.962	0.971	0.891	0.124	0.674	0.759	0.235

Means in the same column with different superscripts differ significantly ($P < 0.05$).
SEM[1], Standard error of mean.

S. cerevisiae. The proportional CH_4 production was estimated to be decreased (11.7%) with high doses of *M. oleifera* extract and *S. cerevisiae* at 72 h. The proportional CH_4 emission was not influenced ($P > 0.05$) by *M. oleifera* extract × *S. cerevisiae* interaction (Table 3).

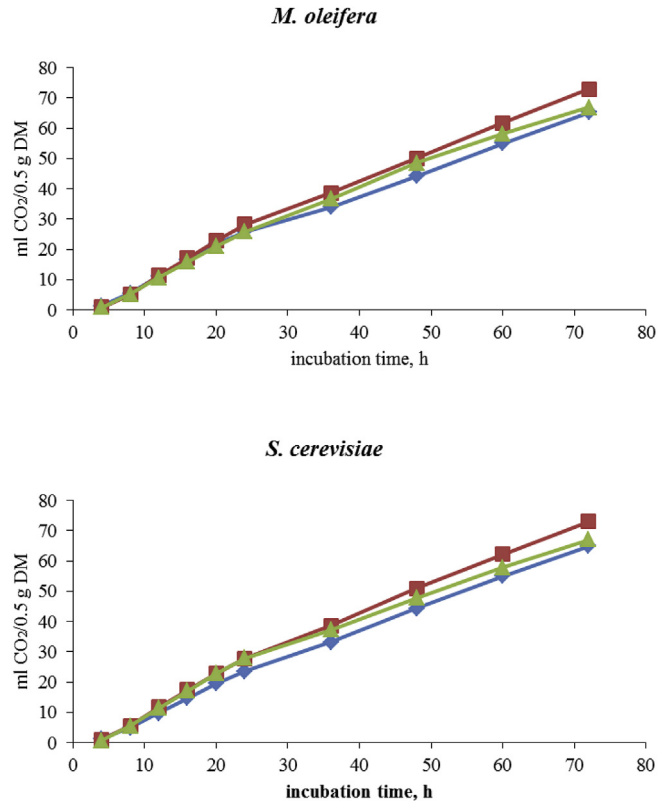

Fig. 3. Ruminal CO_2 production (mL/0.5 g incubated DM) in goats as affected by the dietary inclusion of *M. oleifera* extract [0 (-♦-), 0.6 (-■-), and 1.8 (-▲-) mL/g DM] and *S. cerevisiae* [0 (-♦-), 2.0 (-■-), and 4.0 (-▲-) mg/g DM].

3.4. Ruminal CO_2 production

Fig. 3 depicts *in vitro* ruminal CO_2 emission (mL/0.5 g incubated DM) because of the inclusion of varied levels of *M. oleifera* extract and *S. cerevisiae* in diet fed to goats. No significant differences were estimated on CO_2 production (mL/0.5 g incubated DM) due to the inclusion of *M. oleifera* extract and *S. cerevisiae*. The CO_2 production (mL/0.5 g degraded DM) was increased at varied doses of *M. oleifera* extract and *S. cerevisiae* but results obtained were not significant ($P > 0.05$). The proportional CO_2 production was reduced significantly (quadratic effect, $P = 0.031$) at 72 h of incubation. *M. oleifera* extract × *S. cerevisiae* interaction mitigated the proportional CO_2 production significantly ($P < 0.05$) at 8, 24, 48, and 72 h of incubation period (Table 4).

4. Discussion

Sustainable ruminant husbandry requires diminished influence on natural vegetation, improved animal wellbeing, and regulation of the abundance of rumen fermentation gases (CH_4 and CO_2) emitted into the environment. Anaerobic digestion is an efficient tool to provide cleaner environment by reducing GHG emissions from digestate (Huopana et al., 2013). Moraes et al. (2017) studied the proficient role of anaerobic digestion treatment on the reduction of GHG release from organic waste. CH_4 and CO_2 emission during fermentation in ruminants was reported to cause a loss in dietary energy of 2–12% (Johnson and Johnson, 1995). The improvement of animal performances by mitigating the emission of detrimental gases into the ecosystem is an urgent call of this hour.

According to Liua et al. (2018), it is imperative to understand the influence of diverse methods on varied industries on the socio-economic system to identify the most effective GHG mitigation approaches. Livestock are being regarded as leading responsible factors towards the significant contribution in GHG emission. Proper management system in livestock industries may help reduce the production of detrimental gases (Vasconcelos et al., 2018). The significant mitigation of GHG production from animals is obtained by modifying the ruminant's feed using diversified

supplements. An additive should not only be inexpensive and easily available but also has the potentiality to modify the rumen fermentative mechanism without leaving any residue in the animal products. Considering the emerging public concern of conventional antibiotics and prominent cause for the development of multiple drugs resistance microorganisms, several natural additives have been supplemented into the feeding diets of animals as effectual sources for manipulating ruminal microbial ecosystem. Phytogenic extracts and yeasts cells have been considered as requisite alternatives to conventional auspicious agents that might be supplemented for modulating the rumen fermentative process (Elghandour et al., 2017a,b).

