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Biogas production from prickly pear cactus containing diets supplemented with *Moringa oleifera* leaf extract for a cleaner environmental livestock production

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ABSTRACT

This study presents an experimental investigation of the effect of corn grain replacement with prickly pear cactus and *M. oleifera* leaf extract in ruminal diets. In the control diet, 40 and 70% of corn grain was substituted with prickly pear cactus, while the level of *M. oleifera* leaf extract was varied from 0 to 1.8 mL/g dry matter. A significant interaction between experimental diet and dosage of *M. oleifera* leaf extract was observed for methane, carbon dioxide, and total gas production. An increase in the composition of pear cactus in the diet resulted in a significant decrease in carbon dioxide, methane, as well as the total gas production. A decrease in lag time of gas production was also observed. The asymptotic methane production and the rate of methane production decrease in all the diets with the increase in *M. oleifera* leaf extract doses. Addition of *M. oleifera* leaf extract to the control diet resulted in a decline in carbon dioxide production, while the reverse was the case when *M. oleifera* leaf extract was added to pear cactus containing diet. Therefore, replacement of corn grain with pear cactus and the addition of *M. oleifera* leaf extract resulted in a decrease in the production of greenhouse gases. This is a promising formulation for ecofriendly livestock diet.

1. Introduction

Enteric methane produced during ruminal digestion of feed contributes to up to 12% loss in gross energy (Johnson and Johnson, 1995). Furthermore, methane has global warming potential of 26–28 compared to carbon dioxide and methane emissions from ruminant livestock can have significant impact on livestock's contribution to global warming. Considering significant impacts of methane production on animal performance and greenhouse gas emissions, various approaches including supplementation of yeast products (Elghandour et al., 2017) or salts of organic acids, addition of exogenous enzymes (McGinn et al., 2004) or volatile oils (Hernández et al., 2017) have been explored to reduce methane production. Recently, unconventional feeds and agricultural byproducts in diets of livestock have gained importance (Makkar, 2003) in order to solve the problems connected to shortage and costs of feed ingredients, especially grains. However, information regarding the nutritive values of these feeds is still lacking.

The inclusion of prickly pear cactus (PC; *Optuntia* spp.) offers an alternative approach to replace energy-dense feed ingredient like corn grain because PC exhibits greater dry matter digestibility and high non-fibrous carbohydrate content (617 g/kg DM; Wanderley et al., 2002). Recently, Elghandour et al. (2016) utilized PC as a dietary ingredient in animal diets in semiarid regions. Throughout the tropics, ruminants produce high amounts of methane especially because of low feed quality.

Plant extracts have shown tremendous potential in improving nutritive value of feedstuff for animals in a dose dependent manner

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and these beneficial effects are attributed to the presence of secondary metabolites (Benchaar et al., 2008; Cedillo et al., 2014), which have potential to alter ruminal fermentation (Salem et al., 2014). *M. oleifera* tree, also recognized as 'drumstick tree', is grown in most tropical areas and is of extreme economic and medicinal importance (Makkar and Becker, 1995). Previous studies have suggested *M. oleifera* as an option to replace for example soybean meal and rapeseed meal for ruminants (Soliva et al., 2005) and as a feed additive capable of altering ruminal fermentation (Makkar and Becker, 1995). In addition, a better animal performance with *M. oleifera* leaves supplementation probably due to altered ruminal fermentation and microbial population was observed (Kholif et al., 2015). Diet supplemented with extracted and unextracted *M. oleifera* reduced methane production compared with diets containing rapeseed or soybean meal (Soliva et al., 2005) and the effects were attributed to the presence of greater proportion of α-linolenic acid or secondary metabolites such as tannins and saponins. This study examines the effects of different *M. oleifera* leaf extract concentration in experimental diets containing different levels of PC (as a substitute for corn grain) on *in vitro* rumen fermentation parameters such as nutrient digestibility, gas production parameters, and production of greenhouse gases (methane and carbon dioxide).

