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Deciphering the role of *Moringa oleifera* seeds and probiotic bacteria on mitigation of biogas production from ruminants

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Abstract

Maintaining cleaner and more sustainable ecosystems by mitigating greenhouse gas (GHG) emissions from livestock through dietary manipulation is in demand. This study was aimed to assess the effect of *Moringa oleifera* seeds and probiotics (*Pediococcus acidilactici* BX-B122 and *Bacillus coagulans* BX-B118) as feed supplements on GHG production and fermentation profile from steers and sheep. The treatments included diets containing 0, 6, 12, and 18% of *M. oleifera* seeds meal and a mixture of probiotic bacteria (0.2 ml/g of diet). Total biogas production, CH_4, CO, and H_2S emission from animals (up to 48 h), rumen fermentation profile, and CH_4 conversion efficiency were recorded using standard protocols. Results showed interaction among *M. oleifera* seeds and probiotics on asymptotic biogas production and total biogas production up to 48 h ($P<0.05$). The rate of CH_4 emission in steers was reduced from 0.1694 to 0.0447 ml/h using 6 and 18% of *M. oleifera* seeds ($P<0.05$). Asymptotic CO and the rate of CO production were increased ($P<0.05$) by supplementing different doses of *M. oleifera* seeds and probiotics. Adding 12% of *M. oleifera* seeds and probiotics reduced H_2S production from 0.0675 to 0.0112 ml H_2S/g DM (at 48 h of fermentation) in steers. In sheep, the additives mitigated H_2S production from 0.0364 to 0.0029 ml H_2S/g DM (at 48 h of fermentation), however there were not interaction ($P=0.7744$). In addition, *M. oleifera* seeds and probiotics reduced the pH level and dry matter degradability (DMD) in steers and sheep ($P<0.0001$) showing a positive impact on CH_4:ME and CH_4:OM (in steers) and CH_4:SCFA (in sheep), while the interaction was not significant ($P>0.05$) for CH_4:SCFA (in steers) and CH_4:ME and CH_4:OM (in sheep). In conclusion, the interaction of *M. oleifera* seeds and probiotics in the feeding diet reduced GHG emissions and affected the fermentation profile of steers and sheep.

Keywords Feed additives, Greenhouse gases, *M. Oleifera*, Probiotics, Ruminants, Rumen fermentation

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Introduction

The uncontrolled emission of greenhouse gases (GHG) into the ecosystem is worriment for society. Over time, the variations in the concentrations and proportions of detrimental GHG produced in the atmosphere have caused an unprecedented change in the ecosystem (Lackner et al. 2022; Elghandour et al. 2023). It is estimated that the temperature of the globe might increase by 4 °C in the following decades due to GHG emissions (IPCC 2014). In the current scenario, the agriculture industry and deforestation contribute about 25% of total GHG released into the atmosphere (Ahmed et al. 2020). Livestock industries are considered a source of GHG emissions, contributing approximately 15% of total anthropogenic production (Khusro et al. 2022a). The socio-economic and environmental impact of GHG emissions from animals is expected to increase worldwide in the coming years; thus, its mitigation are an urgently needed.

Dietary manipulation of animals is one of the paramount strategies implemented to minimize the emission of GHG from ruminants. Strategies such as the addition to diets of herbal extracts, plants' metabolites (saponins, tannins, essential oils, organosulfides, etc.), probiotics, yeasts, exogenous enzymes, organic acids, ionophores, algae, and metallic nanoparticles into the fodder had shown to reduce the GHG emission from ruminants (Palangi and Lackner 2022). Among the plants, the inclusion of *Moringa oleifera* (Moringaceae) in diets has been studied due to its ample nutritional properties (proteins, minerals, vitamins, amino acids, etc.) and low anti-nutrient contents (tannin, lignin, phytate, etc.) (Su and Chen 2020; Pedraza-Hernández et al. 2021; Magalhães et al. 2021). *M. oleifera* is a rapidly growing perennial softwood plant (5–12 m in height) primarily distributed in tropical and subtropical regions, *M. oleifera* leaves contain high amounts of crude protein, vitamins, minerals, fatty acids, and different phytochemicals, while seeds contain odorless oil which is resistant to autoxidation process (Ebeid et al. 2020). Therefore *M. oleifera* had been included as an additive in animal's diet to improve the productivity and feed utilization (Pedraza-Hernández et al. 2021; Alvarado-Ramírez et al. 2023).

Probiotics are non-pathogenic direct-fed microorganisms extensively used in animal nutrition as additives (Gado et al. 2017) to stimulate the growth of ruminal bacteria and enhance the total bacterial count by providing them certain with nutritional constituents. Although sometimes probiotic bacteria reduce the methanogenesis process by directly inhibiting of the growth of methanogens (Doyle et al. 2019), some probiotics also might inhibit specific bacteria of the rumen that produce secondary metabolites, which reduce the methanogenesis process. Previous reports have critically analyzed the role of probiotic bacteria, mainly lactic acid bacteria, in mitigating of GHG production of ruminants (Doyle et al. 2019).

Because of the role of dietary feed supplements in livestock industries, the present study aimed to assess the potentialities of *M. oleifera* seeds and probiotic bacteria (*Pediococcus acidilactici* BX-B122 and *Bacillus coagulans* BX-B118) as feed additives in the mitigation of biogas [methane (CH_4), carbon monoxide (CO), and hydrogen sulfide (H_2S)] production from steers and sheep but also explore its fermentation profile under in vitro conditions.

Materials and methods

Experimental treatments

The treatments consisted of ruminant diets with the inclusion of 0, 6, 12, and 18% of *M. oleifera* seeds meal and a commercial probiotic product (INSILATO AL@, BIORGANIX MEXICANA S.A. DE C.V, Coahuila, Mexico), which contained probiotic bacteria [*P. acidilactici* BX-B122 (1×10^{11} cfu m/L) and *B. coagulans* BX-B118 (1×10^{11} cfu m/L)] at a dose of 0.2 ml/g of diet. The ingredients of the diet were purchased from a feed store, while *M. oleifera* seeds were obtained from wild trees in the municipality of Iguala de la Independencia Guerrero, Mexico, with an approximate age of 4 years and under the criterion that the pods had to be mature (brown color and open valves). The seeds were subjected to the dehydration process at room temperature (area free from solar radiation and humidity), and subsequently, it was powdered using a forage grinder to generate flour. Mixing of the ingredients, including *M. oleifera* seed flour, was done manually.

Chemical composition of diets

Three representative samples of each diet were obtained were dehydrated at 60 °C for 72 h, and ground in a hammer mill (Thomas Wiley® Laboratory Mill model 4, Thomas Scientific™, Swedesboro, NJ, USA) with a 1 mm sieve. Ash (method ID 942.05) and nitrogen content (N; method ID 954.01) were quantified (g/kg DM) according to the standard methods of the Association of Official Analytical Chemists (AOAC 1997). From the obtained values, the organic matter (OM) and the crude protein (CP) were calculated as follows:

$$\mathrm{OM} = 100 - \mathrm{Ash}$$

$$\mathrm{CP} = N \times 6.25$$

The content of neutral detergent fiber (NDF) and acid detergent fiber (ADF) was estimated using the ANKOM200 Fiber Analyzer (ANKOM Technology Corp., Macedonia, NY, USA) following the methodology of Van Soest et al. (1991). In addition, sodium sulfite and thermostable α-amylase were used in the NDF analysis,

and the NDF and ADF values were expressed without residual ash. The chemical composition of the diets is presented in Table 1.

In vitro incubations

The ruminal content was obtained from 4 steers (430±20 kg BW) and 4 sheep (40±5 kg BW) that were slaughtered in a local slaughterhouse, regulated by the Official Mexican Standard NOM-033-SAG/ZOO-2014, which establishes methods to kill domestic and wild animals. The ruminal contents were transported to the laboratory in air-tight thermoses pre-heated to 39 °C, where it was filtered with four layers of cheesecloth to obtain only ruminal fluid, which was subsequently used as inoculum for fermentation. The nutrient medium was prepared following the methodology described by Goering and Van Soest (1970) and contained buffer solution, macrominerals, microminerals, reducing agent, resazurin, and distilled water. Fermentation was carried out in glass vials (120 ml) containing 500 mg of diet, probiotic doses (only if applicable), 10 ml of ruminal inoculum, and 40 ml of nutrient medium in each vial. Rubber stoppers and aluminum seals were used to seal the vials hermetically. Further, vials were shaken lightly and placed in a water bath at 39 °C for 48 h. In total, three fermentation cycles were carried out, and in each one, there were 51 vials, including the white ones (containing only ruminal inoculum and nutrient medium).

Biogas estimation

Total biogas production was quantified up to 48 h, following the methodology proposed by Theodorou et al. (1994) and using a digital manometer with a precision of ±2% (Manometer model 407,910, Extech® Instruments, Nashua, NH, USA). The biogases (CH_4, CO, and H_2S) were quantified following the methodology of Acosta et al. (2022) using a portable gas detector (Dräger X-am®, model 2500, Dräger, Lübeck, SH, Germany) connected to an external pump (Dräger X-am®, Dräger, Lübeck, SH, Germany). Furthermore, at the end of each measurement, the accumulated biogas was released to avoid over-estimation.

Table 1 Ingredients and chemical composition of diets for ruminants with the inclusion of different concentrations of *M. Oleifera* seeds

Items	Level of M. oleifera seeds (% of diet)			
	0	6	12	18
Ingredients (g/kg diet)				
Maize grain	735	675	615	555
Maize stubble	150	150	150	150
Soybean grain	90	90	90	90
M. oleifera seeds	0	60	120	180
Mineral salt	25	25	25	25
Chemical composition (g/kg DM)				
Organic matter	910	953	954	953
Crude protein	120	100	100	104
Neutral detergent fiber	400	280	280	300
Acid detergent fiber	220	200	200	220
Secondary metabolites (mg/g)				
Tannins	1.7	2.6	3.0	3.0
Saponins	11	13	24	32

Rumen pH and dry matter degradability (DMD)

The contents of the vials were filtered at the end of the fermentation using filter bags with a porosity of 25 µm (Filter bags F57, ANKOM Technology Corp., Macedonia, NY, USA) to obtain the residues of the diets and collect the liquid part in beakers (Alvarado-Ramírez et al. 2023). pH was measured in the collected liquid using a potentiometer with a glass electrode (pH wireless electrode HALO® model HI11102, Hanna® Instruments, Woonsocket, RI, USA), while DMD (%) was estimated after dehydrating and weighing the residue of the diets by measuring the difference between the initial and final weights (Elghandour et al. 2014).