In this context, higher doses of *M. oleifera* extract and *S. cerevisiae* caused non-significant ($P > 0.05$) increment in asymptotic GP *in vitro*. Higher GP indicates the ruminal fermentation of feeds at better extent, causing greater availability of nutrients for livestock (Salem et al., 2014b). *M. oleifera* extract and *S. cerevisiae* successfully provided enough nutrients to ruminal microbiota, resulting in good feed degradability and fermentation. The microbial growth and feed accessibility suggest the fermentation rate of components (Medjekal et al., 2017). Previous report depicted that phytocomponents at diversified doses improved rumen fermentative trait and GP. The administration of *Salix babylonica* extract tended to increase ruminal GP with improved weight gain in growing lambs (Cedillo et al., 2014). The gramineous and leguminous forages reduced ruminal CH_4 emission with advancing harvest date which might be due to the variations in the chemical composition and condensed tannin in forages (Rong-zhen et al., 2016). The increased GP because of *M. oleifera* extract inclusion suggests the presence of fermentable and digestible saccharides in phyotonstituents. In this study, the fractional rate of GP was estimated to be significant due to the supplementation of *M. oleifera* extract. Lag time increased linearly but only *M. oleifera* extract affected it quadratically. The improvement of GP likely induced higher availability of nutrients to animals. The supplementation of *M. oleifera* extract delayed the adaptation strategies of microbes to the feeds, depicting extended lag period and GP. Reduced lag phase indicates its easy contribution for providing significant amount of nutrients (Salem et al., 2007). It should be noteworthy that the variation observed in responses of plants towards GP factors might be because of the concentration and nature of bioactive components, activity towards ruminal microbiota, and other pivotal parameters that may influence the stability of phytocomponents. The genome characteristics of plant species may also be a plausible factor towards alterating *in vitro* GP among distinctly fermented feeds (Elghandour et al., 2017a,b).

The incorporation of *S. cerevisiae* into diet showed increased impact on GP from goats. The inclusion of yeast induces the cellulolytic activity of microbes present in the hindgut, resulting in increased digestion of fibre (Jouany et al., 2009). Lattimer et al. (2005) suggested that the incorporation of yeasts into the dietary feeds enhanced the growth of microbiota and *in vitro* GP. In this study, the short fermentation lag time because of *S. cerevisiae* addition is due to the reason that yeast contains various macromolecules, which are essential to induce cellulolytic microbes for initiating growth and improving its activity (Callaway and Martin, 1997).

CO_2 and CH_4 are emitted during the ruminal fermentative mechanism. In this context, the asymptotic CH_4 emission, rate of CH_4 emission, and lag period decreased non-significantly ($P > 0.05$) with the inclusion of varied doses of *M. oleifera* extract, *S. cerevisiae*, and *M. oleifera* extract × *S. cerevisiae* interaction, which is in fact crucial for the environment. The supplementation of *M. oleifera* extract and *S. cerevisiae* showed no significant ($P > 0.05$) impact on the asymptotic and fractional rate of CO_2 emission, while *S. cerevisiae* decreased the lag time significantly. *M. oleifera* extract × *S. cerevisiae* interaction showed no significant ($P > 0.05$) effect on asymptotic CO_2, fractional rate of CO_2 emission, and lag time. This might be mainly because of the increment in fibre content and decreased non-structural carbohydrates in feeds constituting *M. oleifera* extract. An increase in cell wall composition may mitigate activities of microbes, resulting reduced CO_2 emission and decreased lag period of CO_2 emission. The alteration in detrimental gases production because of the inclusion of *S. cerevisiae* is primarily due to the dose-dependent interactions between yeast and feeding diet (Patra, 2012).

In the present investigation, the fermentation pH and DMD values were found to be non-significantly ($P > 0.05$) affected due to the supplementation of *M. oleifera* extract. The addition of *S. cerevisiae* affected significantly the DMD. *M. oleifera*

Table 4
Effect of *M. oleifera* and *S. cerevisiae* at different concentrations as feed additives on *in vitro* rumen CO_2 production at different incubation period (h).