2. Materials and methods

2.1. Moringa oleifera leaf extract

Moringa oleifera leaves from different young as well as mature trees were randomly collected from the state of Veracruz, Mexico. The fresh leaves were washed with tap water and chopped into pieces of 1–2 cm in length. Extraction was performed with 8 mL of distilled water per g of leaf for 72 h at a temperature between 25 and 30 °C. Thereafter, the extraction mixture was incubated for 1 h at 39 °C and then gauze filtered. The filtrate was kept at 4 °C until further use. A fresh *M. oleifera* leaf extract (MOLE) was prepared every week.

2.2. Experimental diets

Basal experimental diet (P0) used in this study contains oats straw, soybean hulls, barley, wheat bran, corn gluten feed, molasses, and vitamin-mineral premix added at 249, 250, 120, 30, 30, 70, and 1 g/kg dry matter (DM). Prickly pear cactus was incorporated at three levels; P0 (no PC), P75 (PC added at 75 g/kg DM), and P150 (PC added at 150 g/kg DM). The proportion of corn grain was reduced from 250 g/kg DM in the P0 experimental diet to 175 and 100 g/kg DM for P75, and P150. While the organic matter (OM) content was almost similar for all experimental diets, crude protein (CP) content was greater for P0 compared to P75, and P150 substrates. The diets were formulated in triplicate. *M. oleifera* leaf extract was included with experimental substrate at four different concentrations (0, 0.6, 1.2, and 1.8 mL/g DM) for rumen degradation assay in three runs of incubation in different weeks.

2.3. In vitro rumen degradation assay

Rumen fluid was collected from two Holstein steers 3 h after feeding total mixed ration containing equal proportion of alfalfa hay and commercial concentrate mixture. Rumen contents were collected, composited, and strained using 4 layers of cheesecloth. The strained rumen fluid was immediately transported to the lab, maintained at 39 °C using a water bath, and continuously flushed with CO_2 to mimic ruminal milieu and maintain anaerobic conditions. The strained rumen fluid was buffered with 3 vol of pre-warmed artificial saliva (Goering and Van Soest, 1970) without addition of trypticase. The buffered-ruminal fluid was used as an inoculum for *in vitro* rumen degradation assay.

All experiments were run triplicate. Thereby three independent runs in three different weeks were performed. Within a single run, all samples were included in triplicate. Prickly pear cactus was added in two concentrations (0, 75 and 150 g/kg DM) and *M. oleifera* leaf extract in three doses (0, 0.6, 1.2, and 1.8 mL/g DM).

Substrates (0.5 g) containing different concentrations of MOLE were weighed in glass bottles and mixed with 50 mL of buffered-ruminal fluid (40 mL buffer with 10 mL rumen fluid) (Goering and Van Soest, 1970). The headspace of glass bottles was flushed with CO_2 to maintain anaerobic ruminal milieu. Bottles were closed using neoprene plugs and crimp-sealed to avoid leakage of gases produced during fermentation. Incubations were performed at 39 °C for 72 h. The GP was measured at 2, 4, 6, 8, 10, 12, 14, 16, 18, 24, 36, 48 and 72 h with pressure transducer connected to a voltmeter using a syringe assembly as mentioned earlier (Theodorou et al., 1994). Methane and CO_2 were measured at 6, 10, 14, 18, 24, 48, and 72 h using a Gas-Pro detector (Gas analyzer CORWCON model Tetra3, Abingdon, UK). Bottles were placed on ice to stop the fermentation process at the end of 72 h. Thereafter the bottles were uncapped, and pH of the culture fluid was measured using electrodes on a pH meter (Conductronic pH15, Puebla, Mexico). Culture fluid was filtered through glass crucibles to collect residue left after fermentation. The residues were dried at 65 °C overnight for determination of digestible DM.