Calculous

The production (ml/g DM incubated) of total biogas, CH_4, CO, and H_2S was used to estimate the asymptotic gas production, the production rate, and the time of the lag phase before the production of each gas, using the NLIN protocol of the Statistical Analysis System (SAS 2002) and the model proposed by France et al. (2000) as mentioned below:

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

where y is the production (ml/g MS) of total biogas, CH_4, CO, and H_2S at time t (h); b is the asymptotic production (ml/g MS) of total biogas, CH_4, CO, and H_2S; c is the production rate (ml/h) of total biogas, CH_4, CO, and H_2S; and *Lag* is the lag phase (h) before the production of total biogas, CH_4, CO, and H_2S.

The metabolizable energy (ME; MJ/kg DM) and short-chain fatty acids (SCFA; mmol per 200 mg of DM) were calculated with the equations proposed by Menke et al. (2009) and Getachew et al. (2002), respectively. The CH_4 conversion efficiency was estimated based on CH_4 production per unit of SCFA, ME, and MO in mmol/mmol (CH_4:SCFA), g/MJ (CH_4:ME), and ml/g (CH_4:OM), respectively.

Statistical analyses

The variance analysis (ANOVA) model considered the experimental design (completely randomized) with a factorial arrangement (2×4×2), where factor 1 was the source of ruminal inoculum (steer and sheep), factor 2

was the inclusion of *M. oleifera* seeds (0, 6, 12, and 18%), and factor 3 was the addition of probiotic (without and with) in triplicates. The triplicate data of each treatment in each run were calculated as a mean, and the average values obtained were used as the experimental unit of each treatment. The data were analyzed using the GLM procedure of SAS (2002). The last minimum significance (LSD) was used for the comparison of means; it was calculated from the standard error (SE) by Proc Mixed (SAS 2002), considering the error degrees of freedom (DF) from variance analysis (ANOVA) and a $P=0.05$.

Results

Total biogas production and fermentation kinetics

Table 2 shows the total biogas production from steers and sheep by supplementing *M. oleifera* seeds and probiotic bacteria (*P. acidilactici* BX-B122 and *B. coagulans* BX-B118). In steers, the asymptotic biogas production was increased by supplementing different concentrations (6–18%) of *M. oleifera* seeds in the presence of probiotics. *M. oleifera* seeds ($P=0.0381$) and probiotics depicted ($P=0.0021$) increment in asymptotic biogas production (259.7 to 344.7 ml/g DM). However, there were not interaction *M. oleifera* seeds × probiotics ($P=0.8774$) effect on asymptotic biogas production. Similarly, the rate of biogas production was increased (0.039 to 0.048 ml/h) due to the supplementation of varied concentrations of *M. oleifera* seeds in the presence of probiotic bacteria, while the effect was not significant ($P=0.8062$) for *M. oleifera* seeds and significant ($P<0.0001$) for probiotics inclusion. *M. oleifera* seeds × probiotics was not significant ($P=0.5973$) influence on the biogas production rate. The lag period was reduced (6.34 to 4.86 h) at higher concentrations of *M. oleifera* seeds in the presence of probiotics. However, the effect of *M. oleifera* seeds × probiotics interaction on the lag period was not significant ($P=0.7525$). In a like manner, total biogas production (ml total biogas/g DM incubated) was increased ($P<0.05$) from 2 to 48 h at distinct concentrations of *M. oleifera* seeds in the presence of probiotics. However, there were not interaction *M. oleifera* seeds × probiotics ($P=0.0318$) at 2 h of incubation.

The asymptotic biogas production was increased (150.44 to 390.23 ml/g DM) in sheep, due to the supplementation of varied concentrations (6–18%) of *M. oleifera* seeds in the presence of probiotics (Table 2). *M. oleifera* seeds, probiotics, and there was interaction *M. oleifera* seeds × probiotics ($P<0.0001$) for asymptotic biogas production. Similarly, the rate of biogas production was increased (0.0076 to 0.0339 ml/h; $P<0.05$) due to the inclusion of varied concentrations of *M. oleifera* seeds and probiotics. However, *M. oleifera* seeds × probiotics interaction did not affect ($P=0.3095$) the rate of biogas production. The addition of varied concentrations of *M. oleifera* seeds and probiotics also showed no effect ($P>0.05$) on the lag period. *M. oleifera* seeds and probiotics exhibited a significant increment in total biogas production up to 48 h. Maximum biogas production of 333.6 ml total biogas/g DM was estimated using 6% of *M. oleifera* seeds in the presence of probiotics. Overall, *M. oleifera* seeds × probiotics interaction depicted an influence ($P<0.05$) on asymptotic biogas production and total biogas production up to 48 h, while the rate of biogas production ($P=0.1751$) and the duration or onset of the lag period ($P=0.2871$) was not significantly affected.

Figure 1 illustrates total biogas production kinetics from steers and sheep using *M. oleifera* seeds at different concentrations in the presence and absence of probiotics. The results showed higher total biogas production using 6% of *M. oleifera* seeds in the presence of probiotics.

In vitro CH_4 production

Methane production is due to the inclusion of *M. oleifera* seeds and probiotic bacteria, using steers as a source of ruminal inoculum is shown in Table 3. The addition of various concentrations of *M. oleifera* seeds, probiotics, and *M. oleifera* seeds × probiotics interaction revealed an effect ($P<0.05$) on the asymptotic CH_4 emission from steers. The rate of CH_4 emission was reduced ($P<0.05$, 0.1694 to 0.0447 ml/h) using 6 and 18% of *M. oleifera* seeds, while the effect was not significant ($P=0.7246$) due to the addition of probiotics. The supplementation of *M. oleifera* seeds at higher concentrations caused a reduced lag period (21.02 to 8.48 h), however, the effect was not significant ($P=0.1285$), while the presence of probiotics ($P=0.0262$) and *M. oleifera* seeds × probiotics interaction ($P=0.0347$) showed a significant effect on lag period reduction. On the other hand, CH_4 production was increased up to 48 h using various concentrations of *M. oleifera* seeds in the presence of probiotics. However, the production due to *M. oleifera* seeds and probiotics supplementation was estimated to be lower than that of the control.

On the contrary, using sheep as a source of ruminal inoculum, the addition of various concentrations of *M. oleifera* seeds, probiotics, and *M. oleifera* seeds × probiotics interaction exhibited an increment ($P<0.05$) in CH_4 production up to 48 h to the control. Overall, the interaction of *M. oleifera* seeds × probiotics affected the CH_4 production ($P<0.05$). Figure 2 illustrates CH_4 production from steers and sheep using *M. oleifera* seeds at different concentrations in the presence and absence of probiotics. The results revealed a low CH_4 production using 18% of *M. oleifera* seeds in the presence of probiotics.

In vitro CO production

Carbon monoxide production from ruminants due to the addition of *M. oleifera* seeds and probiotic bacteria, using steers and sheep as a source of ruminal inoculum

Table 2 Parameters and total biogas production from steers and sheep as a source of inoculum using different concentrations of *M. Oleifera* seeds in the presence or absence of probiotic (*P. acidilactici* BX-B122 and *B. coagulans* BX-B118)

Rumen inoculum source (RIS)	Moringa seed % (MSP)	Probiotic bacteria (PB)	Total Biogas production					
			Parameters[1]			mL total biogas/g DM incubated		
			b	c	Lag	2 h	24 h	48 h
Steers	0	Without	278.87	0.0390	4.87	27.60	193.06	269.82
		With	318.90	0.0467	4.94	33.81	275.88	307.91
	6	Without	299.10	0.0399	6.34	26.40	193.84	288.25
		With	344.67	0.0450	5.57	45.77	282.33	333.62
	12	Without	274.97	0.0396	6.24	25.16	177.99	264.71
		With	298.27	0.0464	5.14	32.80	257.61	289.79
	18	Without	259.73	0.0394	6.29	23.89	167.72	250.42
		With	297.47	0.0481	4.86	32.75	265.94	288.02
		[2]LSD 0.05=	27.70	0.0022	1.19	3.66	9.99	22.54
		[2]SEM	14.142	0.00133	0.711	2.183	5.965	13.474
		MSP	0.0381	0.8062	0.5177	0.0106	0.0035	0.0361
		Linear	0.1708	0.4919	0.3598	0.2912	0.0093	0.1641
		Quadratic	0.8644	0.8194	0.4774	0.7829	0.1480	0.8800
		PB	0.0021	< 0.0001	0.1279	< 0.0001	< 0.0001	0.0015
		MSP × PB	0.8774	0.5973	0.7525	0.0318	0.4463	0.8981
Sheep	0	Without	163.33	0.0155	3.49	27.00	69.82	155.37
		With	212.80	0.0379	4.11	32.32	162.71	201.94
	6	Without	176.67	0.0128	5.00	27.45	76.37	146.07
		With	390.23	0.0302	4.06	60.41	254.49	370.53
	12	Without	150.44	0.0095	3.75	28.12	75.56	140.32
		With	315.67	0.0255	2.02	57.45	191.01	294.67
	18	Without	158.03	0.0076	3.60	27.14	60.75	150.36
		With	389.50	0.0339	2.26	56.18	275.98	374.13
		[2]LSD 0.05=	22.60	0.0049	1.21	1.35	24.04	23.30
		[2]SEM	13.487	0.00293	0.722	0.805	14.344	13.901
		MSP	< 0.0001	0.0446	0.1083	< 0.0001	0.0052	< 0.0001
		Linear	< 0.0001	0.0592	0.2436	< 0.0001	0.0022	< 0.0001
		Quadratic	0.8571	0.0263	0.4497	< 0.0001	0.4777	0.8091
		PB	< 0.0001	< 0.0001	0.1148	< 0.0001	< 0.0001	< 0.0001
		MSP × PB	< 0.0001	0.3095	0.4103	< 0.0001	0.0021	< 0.0001
[2]LSD 0.05=			23.16	0.0038	1.20	2.76	18.99	22.94
[2]Pooled SEM			13.818	0.00227	0.717	1.645	10.985	13.689
P value								
RIS			< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
MSP			< 0.0001	0.0656	0.1753	< 0.0001	0.0031	< 0.0001
Linear			0.0021	0.1286	0.8416	0.0002	0.0337	0.0024
Quadratic			0.9994	0.0266	0.9669	0.0025	0.2186	0.7789
PB			< 0.0001	< 0.0001	0.0272	< 0.0001	< 0.0001	< 0.0001
RIS × MSP			< 0.0001	0.0422	0.2513	< 0.0001	0.0010	0.0001
RIS × PB			< 0.0001	< 0.0001	0.9509	< 0.0001	< 0.0001	< 0.0001
MSP × PB			0.0002	0.1751	0.2871	< 0.0001	0.0003	< 0.0001
RIS × MSP × PB			0.0002	0.6103	0.9483	< 0.0001	0.0062	0.0002