M. oleifera extract (mL/g DM)	*S. cerevisiae* (mg/g DM)	mL CO_2/0.5 g dry matter incubated				mL CO_2/0.5 g dry matter degraded				Proportional CO_2 production			
		8	24	48	72	8	24	48	72	8	24	48	72
0	0	4.3	26.6	43.4	62.7	4.7	29.4	48.0	68.9	13.2	40.2	52.4	65.2
0	2	5.1	29.9	54.0	79.3	7.4	43.0	77.7	114.1	11.5	35.4	47.2	59.7
0	4	4.8	22.8	31.2	46.9	6.5	32.0	43.8	65.6	11.5	30.6	37.6	50.7
0.6	0	4.7	31.2	58.3	84.3	6.5	42.7	79.5	115.0	10.7	37.1	49.3	62.3
0.6	2	6.4	32.2	56.0	80.6	9.0	45.5	79.3	114.1	15.8	47.7	56.8	71.3
0.6	4	4.0	23.9	42.0	61.4	5.5	32.8	57.6	84.2	10.3	33.2	44.7	57.6
1.8	0	4.0	18.5	41.3	60.2	5.3	24.6	54.4	79.5	9.3	26.8	39.3	50.3
1.8	2	8.3	38.0	73.2	99.5	10.9	50.0	96.4	131.0	12.1	35.5	50.1	63.6
1.8	4	7.8	37.0	67.0	86.6	11.3	53.9	97.7	126.5	13.8	38.8	52.9	62.3
Pooled SEM[1]		1.21	5.15	8.51	11.58	1.68	7.17	11.9	16.13	0.84	1.89	2.28	2.53
Additive effect:													
Extract													
Linear		0.232	0.497	0.138	0.229	0.196	0.409	0.114	0.187	0.747	0.514	0.585	0.959
Quadratic		0.618	0.963	0.967	0.82	0.715	0.849	0.851	0.703	0.702	0.035	0.16	0.084
S. cerevisiae													
Linear		0.45	0.717	0.933	0.784	0.303	0.435	0.713	0.838	0.481	0.851	0.509	0.465
Quadratic		0.242	0.269	0.169	0.158	0.216	0.223	0.139	0.122	0.104	0.03	0.056	0.031
Extract × *S. cerevisiae*		0.774	0.588	0.553	0.671	0.768	0.563	0.49	0.58	0.026	0.007	0.014	0.039

Means in the same column with different superscripts differ significantly ($P < 0.05$).
SEM[1], Standard error of mean.

extract × *S. cerevisiae* interaction revealed significant influence on DMD too. Findings of the present study partially support the results of Elghandour et al. (2017a,b) who revealed that plant leaves incorporation caused reduction and increment in the ruminal pH and *in vitro* DMD values. The impact of yeasts on pH depends on the fermentative feed. Previous study demonstrated increased cecal pH in horses fed the yeast cells with respect to control diet (Hall and Miller-Auwerda, 2005).

The inclusion of *S. cerevisiae* with respect to *M. oleifera* extract depicted the improved rate of GP in a time dependent manner. The proportional CH_4 production was estimated to be decreased ($P > 0.05$) at high doses of *M. oleifera* extract and *S. cerevisiae* at 72 h of incubation. *M. oleifera* extract × *S. cerevisiae* interaction mitigated the proportional CO_2 production significantly ($P < 0.05$) at 8, 24, 48, and 72 h of incubation period. Our finding favours the previous report of Polyorach et al. (2014) who revealed increased GP and decreased CH_4 emission due to the supplementation of dose dependent ration. This might be because of an improved proportion of ration protein, which alters the concentration of short chain fatty acids and releases lower level of acetic acid and more amount of propionic acid (Iqbal et al., 2008). Elghandour et al. (2014) estimated improved CH_4 emission due to the supplementation of lower doses of yeasts into the feeding diet. However, higher dose of yeast mitigated CH_4 emission. Authors indicated that yeasts might induce the acetogens for competing or co-metabolizing H_2 with methanogens, causing reduction in GHG production. Polyorach et al. (2014) demonstrated that CH_4 emission was mitigated when animal feed was supplemented with yeast fermented cassava chip protein in lieu of soybean meal. Martin and Nisbet (1992) demonstrated an improved CH_4 emission after dietary supplementation. Variations observed in our finding and previous reports might be because of the distinct type of strains and nature of rations used (Patra, 2012).

5. Conclusions

The supplementation of *M. oleifera* extract (1.8 mL/g DM) and *S. cerevisiae* (4 mg/g DM) exhibited improvement in asymptotic GP from 88.8 to 147.5 mL/g DM. The fractional rate of GP (0.069) and lag time (1.32–3.99 h) were increased too due to the supplementation of these additives. *In vitro* CH_4 (11.7%) and proportional CO_2 (50.3%) emission from goats were mitigated at higher doses of additives with respect to the control diet. Supplementing dietary feeds of goats with *M. oleifera* extract and *S. cerevisiae* could be valuable resources of sustainability and can undeniably be utilized as valuable cleaner product or feedstuff for ecosystem and livestock by avoiding the ruminal gases (CH_4 and CO_2) produced anaerobically at greater extent. In view of the colossal side effects of conventional antibiotics, *M. oleifera* extract and *S. cerevisiae* may be considered as requisite additives for animals and can undeniably manage socio-economic aspects in livestock industries. *In vivo* studies are essential to understand the mechanism of action of *M. oleifera* extract and *S. cerevisiae* at varied concentrations on fermentation kinetics and nutrients digestibility in goats.

Conflicts of interest

None declared.

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