2.4. Chemical analysis

Ash, acid detergent fiber (ADF), crude protein (CP), ether extract (EE), lignin and DM were quantified according to the AOAC methods (1997). The quantification ADF and Neutral detergent fiber (NDF) was performed according to Van Soest et al. (1991). The gas volumes recorded were fitted using NLIN procedure of SAS (SAS, 2002) based on model described earlier (France et al., 2000). *In vitro* metabolizable energy (ME) and OM digestibility were obtained as previously described by Menke et al. (1979). Production of microbial biomass (MCP) and the partitioning factor at 24 h of fermentation (PF_{24}) were measured according to the report of Blümmel et al. (1997). The total gas volume measured after 24 h was presented as gas yield (GY_{24}) per gram of digestible DM. Quantification of short chain fatty acids (SCFA) was based on calculations described earlier (Getachew et al., 2002).

2.5. Statistical analyses

A completely randomize design was used to analyzed the data of biogas, methane and carbon dioxide production and degradability using the generalized linear model procedure (SAS, 2002). Data of each one of the 3 runs within the same sample (total mixed ration) of the same level of prickly pear cactus (PC) and *M. oleifera* leaf extract doses (MOLE) were averaged and used as an experimental unit. Each run was used as an experimental unit according to the following model:

$$Y_{ijk} = \mu + D_i + M_j + (S \times M)_{ij} + e_{ijk}$$

where: Y_{ijk} represents result of the *i*th experimental diet (D_i) with *j*th level of MOLE (M_j); μ is the general mean; $(D \times M)_{ij}$ is the interaction between experimental diet and MOLE dose; e_{ijk} the experimental error. Contrast statement was used to observe linear or quadratic trend in various fermentation parameters in response to increasing inclusion levels of PC and MOLE. Results were discussed as significant at 5% level of significance, while tendencies

Table 1
In vitro gas production kinetics in response to experimental diets with three different levels of prickly pear cactus and four different levels *Moringa oleifera* leaf extract (MOLE).

Ration[a]	MOLE (mg/g DM)	Gas production (mL/g DM)[b]		
		b	*c*	*Lag*
P0	0	281	0.030	1.92
	0.6	281	0.035	1.97
	1.2	270	0.040	1.98
	1.8	265	0.036	1.95
P75	0	292	0.028	1.86
	0.6	253	0.027	1.75
	1.2	277	0.028	1.72
	1.8	283	0.028	1.83
P150	0	262	0.027	1.84
	0.6	297	0.030	2.15
	1.2	242	0.024	1.80
	1.8	288	0.027	2.20
Pooled SEM[c]		11.2	0.0030	0.109
Ration effect				
Linear		0.820	0.002	0.044
Quadratic		0.664	0.025	0.080
MOLE effect				
Linear		0.988	0.424	0.191
Quadratic		0.065	0.718	0.208
Ration × MOLE		0.019	0.424	0.275

[a] P75, prickly pear cactus was included in the experimental diet at 75 g/kg DM; P150, prickly pear cactus was included in the experimental diet at 150 g/kg DM.
[b] *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).
[c] SEM standard error of the mean.

were considered between 5 and 10% level of significance.

3. Results

Organic matter, CP, NDF, ADF, and EE content of P0 were 964, 130, 356, 121, and 24 g/kg DM, respectively. The inclusion of PC by replacing corn grain reduced the concentration of CP to 119 and 113 g/kg DM for P75 and P150. The organic matter and EE content averaged around 950 and 23 g/kg DM with inclusion of PC, indicating no significant effect. The NDF and ADF contents were similar for P0 and P150; however, NDF and ADF concentrations were higher for P75 compared to P0 and P150.

The interaction between diet and MOLE has a significant effect on asymptotic GP ($P = 0.02$), but no effect on the rate of GP ($P = 0.42$) or lag phase ($P = 0.28$), in response to various treatments. The rate of GP ($P < 0.01$) and lag phase ($P = 0.04$) linearly decreased with the inclusion of PC. No treatment effects were observed with MOLE inclusion on gas kinetics (Table 1).