[1]*b* = asymptotic biogas total production (ml/g DM); *c* = rate biogas total production (ml/h); *Lag* = initial delay before gas total production begins (h)

[2]LSD = last significant difference; SEM = standard error of mean

is shown in Table 4. Findings showed that various concentrations of *M. oleifera* seeds, probiotics, and *M. oleifera* seeds × probiotics interaction depicted an increment ($P<0.05$) in asymptotic CO production (0.007 to 1.026 ml/g DM), lag period (0.0135 to 0.1710 h), and CO production (up to 48 h; 0.0076 to 0.9367 ml CO/g DM incubated) from steers. In contrast, the rate of CO production was decreased ($P<0.05$, 0.0008 to 0.0003 ml/h)

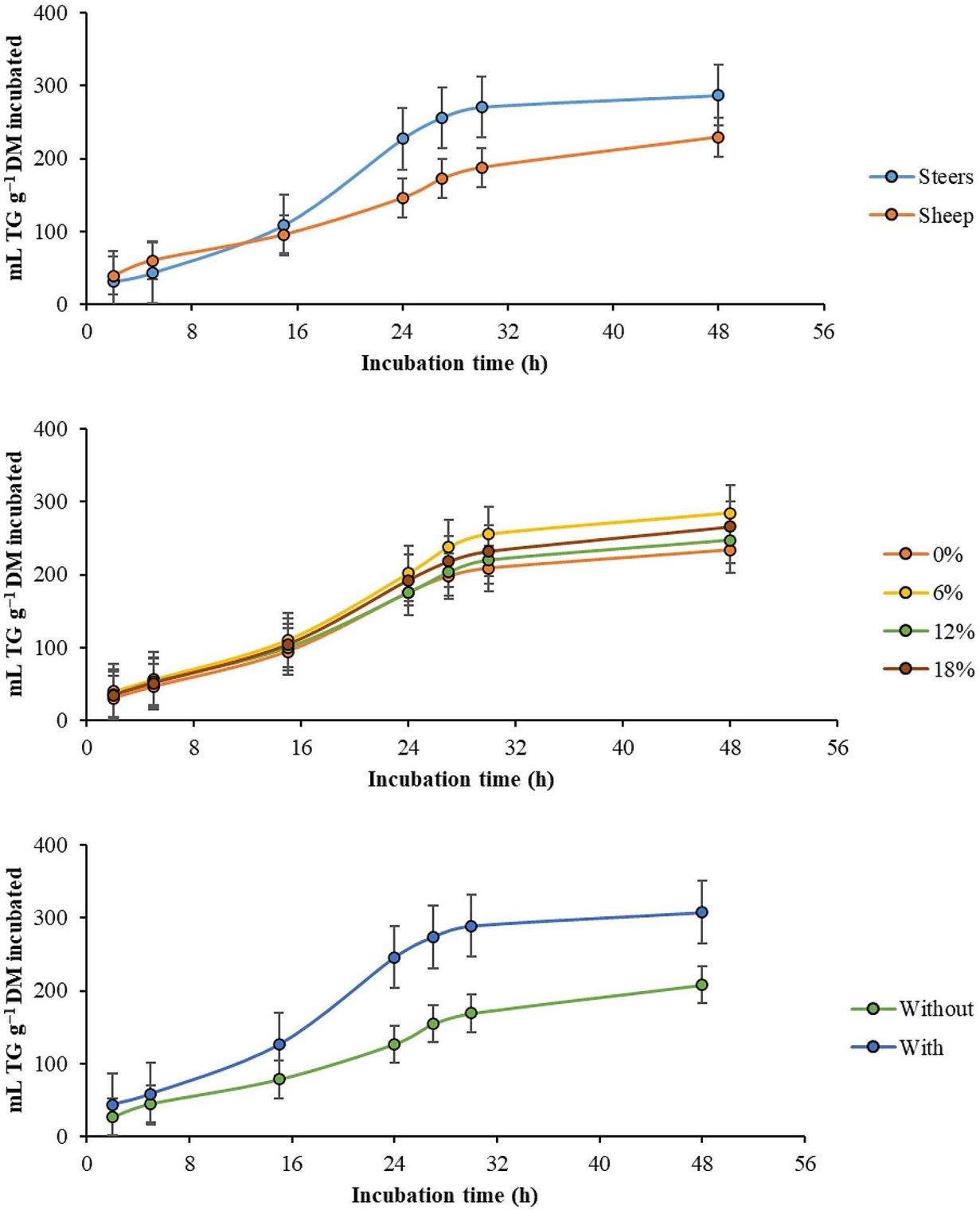

Fig. 1 Kinetics of ruminal total gas (TG) production from steers and sheep as a source of inoculum using different concentrations of *M. oleifera* seeds in the presence or absence of probiotic (*P. acidilactici* BX-B122 and *B. coagulans* BX-B118)

Table 3 Parameters and CH_4 production from steers and sheep as a source of inoculum using different concentrations of *M. Oleifera* seeds in the presence or absence of probiotic (*P. acidilactici* BX-B122 and *B. coagulans* BX-B118)

Rumen inoculum source (RIS)	Moringa seeds % (MSP)	Probiotic bacteria (PB)	CH_4 production								
			Parameters[2]			ml CH_4/g DM incubated			ml CH_4/100 ml biogas total		
			B	c	Lag	2 h	24 h	48 h	2 h	24 h	48 h
Steers	0	Without	60.63	0.0687	16.91	0.299	27.002	60.193	1.08	13.83	21.67
		With	20.79	0.0260	14.18	0.000	3.870	15.691	0.00	1.34	5.13
	6	Without	16.21	0.0648	18.73	0.264	4.626	15.869	1.00	2.38	5.54
		With	28.52	0.0610	18.75	0.000	7.042	28.352	0.00	2.47	8.46
	12	Without	43.12	0.0839	19.40	0.211	12.530	43.264	0.83	7.08	16.33
		With	17.69	0.1694	19.40	0.000	7.836	17.470	0.00	3.03	6.01
	18	Without	18.82	0.1064	21.02	0.219	4.880	19.159	0.92	2.92	7.58
		With	21.41	0.0447	8.48	0.000	14.615	21.381	0.00	5.50	7.42
		[2]LSD 0.05=	9.61	0.0377	3.69	0.026	5.07	7.74	0.085	2.44	2.43
		[2]SEM	5.743	0.02252	2.199	0.0155	3.0318	5.6258	0.051	1.456	1.451
		MSP	0.0102	0.0160	0.1285	0.0395	0.0442	0.0230	0.1251	0.0202	0.0011
		Linear	0.0025	0.2281	0.7230	0.0204	0.0790	0.0063	0.1220	0.0338	0.0009
		Quadratic	0.9991	0.0041	0.0402	0.0917	0.3725	0.7991	0.0776	0.5136	0.5746
		PB	0.0069	0.7246	0.0262	< 0.0001	0.0863	0.0030	< 0.0001	0.0039	< 0.0001
		MSP × PB	0.0011	0.0223	0.0347	0.0395	0.0004	0.0004	0.1251	0.0005	< 0.0001
Sheep	0	Without	18.32	0.0254	17.05	0.089	3.535	13.865	0.33	4.96	8.79
		With	37.55	0.0388	24.45	0.000	5.202	38.395	0.00	3.44	18.89
	6	Without	11.53	0.0110	13.15	0.000	0.995	9.950	0.00	1.25	6.25
		With	86.57	0.0964	24.14	0.000	8.256	86.545	0.00	3.21	23.24
	12	Without	7.53	0.0037	21.95	0.000	0.567	6.937	0.00	0.75	4.83
		With	34.81	0.0819	26.96	0.000	4.332	34.036	0.00	2.26	11.52
	18	Without	8.66	0.0096	24.14	0.000	0.589	9.378	0.00	0.98	6.21
		With	30.61	0.1392	23.63	0.000	5.267	30.275	0.00	1.89	8.20
		[2]LSD 0.05=	8.02	0.0293	3.43	0.03	2.09	9.127	0.099	1.14	2.23
		[2]SEM	4.786	0.01759	2.048	0.0157	1.2471	5.4459	0.059	0.678	1.624
		MSP	< 0.0001	0.1395	0.0383	0.0266	0.2616	0.0002	0.0266	0.0028	0.0003
		Linear	0.1022	0.0287	0.1456	0.0121	0.2651	0.2641	0.0121	0.0009	0.0009
		Quadratic	0.5371	0.5029	0.2456	0.1220	0.2833	0.6045	0.1220	0.0400	0.1149
		PB	< 0.0001	< 0.0001	0.0011	0.0628	0.0002	< 0.0001	0.0628	0.1565	< 0.0001
		MSP × PB	< 0.0001	0.0338	0.0750	0.0266	0.2016	0.0003	0.0266	0.0877	0.0023
[2]LSD 0.05=			8.85	0.034	3.56	0.026	3.89	9.28	0.092	1.904	2.576
[2]Pooled SEM			5.286	0.02020	2.125	0.0156	2.3181	5.5366	0.055	1.136	1.540
P value											
RIS			0.6943	0.0108	< 0.0001	< 0.0001	< 0.0001	0.7203	< 0.0001	0.0001	0.1221
MSP			0.0004	0.0190	0.0787	0.0011	0.0409	0.0023	0.0036	0.0005	< 0.0001
Linear			0.0005	0.0191	0.4419	0.0006	0.0372	0.0044	0.0030	0.0006	< 0.0001
Quadratic			0.6884	0.0342	0.0197	0.0213	0.2129	0.8571	0.0191	0.1311	0.3952
PB			0.0001	0.0014	0.3740	< 0.0001	0.8560	0.0002	< 0.0001	0.0211	0.0676
RIS × MSP			< 0.0001	0.0251	0.0376	0.5699	0.0395	< 0.0001	0.4270	0.1527	0.0002
RIS × PB			< 0.0001	0.0003	< 0.0001	< 0.0001	0.0012	< 0.0001	< 0.0001	0.0009	< 0.0001
MSP × PB			< 0.0001	0.0188	0.0024	0.0011	< 0.0001	< 0.0001	0.0036	< 0.0001	< 0.0001
RIS × MSP × PB			0.0259	0.0128	0.6617	0.5699	0.0008	0.0129	0.4270	0.0031	< 0.0001

[1]*b* = asymptotic CH_4 production (ml/g DM); *c* = rate CH_4 production (ml/h); *Lag* = initial delay before CH_4 production begins (h)

[2] LSD = last minimum difference; SEM = standard error of mean

due to the inclusion of *M. oleifera* seeds in the presence of probiotics. Likewise, in sheep, asymptotic CO production (0.1626 to 1.4293 ml/g DM) as well as CO production (0.1538 to 1.4085 ml/g DM incubated) were estimated to be increased ($P<0.05$) by supplementing varied concentrations of *M. oleifera* seeds in the presence of probiotics.

Figure 3 shows CO production (ml/g DM) from steers and sheep using *M. oleifera* seeds at different concentrations in the presence and absence of probiotics. Results

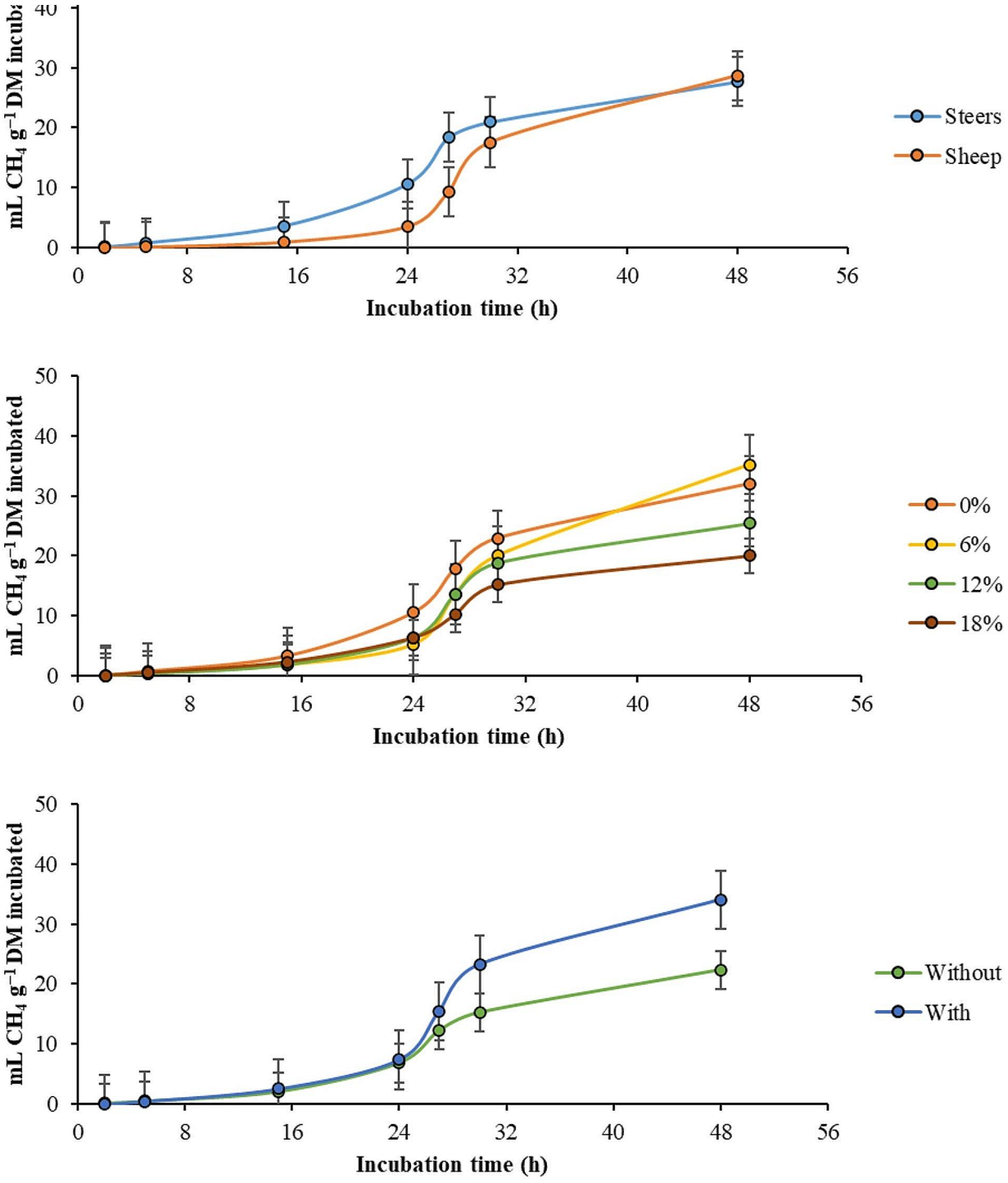

Fig. 2 Kinetics of ruminal CH_4 production from steers and sheep as a source of inoculum using different concentrations of *M. oleifera* seeds in the presence or absence of probiotic (*P. acidilactici* BX-B122 and *B. coagulans* BX-B118)

Table 4 Parameters and CO production from steers and sheep as a source of inoculum using different concentrations of *M. Oleifera* seeds in the presence or absence of probiotic (*P. acidilactici* BX-B122 and *B. coagulans* BX-B118)

Rumen inoculum source (RIS)	Moringa seeds % (MSP)	Probiotic (PB)	CO production					
			Parameters[1]			ml CO/g DM incubated		
			B	c	Lag	2 h	24 h	48 h
Steers	0	Without	0.0125	0.0005	0.0135	0.00006	0.00607	0.01231
		With	0.2086	0.0003	0.1519	0.00109	0.06930	0.19127
	6	Without	0.0077	0.0006	0.0168	0.00006	0.00278	0.00758
		With	0.3573	0.0004	0.1710	0.00287	0.09448	0.33767
	12	Without	0.0087	0.0006	0.0164	0.00005	0.00360	0.00857
		With	0.5573	0.0004	0.1660	0.00146	0.15051	0.52304
	18	Without	0.0070	0.0008	0.0194	0.00003	0.00232	0.00689
		With	1.0263	0.0003	0.1365	0.00339	0.38647	0.93670
		[2]LSD 0.05=	0.0737	0.00007	0.006	0.0005	0.032	0.055
		[2]SEM	0.04378	0.00004	0.00436	0.000292	0.019079	0.040344
		MSP	< 0.0001	0.0466	0.0072	0.0032	< 0.0001	< 0.0001
		Linear	< 0.0001	0.0066	0.2938	0.0013	< 0.0001	< 0.0001
		Quadratic	0.4316	0.6966	0.0109	0.1446	0.0314	0.5564
		PB	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
		MSP × PB	< 0.0001	0.0149	0.0028	0.0028	< 0.0001	< 0.0001
Sheep	0	Without	0.3284	0.0003	0.1903	0.00099	0.05651	0.24756
		With	0.7805	0.0011	0.2302	0.00008	0.18947	0.76709
	6	Without	0.2318	0.0004	0.5682	0.00020	0.02033	0.20445
		With	1.4293	0.0012	0.2353	0.00018	0.29208	1.40849
	12	Without	0.1626	0.0006	0.5871	0.00011	0.00944	0.15379
		With	0.7984	0.0012	0.2340	0.00014	0.16364	0.78836
	18	Without	0.2650	0.0012	0.2340	0.00007	0.00452	0.24315
		With	1.3513	0.0003	0.1903	0.00014	0.41464	1.34915
		[2]LSD 0.05=	0.154	0.0002	0.292	0.00007	0.027	0.089
		[2]SEM	0.09196	0.00013	0.17447	0.000041	0.027834	0.089242
		MSP	0.0025	0.4936	0.4949	< 0.0001	0.0031	0.0016
		Linear	0.0140	0.8095	0.9914	< 0.0001	0.0067	0.0052
		Quadratic	0.0227	0.1588	0.2056	< 0.0001	0.0044	0.0327
		PB	< 0.0001	0.0013	0.1812	< 0.0001	< 0.0001	< 0.0001
		MSP × PB	0.0024	< 0.0001	0.5901	< 0.0001	0.0005	0.0028
[2]LSD 0.05=			0.121	0.00015	0.0207	0.00035	0.04	0.116
[2]Pooled SEM			0.07202	0.00009	0.12341	0.000208	0.023862	0.069252
P value								
RIS			< 0.0001	< 0.0001	0.0011	< 0.0001	< 0.0001	< 0.0001
MSP			< 0.0001	0.2657	0.4327	0.0099	< 0.0001	< 0.0001
Linear			< 0.0001	0.2297	0.9872	0.0233	< 0.0001	< 0.0001
Quadratic			0.0132	0.1364	0.1738	0.0294	0.0003	0.0236
PB			< 0.0001	0.3654	0.7934	< 0.0001	< 0.0001	< 0.0001
RIS × MSP			0.0006	0.5079	0.5400	< 0.0001	0.0118	0.0011
RIS × PB			0.0001	< 0.0001	0.0165	< 0.0001	0.0057	< 0.0001
MSP × PB			< 0.0001	< 0.0001	0.6314	< 0.0001	< 0.0001	< 0.0001
RIS × MSP × PB			0.0015	< 0.0001	0.5354	0.0123	0.0669	0.0020

[1]*b* = asymptotic CO production (ml/g DM); *c* = rate CO production (ml/h); *Lag* = initial delay before CO production begins (h)