Methane production (mL/g DM), corrected for DM, along with proportion of methane production was linearly and quadratically reduced with inclusion of PC ($P < 0.01$; Table 2). MOLE dosage has a significant linear and quadratic effect on total methane produced from degraded DM, and the proportion of total gas produced. Similarly, methane production was significant affected by the interaction effects between diet and MOLE dosage.

The MOLE addition to the experimental diet has no significant effect on GP (Fig. 1). An increase in MOLE dosage resulted in a decrease in the asymptotic methane production (quadratic effect, $P = 0.002$) and the production rate of methane ($P < 0.05$) in all diets (Fig. 2). The interactions of diet type and MOLE dosage significantly affect ($P < 0.01$) both methane and proportional methane production (Table 2). P0 diet supplemented with MOLE resulted in a decrease in carbon dioxide production ($P < 0.05$), but increased (linear effect, $P = 0.025$) in the PC containing diets supplemented with MOLE (Fig. 3). The interactions between diet type and MOLE dosage has a significantly influence ($P < 0.01$) on carbon dioxide production (Table 3). However, the asymptotic GP and the gas production lag time were not affected by MOLE in PC containing diets.

By replacing corn grain with PC in the experimental diets, a linear and quadratic increase ($P < 0.05$) in fermentation pH, and PF_{24} was observed (Table 4). However, the metabolizable energy, OM, SCFA, MCP and GY_{24} decreased (linear and quadratic effect, $P < 0.05$). Meanwhile, inclusion of MOLE into the experimental diets has no significant effect on metabolizable energy, OM, SCFA, MCP and GY_{24}.

Table 2
In vitro methane production in response to experimental diets with three different levels of prickly pear cactus and four different levels *Moringa oleifera* leaf extract (MOLE).

Ration[a]	MOLE (mg/g DM)	Methane production								
		mL/g of DM incubated			mL/g of DM degraded			methane production, % total gas production		
		6 h	24 h	48 h	6 h	24 h	48 h	6 h	24 h	48 h
P0	0	8.8	27.9	42.5	13.6	43.4	66.1	18.4	19.0	19.7
	0.6	5.7	19.4	31.6	8.9	30.1	49.0	10.4	11.9	13.8
	1.2	4.4	15.1	24.8	6.8	23.2	38.2	7.9	9.2	10.9
	1.8	6.6	21.2	32.7	9.8	31.5	48.4	12.6	13.8	15.1
P75	0	4.3	14.0	21.9	6.5	21.3	33.3	11.2	11.3	11.3
	0.6	3.6	12.1	19.3	5.6	18.6	29.6	10.9	11.0	11.2
	1.2	3.4	11.4	18.0	5.4	18.0	28.4	9.7	9.8	9.9
	1.8	3.5	11.7	18.8	5.3	17.9	28.7	8.0	8.5	9.0
P150	0	3.6	11.7	17.9	5.6	18.0	27.6	9.0	9.2	9.4
	0.6	3.7	12.0	18.8	5.5	18.1	28.4	6.8	7.3	7.8
	1.2	3.5	11.4	17.5	5.4	17.4	26.8	11.4	11.2	11.1
	1.8	4.6	14.8	22.6	7.0	22.4	34.2	10.0	10.1	10.2
Pooled SEM[b]		0.39	1.14	1.59	0.61	1.80	2.51	1.03	0.93	0.87
Diet effect										
Linear		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.007	0.003	<0.001
Quadratic		0.003	<0.001	<0.001	0.003	<0.001	<0.001	0.013	0.001	0.001
MOLE effect										
Linear		0.061	0.056	0.053	0.033	0.029	0.026	0.008	0.009	0.015
Quadratic		0.002	0.002	0.002	0.004	0.004	0.004	0.024	0.014	0.011
Ration × MOLE		0.003	0.003	0.003	0.003	0.002	0.003	0.009	0.008	0.002

[a] P75, prickly pear cactus was included in the experimental diet at 75 g/kg DM; P150, prickly pear cactus was included in the experimental diet at 150 g/kg DM.
[b] SEM standard error of the mean.