[2]LSD, last minimum difference; SEM = standard error of mean

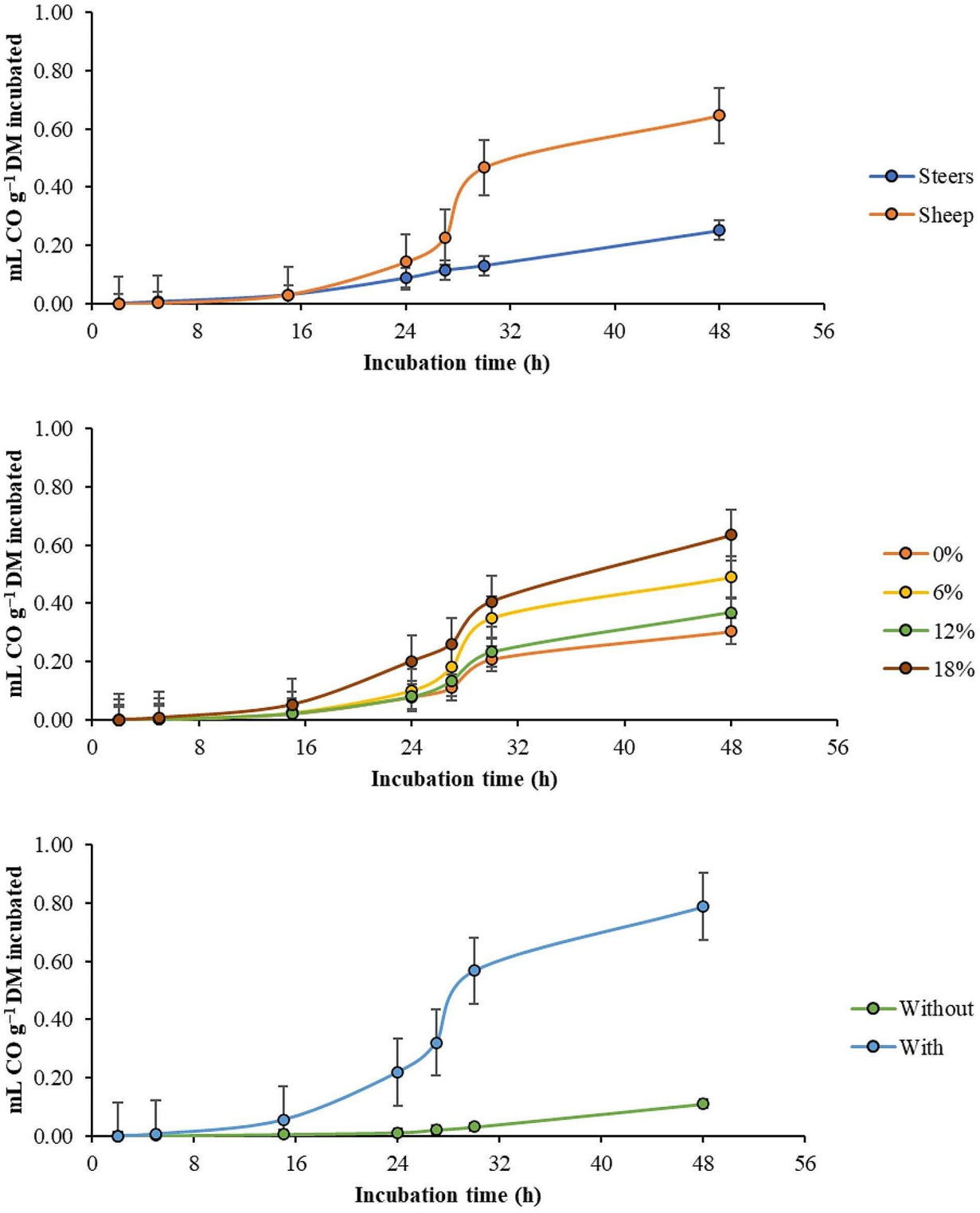

Fig. 3 Kinetics of ruminal CO production from steers and sheep as a source of inoculum using different concentrations of *M. oleifera* seeds in the presence or absence of probiotic (*P. acidilactici* BX-B122 and *B. coagulans* BX-B118)

showed low CO production using 12% of *M. oleifera* seeds in the presence of probiotics.

In vitro H_2S production

Hydrogen sulfide production from ruminants due to the supplementation of *M. oleifera* seeds and probiotic bacteria, using steers and sheep as a source of ruminal inoculum is shown in Table 5. In steers, asymptotic H_2S production was decreased from 0.0672 to 0.0114 ml/g DM, but *M. oleifera* seeds × probiotics interaction was not significant ($P=0.3390$). Similarly, *M. oleifera* seeds × probiotics interaction depicted no significant effect on the biogas production rate ($P=0.2977$) and lag period ($P=0.2952$). Adding 12% of *M. oleifera* seeds along with probiotics revealed a reduction in H_2S production from 0.06745 to 0.01116 ml H_2S/g DM incubated at 48 h, however there were not interaction *M. oleifera* seeds × probiotics ($P=0.4490$).

In sheep, the inclusion of *M. oleifera* seeds with probiotics exhibited a reduction in asymptotic H_2S production from 0.0363 to 0.0029 ml/g DM. However, the influence of *M. oleifera* seeds × probiotics interaction was not significant ($P=0.8631$). The interaction showed no significant ($P=0.1557$) effect on the biogas production rate, but the lag period was reduced ($P=0.0044$) from 0.1534 to 0.0026 h. On the other hand, the inclusion of *M. oleifera* seeds along with probiotics in the diet caused mitigation in H_2S emission from 0.03636 to 0.00293 ml/g DM up to 48 h, but the interaction was not significant ($P=0.7744$).

Figure 4 estimates H_2S production from steers and sheep using *M. oleifera* seeds at different concentrations in the presence and absence of probiotics. Results showed low H_2S production using 18% of *M. oleifera* seeds in the presence of probiotics.

Fermentation profile and CH_4 conversion efficiency

In steers, the supplementation of varied concentrations of *M. oleifera* seeds in the presence of probiotics resulted in a reduction ($P<0.0001$) in pH from 7.11 to 6.42. The DMD was reduced from 85.82 to 60.68% using 12% *M. oleifera* seeds in the presence of probiotics, but the interaction was not significant ($P=0.9706$). The supplementation of *M. oleifera* seeds along with the probiotics increased SCFA (3.70 to 6.35 mmol/g DM) and ME (5.82 to 7.18 MJ/kg DM). *M. oleifera* seeds × probiotics did not significant affected ($P=0.3368$) the CH_4:SCFA, but it affected ($P=0.0004$) the CH_4:ME and CH_4:OM (Table 6).

In sheep, including of *M. oleifera* seeds and probiotics yielded reduced the pH ($P=0.0032$) and DMD ($P=0.0208$) from 6.95 to 6.18 and 72.94 to 61.40%, respectively. The SCFA and ME were increased from 1.33 to 6.11 mmol/g DM and 4.60 to 7.05 MJ/kg DM, respectively, using 18% *M. oleifera* seeds along with probiotics. *M. oleifera* seeds × probiotics interaction exerted a significant effect ($P=0.0022$) on CH_4:SCFA, but the effect was not significant for CH_4:ME ($P=0.2044$) and CH_4:OM ($P=0.1994$) (Table 6).

Discussion

The anthropogenic GHG emissions have become a pivotal topic globally because of their detrimental impact on climate change and global warming. In the coming years, the release of GHG will exhibit significant ecological and socio-economic effects worldwide due to the significant rise in temperature. Since livestock is one of the prime contributors towards increments in GHG release, followed by a change in the earth's climate (Mangar et al. 2022), it is imperative to minimize GHG emissions from livestock by developing alternative feed resources.

The volume of biogas produced from livestock depends on the nature of feed digestion and the fermentation process. Some feed additives affect animal biogas emissions (Santillán et al. 2023). A plethora of dietary supplements, such as the inclusion of plants and probiotics, have been tested to investigate their roles in the rate of biogas production from ruminants and non-ruminants (Khusro et al. 2022a).

In the present study, supplementing different concentrations (6–18%) of *M. oleifera* seeds along with probiotics (*P. acidilactici* BX-B122 and *B. coagulans* BX-B118) in diets increased the total biogas production of steers and sheep. The increase in biogas production from steers and sheep shows the availability and digestibility of diets. Similarly, Pedraza-Hernandez et al. (2019) found that including different doses of *M. oleifera* extract increased the rate of in vitro total biogas production from goats. On the contrary, Elghandour et al. (2017) and Mangar et al. (2022) observed a reduction in total biogas production from dairy calves and cows, respectively, after the supplementation of *M. oleifera* as a feed additive. The potential effects of *M. oleifera* inclusion on biogas production might depend on factors such as genetic differences, soil fertility, nutritional content of the plant, and type of livestock used (Fritsche et al. 2017). In another study, Abdelbagi et al. (2021) estimated increased biogas production from steers by supplementing probiotics as additives. Likewise, Elghandour et al. (2018) observed higher horse biogas production by adding probiotics to an oat straw-containing diet. The increment in biogas production is mainly associated with better microbial fermentation, followed by a nutrient digestibility enhancing.

The mitigation of CH_4 emissions from ruminants and non-ruminants is the main target of veterinarians because livestock causes approximately 35–40% of the CH_4 emissions (Vohra et al. 2016; Khusro et al. 2022b). In ruminants, 90% of CH_4 emissions are derived from enteric fermentation (Doyle et al. 2019). Among the different types of GHG produced, the CH_4 ranks second

Table 5 Parameters and H_2S production from steers and sheep as a source of inoculum using different concentrations of *M. Oleifera* seeds in the presence or absence of probiotic (*P. acidilactici* BX-B122 and *B. coagulans* BX-B118)

Rumen inoculum source (RIS)	Moringa seeds % (MSP)	Probiotic bacteria (PB)	H_2S production					
			Parameters[1]			ml H_2S/g DM incubated		
			b	c	Lag	2 h	24 h	48 h
Steers	0	Without	0.0630	0.0006	0.1494	0.00011	0.03328	0.06314
		With	0.0091	0.0006	0.1579	0.00002	0.00316	0.00895
	6	Without	0.0672	0.0000	0.0159	0.00011	0.03342	0.06745
		With	0.0114	0.0001	0.0209	0.00007	0.00242	0.01166
	12	Without	0.0622	0.0006	0.1576	0.00010	0.03069	0.06194
		With	0.0114	0.0006	0.1575	0.00004	0.00273	0.01116
	18	Without	0.0584	0.0000	0.0198	0.00010	0.02892	0.05860
		With	0.0126	0.0001	0.0166	0.00007	0.00537	0.01230
		[2]LSD 0.05=	0.005	0.00002	0.005	0.00002	0.001	0.005
		[2]SEM	0.00281	0.00001	0.00321	0.000010	0.000849	0.003083
		MSP	0.5577	< 0.0001	< 0.0001	0.1514	0.2953	0.5649
		Linear	0.8526	< 0.0001	< 0.0001	0.0870	0.2228	0.8505
		Quadratic	0.6732	< 0.0001	< 0.0001	0.7485	0.2026	0.7669
		PB	< 0.0001	0.0321	0.2798	< 0.0001	< 0.0001	< 0.0001
		MSP × PB	0.3390	0.2977	0.2952	0.0241	0.0021	0.4490
Sheep	0	Without	0.0363	0.0003	0.1534	0.00011	0.01204	0.03636
		With	0.0027	0.0003	0.1347	0.00000	0.00011	0.00271
	6	Without	0.0350	0.0000	0.0025	0.00011	0.01317	0.03418
		With	0.0038	0.0000	0.0028	0.00000	0.00017	0.00376
	12	Without	0.0360	0.0004	0.1390	0.00012	0.01303	0.03283
		With	0.0029	0.0003	0.1670	0.00000	0.00010	0.00293
	18	Without	0.0356	0.0000	0.0027	0.00011	0.01047	0.03518
		With	0.0042	0.0000	0.0026	0.00000	0.00024	0.00418
		[2]LSD 0.05=	0.003	0.00002	0.009	0.000003	0.0008	0.003
		[2]SEM	0.00169	0.00001	0.00533	0.000002	0.000468	0.001928
		MSP	0.9913	< 0.0001	< 0.0001	0.6740	0.0483	0.7832
		Linear	0.8310	< 0.0001	< 0.0001	0.8870	0.1456	0.9394
		Quadratic	0.8861	< 0.0001	< 0.0001	0.2325	0.0522	0.3163
		PB	< 0.0001	0.0394	0.5428	< 0.0001	< 0.0001	< 0.0001
		MSP × PB	0.8631	0.1557	0.0044	0.6740	0.0320	0.7744
[2]LSD 0.05=			0.004	0.00002	0.007	0.00001	0.001	0.004
[2]Pooled SEM			0.00232	0.00001	0.00440	0.000007	0.000686	0.002571
P value								
RIS			< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
MSP			0.7338	< 0.0001	< 0.0001	0.1402	0.1400	0.6915
Linear			0.9604	< 0.0001	< 0.0001	0.0786	0.0737	0.9040
Quadratic			0.7712	< 0.0001	< 0.0001	0.9363	0.8808	0.7716
PB			< 0.0001	0.8905	0.2755	< 0.0001	< 0.0001	< 0.0001
RIS × MSP			0.5956	< 0.0001	0.2381	0.1375	0.2016	0.5473
RIS × PB			< 0.0001	0.0028	0.9655	< 0.0001	< 0.0001	< 0.0001
MSP × PB			0.3862	0.0589	0.0254	0.0170	< 0.0001	0.4504
RIS × MSP × PB			0.4719	0.5798	0.0012	0.0151	0.0450	0.5931

[1]*b* = asymptotic H_2S production (ml/g DM); *c* = rate H_2S production (ml/h); *Lag* = initial delay before H_2S production begins (h)

[2]LSD = last minimum difference; SEM = standard error of mean

after CO_2 and absorbs more energy than CO_2, with a global warming potential of ~28 (Króliczewska et al. 2023). Thus, an innovative approach is required to reduce CH_4 production from ruminants to ensure a cleaner ecosystem. In this regard, in the present context, adding different doses of *M. oleifera* seeds, probiotics, and *M. oleifera* seeds × probiotics interaction revealed a significant ($P<0.05$) effect on asymptotic CH_4 emission from steers. The rate of CH_4 emission was significantly ($P<0.05$) reduced using 6 and 18% of *M. oleifera* seeds,

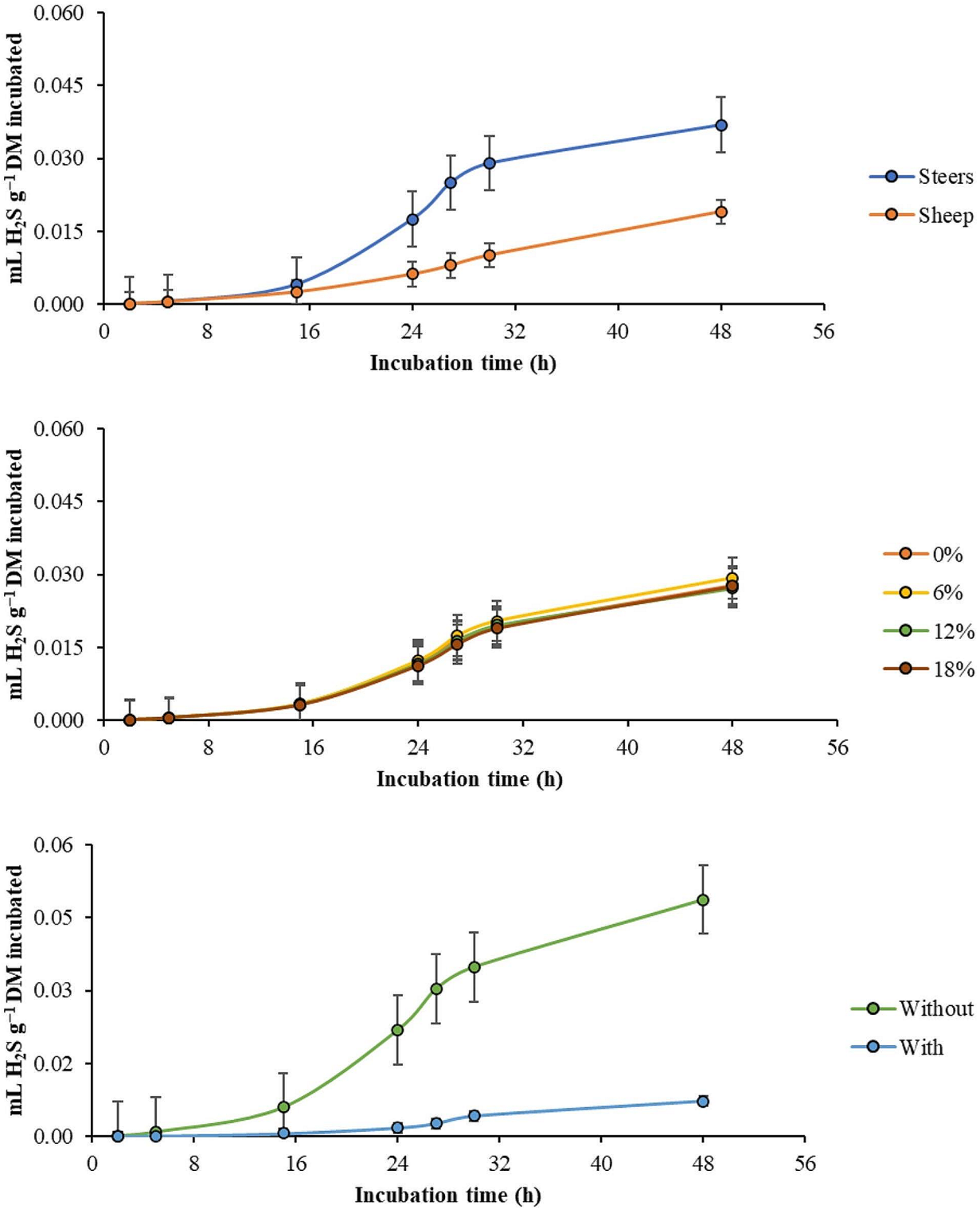

Fig. 4 Kinetics of ruminal H_2S production from steers and sheep as a source of inoculum using different concentrations of *M. oleifera* seeds in the presence or absence of probiotic (*P. acidilactici* BX-B122 and *B. coagulans* BX-B118)

Table 6 Rumen fermentation profile and CH_4 conversion efficiency from steers and sheep as a source of inoculum using different concentrations of *M. Oleifera* seeds in the presence or absence of probiotic (*P. acidilactici* BX-B122 and *B. coagulans* BX-B118)

Rumen inoculum source (RIS)	Moringa seeds % (MSP)	Probiotic bacteria (PB)	Rumen fermentation profile[1]				CH_4 conversion efficiency[2]		
			pH	DMD (%)	SCFA (mmol/g DM)	ME (MJ/kg DM)	CH_4:SCFA (mmol/mmol)	CH_4:ME (g/MJ)	CH_4:OM (ml/g)
Steers	0	Without	7.11	85.26	4.26	6.11	181.41	20.47	29.18
		With	6.42	61.50	6.10	7.05	17.18	2.48	4.18
	6	Without	6.98	85.69	4.28	6.12	31.15	3.51	5.00
		With	6.42	62.26	6.35	7.18	31.91	4.55	7.61
	12	Without	7.35	82.63	3.93	5.94	92.94	9.83	13.54
		With	6.51	60.68	5.75	6.87	39.29	5.29	8.47
	18	Without	7.13	85.82	3.70	5.82	38.28	3.90	5.27
		With	6.66	62.42	5.95	6.97	71.21	9.75	15.79
		[3]LSD 0.05=	0.003	3.398	0.216	32.03	0.111	3.743	5.489
		[3]SEM	0.019	2.027	0.129	19.112	0.066	2.233	3.275
		MSP	< 0.0001	0.6112	0.0027	0.0201	0.0027	0.0318	0.0442
		Linear	< 0.0001	0.7198	0.0141	0.0332	0.0133	0.0540	0.0790
		Quadratic	< 0.0001	0.2505	0.1647	0.5194	0.1636	0.4230	0.3725
		PB	< 0.0001	< 0.0001	< 0.0001	0.0036	< 0.0001	0.0248	0.0862
		MSP × PB	< 0.0001	0.9706	0.3315	0.0005	0.3368	0.0004	0.0004
Sheep	0	Without	6.84	72.93	1.53	4.70	65.59	3.48	3.82
		With	6.61	70.72	3.59	5.76	46.85	4.19	5.62
	6	Without	6.95	72.94	1.67	4.78	16.51	0.96	1.08
		With	6.63	67.29	5.63	6.81	41.75	5.60	8.92
	12	Without	6.92	69.81	1.66	4.77	9.91	0.55	0.61
		With	6.49	61.40	4.22	6.09	29.51	3.30	4.68
	18	Without	6.92	72.39	1.33	4.60	8.28	0.39	0.42
		With	6.18	63.35	6.11	7.05	24.55	3.44	5.69
		[3]LSD 0.05=	0.101	1.778	0.535	15.126	0.275	1.461	2.261
		[3]SEM	0.060	1.061	0.319	9.025	0.164	0.872	1.349
		MSP	0.0079	0.0001	0.0053	0.0018	0.0054	0.0981	0.2473
		Linear	0.0097	0.0018	0.0022	0.0004	0.0023	0.0427	0.2362
		Quadratic	0.2205	0.0003	0.4773	0.0496	0.4877	0.2265	0.3030
		PB	< 0.0001	< 0.0001	< 0.0001	0.1165	< 0.0001	0.0004	0.0001
		MSP × PB	0.0032	0.0208	0.0021	0.1029	0.0022	0.2044	0.1994
[3]LSD 0.05=			0.074	2.712	0.407	25.048	0.21	2.841	4.198
[3]Pooled SEM			0.044	1.618	0.243	14.945	0.125	1.695	2.505
P value									
RIS			< 0.0001	< 0.0001	< 0.0001	0.0001	< 0.0001	< 0.0001	< 0.0001
MSP			0.0273	0.0078	0.0021	0.0003	0.0022	0.0117	0.0403
Linear			0.4765	0.1696	0.0259	0.0004	0.0274	0.0100	0.0349
Quadratic			0.0044	0.0031	0.2312	0.1427	0.2375	0.2300	0.2190
PB			< 0.0001	< 0.0001	< 0.0001	0.0239	< 0.0001	0.5116	0.8399
RIS × MSP			< 0.0001	0.1578	0.0013	0.1565	0.0013	0.0494	0.0394
RIS × PB			< 0.0001	< 0.0001	< 0.0001	0.0006	< 0.0001	0.0004	0.0011
MSP × PB			0.0048	0.5582	0.0002	< 0.0001	0.0002	< 0.0001	< 0.0001
RIS × MSP × PB			< 0.0001	0.2992	0.0080	0.0039	0.0081	0.0009	0.0009