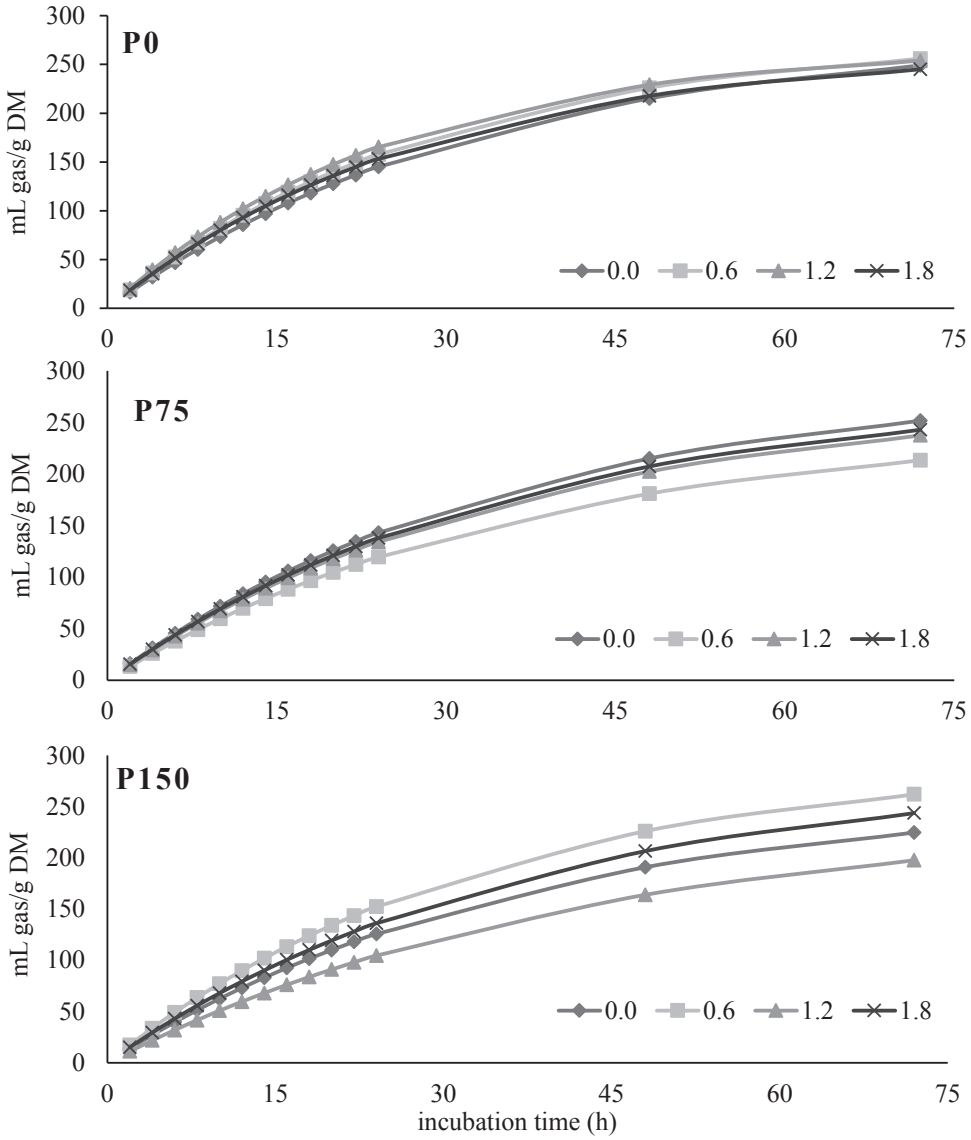

Fig. 1. Effect of experimental diets with different inclusion levels of prickly pear cactus and *Moringa oleifera* leaf extract, at 0, 0.6, 1.2 and 1.8 mL/g DM, on *in vitro* gas production (mL/g DM). Experimental diets were characterized by replacing corn grain by 0, 75, and 150 g/kg DM prickly pear cactus for P0, P75, and P150, respectively.