[1]pH = ruminal pH; DMD = dry matter degradability; SCFA = short-chain fatty acids; ME = metabolizable energy

[2]CH_4:SCFA = methane: short-chain fatty acids ratio; CH_4:ME = methane: metabolizable energy ratio; CH_4:OM = methane: organic matter ratio

[3]LSD = last minimum difference; SEM = standard error of mean

while the effect was not significant ($P = 0.7246$) due to the addition of probiotics. Including *M. oleifera* seeds, probiotics, and *M. oleifera* seeds × probiotics interaction exhibited a significant increment in CH_4 production up to 48 h in sheep. Similar observations were estimated by Pedraza-Hernández et al. (2019), Dong et al. (2019), and Mangar et al. (2022), who suggested the utilization of *M. oleifera* as feed additives to mitigate CH_4 emission

from goats and cows. The reduction in CH_4 production might be because of the cell plant wall content that might decrease the microbial action, thereby causing reduced emissions of CH_4 (Elghandour et al. 2017). However, Elghandour et al. (2018) depicted increased CH_4 production in horses after adding probiotic to the feeding diet. Overall, the source, concentrations, and strains of probiotics are factors that could affect the emission of CH_4 from animals (Vohra et al. 2016).

Carbon monoxide is an indirect GHG because it has the potential to react with other molecules (i.e. the hydroxyl radical, OH*) present in the air and create another GHG, mainly CO_2. It generally causes a lower absorption of energy in the infrared region. However, it enhances global warming by reacting with certain chemical species in the atmosphere, thereby increasing the amount of primary GHG and modulating CH_4 and ozone production (Sobieraj et al. 2022). Including different levels of *M. oleifera* seeds, probiotics, and *M. oleifera* seeds × probiotics promoted increments of CO production from steers. However, the present study´s findings differ from the reports of Santillán et al. (2023), who demonstrated that CO emissions could be reduced in horses fed diets with plant leaf extract.

In livestock, H_2S is known as a toxic signalling molecule after NO (nitric oxide) and CO (Shah et al. 2020). The anaerobic digestion of organic materials through the action of sulphate-reducing bacteria releases H_2S in the ecosystem. The gut bacteria cause the metabolism of dietary SO_4^{2-} (sulfate) and produce H_2S in animals, which is rapidly absorbed through the intestinal wall and exhibits toxicological effects (Pal et al. 2018). Since the accumulation of H_2S gas causes poliomyelitis in ruminants (Binversie et al. 2016), it is imperative to regulate the synthesis of H_2S in the rumen. In the rumen, H_2S (sulphide) production depends on the amount of SO_4^{2-} in the diet. Ruminal microbes use sulphur or SO_4^{2-}, which is present in the diet, to synthesize H_2S. A competitive relationship is observed among methanogens and sulphide-reducing bacteria to require H^+ for the metabolic process. Correspondingly, sulphide-reducing bacteria reduce SO_4^{2-} to H_2S, and methanogens reduce CO_2 to CH_4 in the rumen (Shah et al. 2020). Depicted that the inclusion of sulphur in the diet of steers enhanced H_2S production (Drewnoski et al. 2012), the addition of S-containing amino acid and $SO4^{2-}$ in the diet of swine might mitigate the H_2S production (Sutton et al. 1999). Santillán et al. (2023) found a reduction of H_2S emission from equines by supplementing *M. oleifera* plant extract in the diet.

Saksrithai and King (2018) summarized extensively the potential role of different additives in reducing the emission of H_2S from poultry and animals. In the line with prior reports, the present investigation revealed reduction in H_2S production from steers and sheep due to the inclusion of *M. oleifera* seeds along with probiotics in the diet.

Plants are sources of saponins, tannins, flavonoids, and other metabolites, which directly or indirectly could mitigate ruminants' digestion-associated biogas emission, mainly CH_4 (Króliczewska et al. 2023). Saponins are known to inhibit the growth of ciliate protozoa present in the rumen (Hartinger et al. 2018) and reduce the production of CH_4 indirectly through the defaunation process (removal of protozoa from the rumen), which is known to disrupt the protozoan cell membrane in the rumen.

A hydrophilic sugar moiety and a hydrophobic steroid or triterpenoid aglycone are saponins' components that allow to the formation of complexes with sterols of cell membranes, leading to cell death (Patra and Saxena 2009). Additionally, saponins affect CH_4 emission by reducing the viability of methanogens and deactivating methanogenesis-associated genes, slowing down the methanogenesis process. Saponin also affects specific microbes in the rumen and alters biochemical mechanisms in the rumen (Ramos-Morales et al. 2017).

Tannins are another secondary polyphenolic plant metabolites that affect the rumen ecosystem (Broucek 2018). Tannins cause indirect inhibition of hydrogen-producing microbes and direct inhibition of methanogenic microbes in rumen (Kumar et al. 2014). Anti-methanogenic properties of tannins may be bactericidal or bacteriostatic and may depend on the type of bacterial species present in the rumen (Vasta et al. 2019). Overall, the anti-methanogenic traits of tannins rely on the binding of tannins to protein through the interaction of phenolic hydroxyl groups with amino acid residues by hydrogen bonds and hydrophobic interactions (Vasta et al. 2019); similarly, flavonoids decrease the viability of protozoa and methanogens, and thus, inhibit the methanogenesis process in the rumen by absorbing H_2 after the breakdown of their carbon ring structures (Oskoueian et al. 2013).

Probiotics (lactobacilli, bacilli, pediococci, lactococci, bifidobacteria, and propionibacteria) are known to affect the ruminal fermentation process and improve animals' health by controlling the gastro-intestinal microflora (Tavendale et al. 2005). Probiotics present in the rumen increase feed efficiency, which may decrease the production of GHG, particularly CH_4 emissions (Islam and Lee 2019). Since the increase in propionate production and reduction in CH_4 emission are co-related (Haque 2018), probiotics can help promote fermentation mechanisms to release hydrogen-based propionate. However, probiotic bacteria affect methanogenesis in ruminants by other possible mechanisms such as (1) Shifting of the ruminal fermentation process so that there is a prominent reduction in CH_4 emission, (2) Directly inhibiting the methanogens present in the rumen, and (3) Inhibiting H_2 or

methyl-containing compounds producing specific bacterial species present in the rumen that are responsible for the methanogenesis process (Doyle et al. 2019).

In the present study, steers and sheep showed a reduction in pH and DMD because of the addition of 6 and 18% of *M. oleifera* seeds. In addition, the supplementation of *M. oleifera* seeds along with probiotics increased SCFA and ME and reduced the rate of CH_4 emission.

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Code Availability
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Declarations

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Competing interests
There is no conflict of interest.

Ethics approval
The ruminal contents of sheep and steers were taken from the slaughterhouse of Toluca, Estado de Mexico, Mexico.

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References

Abdelbagi M, Ridwan R, Fidriyanto R, Jayanegara A (2021) Effects of probiotics and encapsulated probiotics on enteric methane emission and nutrient digestibility *in vitro*. IOP Conf Ser: Earth Environ Sci 788:012050. https://doi.org/10.1088/1755-1315/788/1/012050

Acosta JAD, Elghandour MM, Mariezcurrena-Berasain MD, Adegbeye MJ, Fajemisin AN, Pliego AB et al (2022) Effect of mid-term dietary administration of the *Caesalpinia coriaria* extract on the sustainable mitigation of equine fecal methane, carbon monoxide and hydrogen sulfide production. J Equine Vet Sci 115:104021. https://doi.org/10.1016/j.jevs.2022.104021

Ahmed J, Almeida E, Aminetzah D, Denis N, Henderson K, Katz J et al (2020) Agriculture and Climate Change: reducing emissions through Improved Farming practices. McKinsey and Company, New York, USA, pp 1–45

Alvarado-Ramírez ER, Maggiolino A, Elghandour MMMY, Rivas-Jacobo MA, Ballesteros-Rodea G, Palo PD et al (2023) Impact of co-ensiling of maize with *Moringa oleifera* on the production of greenhouse gases and the characteristics of fermentation in ruminants. Animals 13(4):764. https://doi.org/10.3390/ani13040764

AOAC (1997) Association of Official Analytical Chemists, 16th edn. Association of Official Analytical Chemists International, Arlington, VA, USA

Binversie EY, Ruiz-Moreno M, Carpenter A, Heins B, Crawford G, DiCostanzo A et al (2016) Effects of dietary roughage and sulfur in diets containing corn dried distillers grains with solubles on hydrogen sulfide production and fermentation by rumen microbes *in vitro*. J Anim Sci 94:3883–3893. https://doi.org/10.2527/jas.2016-0502

Broucek J (2018) Options to methane production abatement in ruminants: a review. J Anim Plant Sci 28:348–364

Dong L, Zhang T, Diao Q (2019) Effect of dietary supplementation of *Moringa oleifera* on the production performance and fecal methanogenic community of lactating dairy cows. Animals 9:262. https://doi.org/10.3390/ani9050262

Doyle N, Mbandlwa P, Kelly WJ, Attwood G, Li Y, Ross RP et al (2019) Use of lactic acid bacteria to reduce methane production in ruminants, a critical review. Front Microbiol 10:2207. https://doi.org/10.3389/fmicb.2019.02207

Drewnoski M, Richter E, Hansen S (2012) Dietary sulfur concentration affects rumen hydrogen sulfide concentrations in feedlot steers during transition and finishing. J Anim Sci 90:4478–4486. https://doi.org/10.2527/jas.2012-5078

Ebeid HM, Mengwei L, Kholif AE, Hassan FU, Lijuan P, Xin L et al (2020) *Moringa oleifera* oil modulates rumen microflora to mediate *in vitro* fermentation kinetics and methanogenesis in total mix rations. Curr Microbiol 77:1271–1282. https://doi.org/10.1007/s00284-020-01935-2

Elghandour MM, Chagoyán JCV, Salem AZM, Kholif AE, Castañeda JSM, Camacho LM et al (2014) Effects of Saccharomyces cerevisiae at direct addition or pre-incubation on in Vitro gas production kinetics and degradability of four fibrous feeds. Ital J Anim Sci 13:3075. https://doi.org/10.4081/ijas.2014.3075

Elghandour MM, Salem AZM, Khusro A, Cipriano-Salazar M, Olivares-Pérez J, Barros-Rodriguez MA et al (2017) Assessment of some browse tree leaves on gas production and sustainable mitigation of CH_4 and CO_2 emissions in dairy calves at different age. J Clean Prod 162:1192–1199. https://doi.org/10.1016/j.jclepro.2017.06.029

Elghandour MM, Khusro A, Greiner R, Salem AZM, de la Fuente JL, Márquez-Molina O et al (2018) Horse fecal methane and carbon dioxide production and fermentation kinetics influenced by *Lactobacillus farciminis*–supplemented diet. J Equine Vet 62:98–101. https://doi.org/10.1016/j.jevs.2017.12.006

Elghandour MM, Maggiolino A, Alvarado-Ramírez ER, Hernández-Meléndez J, Rivas-Cacerese RR, Hernández-Ruiz PE et al (2023) Marine microalgae as a nutritive tool to mitigate ruminal greenhouse gas production: *in vitro* fermentation characteristics of fresh and ensiled maize (*Zea mays* L.) forage. Vet Sci 10:556. https://doi.org/10.3390/vetsci10090556

France J, Dijkstra J, Dhanoa MS, Lopez S, Bannink A (2000) Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed *in vitro*: derivation of models and other mathematical considerations. Br J Nutr 83:143–150. https://doi.org/10.1017/S0007114500000180

Fritsche S, Wang X, Jung C (2017) Recent advances in our understanding of tocopherol biosynthesis in plants: an overview of key genes, functions, and breeding of vitamin E improved crops. Antioxidants 6:99. https://doi.org/10.3390/antiox6040099

Gado HM, Khusro A, Salem AZM (2017) Role of probiotics in animal nutrition. Anim Rev 4:8–20

Getachew G, Makkar HPS, Becker K (2002) Tropical browses: contents of phenolic compounds, *in vitro* gas production and stoichiometric relationship between short chain fatty acid and *in vitro* gas production. J Agric Sci 139:341–352. https://doi.org/10.1017/S0021859602002393

Goering MK, Van Soest PJ (1970) Forage Fibre Analysis (Apparatus, reagents, procedures and some applications. Agricultural Research Service USDA: Washington, DC, USA, pp 1–24

Haque MN (2018) Dietary manipulation: a sustainable way to mitigate methane emissions from ruminants. J Anim Sci Technol 60:15. https://doi.org/10.1186/s40781-018-0175-7

Hartinger T, Gresner N, Südekum KH (2018) Does intra-ruminal nitrogen recycling waste valuable resources? A review of major players and their manipulation. J Anim Sci Biotechnol 9:33. https://doi.org/10.1186/s40104-018-0249-x

IPCC (2014) Climate Change 2014: contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change: Mitigation of Climate Change. Cambridge University Press, Cambridge, New York, pp 1–3

Islam M, Lee SS (2019) Advanced estimation and mitigation strategies: a cumulative approach to enteric methane abatement from ruminants. J Anim Sci Technol 61:122–137. https://doi.org/10.5187/jast.2019.61.3.122

Khusro A, Aarti C, Elghandour MMMY, Adegbeye MJ, Mellado M, Barbabosa-Pliego A et al (2022a) Dietary manipulation to mitigate greenhouse gas emission from livestock. Handbook of Climate Change Mitigation and Adaptation. Springer International Publishing, Cham, pp 2537–2575

Khusro A, Sahibzada MUK, Khan SU, Rajakrishnan R, Elghandour MMMY, Salem AZM et al (2022b) Anti-methanogenic traits of safflower oil compounds against Methyl-Coenzyme M reductase receptor in equines: an *in silico* docking analysis. J Equine Vet 113:103938. https://doi.org/10.1016/j.jevs.2022.103938

Króliczewska B, Pecka-Kiełb E, Bujok J (2023) Strategies used to reduce methane emissions from ruminants: controversies and issues. Agriculture 13:602. https://doi.org/10.3390/agriculture13030602

Kumar S, Choudhury PK, Carro MD, Griffith GW, Dagar SS, Puniya M et al (2014) New aspects and strategies for methane mitigation from ruminants. Appl Microbiol Biotechnol 98:31–44. https://doi.org/10.1007/s00253-013-5365-0

Lackner M, Sajjadi B, Chen WY (2022) Handbook of Climate Change Mitigation and Adaptation, Springer, 3rd, edition, https://doi.org/10.1007/978-3-030-72579-2

Magalhães ERB, de Menezes NNF, Silva FL, Garrido JWA, Sousa MADSB, dos Santos ES (2021) Effect of oil extraction on the composition, structure, and coagulant effect of *Moringa oleifera* seeds. J Clean Prod 279:123902. https://doi.org/10.1016/j.jclepro.2020.123902

Mangar A, Muetzel S, Malik A, Bhuker A, Mor V, Molenaar A et al (2022) *Moringa oleifera* L.: a potential plant for greenhouse gas mitigation in temperate agriculture systems. Agriculture 12:1116. https://doi.org/10.3390/agriculture12081116

Menke KH, Raab L, Salewski A, Steingass H, Fritz D, Schneider W (2009) The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor *in vitro*. J Agric Sci 93:217–222. https://doi.org/10.1017/S0021859600086305

Oskoueian E, Abdullah N, Oskoueian A (2013) Effects of flavonoids on rumen fermentation activity, methane production, and microbial population. BioMed Res Int 2013:349129. https://doi.org/10.1155/2013/349129

Pal VK, Bandyopadhyay P, Singh A (2018) Hydrogen sulfide in physiology and pathogenesis of bacteria and viruses. IUBMB Life 70:393–410

Palangi V, Lackner M (2022) Management of enteric methane emissions in ruminants using feed additives: a review. Animals 12:3452. https://doi.org/10.3390/ani12243452

Patra AK, Saxena J (2009) The effect and mode of action of saponins on the microbial populations and fermentation in the rumen and ruminant production. Nutr Res Rev 22:204–219. https://doi.org/10.1017/S0954422409990163

Pedraza-Hernández J, Elghandour MM, Khusro A, Camacho-Diaz LM, Vallejo LH, Barbabosa-Pliego A et al (2019) Mitigation of ruminal biogases production from goats using *Moringa oleifera* extract and live yeast culture for a cleaner agriculture environment. J Clean Prod 234:779–786. https://doi.org/10.1016/j.jclepro.2019.06.126

Pedraza-Hernández J, Elghandour MMMY, Khusro A, Salem MZM, Camacho-Diaz LM, Barbabosa-Pliego A et al (2021) Assessment on bioactive role of *Moringa oleifera* leaves as anthelmintic agent and improved growth performance in goats. Trop Anim Health Prod 53:318. https://doi.org/10.1007/s11250-021-02745-9

Ramos-Morales E, de la Fuente G, Duval S, Wehrli C, Bouillon M, Lahmann M et al (2017) Antiprotozoal effect of saponins in the rumen can be enhanced by chemical modifications in their structure. Front Microbiol 8:399. https://doi.org/10.3389/fmicb.2017.00399

Saksrithai K, King AJ (2018) Controlling hydrogen sulfide emissions during poultry productions. J Anim Res Nutr 3:1–14. https://doi.org/10.21767/2572-5459.100040

Santillán MKG, Khusro A, Salem AZ, Pliego AB, Elghandour MM (2023) Potential and valorization of *Salix babylonica* waste leaf extract to mitigate equine fecal production of methane, carbon monoxide, and hydrogen sulfide. Waste Biomass Valori 14:1079–1095. https://doi.org/10.1007/s12649-022-01926-3

SAS (Statistical Analysis System) (2002) Institute, Cary SAS. User's guide: statistics, Version 9.0. SAS Institute, Cary, NC, USA

Shah AM, Ma J, Wang Z, Hu R, Wang X, Peng Q et al (2020) Production of hydrogen sulfide by fermentation in rumen and its impact on health and production of animals. Processes 8(9):1169. https://doi.org/10.3390/pr8091169

Sobieraj K, Stegenta-Dąbrowska S, Luo G, Koziel JA, Białowiec A (2022) Carbon monoxide fate in the environment as an inspiration for biorefinery industry: A review. Front Environ. https://doi.org/10.3389/fenvs.2022.822463

Su B, Chen X (2020) Current status and potential of *Moringa oleifera* leaf as an alternative protein source for animal feeds. Front Vet Sci 7:53. https://doi.org/10.3389/fvets.2020.00053

Sutton AL, Kephart KB, Verstegen MWA, Canh TT, Hobbs PJ (1999) Potential for reduction of odors compounds in swine manure through diet modification. J Anim Sci 77:430–439. https://doi.org/10.2527/1999.772430x

Tavendale MH, Meagher LP, Pacheco D, Walker N, Attwood GT, Sivakumaran S (2005) Methane production from *in vitro* rumen incubations with *Lotus pedunculatus* and *Medicago sativa*, and effects of extractable condensed tannin fractions on methanogenesis. Anim Feed Sci Technol 123–124:403–419. https://doi.org/10.1016/j.anifeedsci.2005.04.037

Theodorou MK, Williams BA, Dhanoa MS, McAllan AB, France JA (1994) Simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim Feed Sci Technol 48:185–197. https://doi.org/10.1016/0377-8401(94)90171-6

Van Soest PV, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J Dairy Sci 74:3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Vasta V, Daghio M, Cappucci A, Buccioni A, Serra A, Viti C et al (2019) Plant polyphenols and rumen microbiota responsible for fatty acid biohydrogenation, fiber digestion, and methane emission: experimental evidence and methodological approaches. J Dairy Sci 102:3781–3804. https://doi.org/10.3168/jds.2018-14985

Vohra A, Syal P, Madan A (2016) Probiotic yeasts in livestock sector. Anim Feed Sci Technol 219:31–47. https://doi.org/10.1016/j.anifeedsci.2016.05.019

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