4. Discussion

4.1. Biogas production

The interactions between experimental diets and MOLE doses reveal that diet composition, as well as MOLE concentrations affect fermentation parameters related to GP in synergistic or antagonistic manner. Furthermore, it can be assumed that alterations of the metabolic processes in the rumen depend on diet composition. The observed short lag time without affecting GP when corn grain was replaced by PC reflects high initial activity of the ruminal microflora for diets with PC. The nutrient availability with experimental diets is associated with GP profiles (Elghandour et al., 2015) and it could be concluded that diets containing PC did not provide the ruminal environment with nutrients or fermentable material for significant microbial activity at the beginning of the incubation. PC was reported to contain per dry matter 50% of non-fibrous carbohydrates (Costa et al., 2009). It seems that this amount of non-fibrous carbohydrates was sufficient to promote the fermentation process, but not to have a significant effect on GP. Therefore, it could be assumed that replacement of corn grain with PC will not affect the nutritive value of diets fed to ruminant livestock.

Secondary metabolites are present in MOLE and it was expected that addition of MOLE would increase GP. It is vivid that addition of *Salix babylonica* extracts in comparable amounts to those used in this study to experimental diets resulted in higher GP and a decrease in the lag time of gas production (Salem et al., 2014). However, such an increase in GP was not observed in this study. Either the nature or the amount of the secondary metabolites introduced into the diets by MOLE addition might explain this observation (Bodas et al., 2012).

4.2. Methane and carbon dioxide production

Reduction strategies for methane production from the livestock sector are always desirable from an environmental perspective. The reduction in methane production in the presence of PC reveals that replacing corn grain by PC might be an effective strategy towards reducing environmental footprint from livestock population. The effects observed on methane production might be due to a

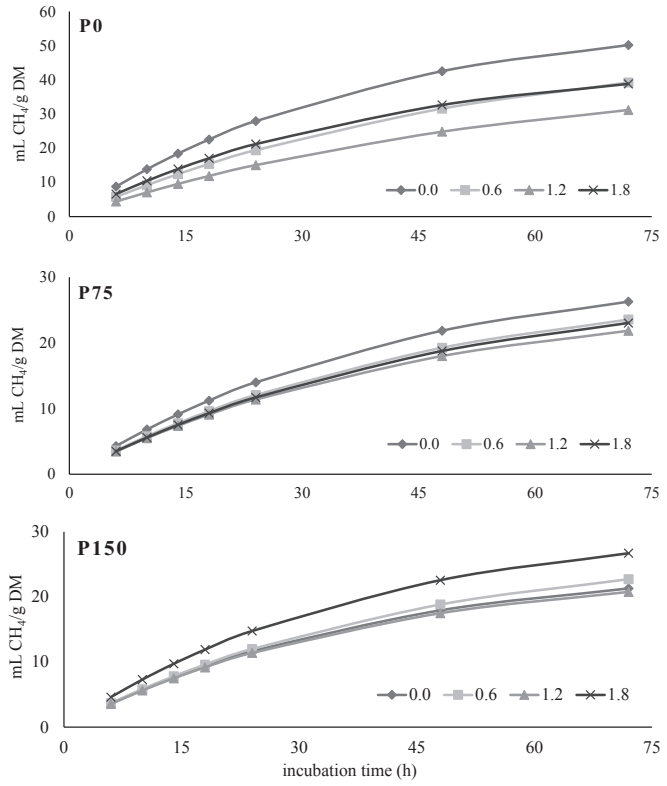

Fig. 2. Effect of experimental diets with different inclusion levels of prickly pear cactus, and *Moringa oleifera* leaf extract, at 0, 0.6, 1.2 and 1.8 mL/g DM, on *in vitro* methane production. Experimental diets were characterized by replacing corn grain by 0, 75, and 150 g/kg DM prickly pear cactus for P0, P75, and P150, respectively.

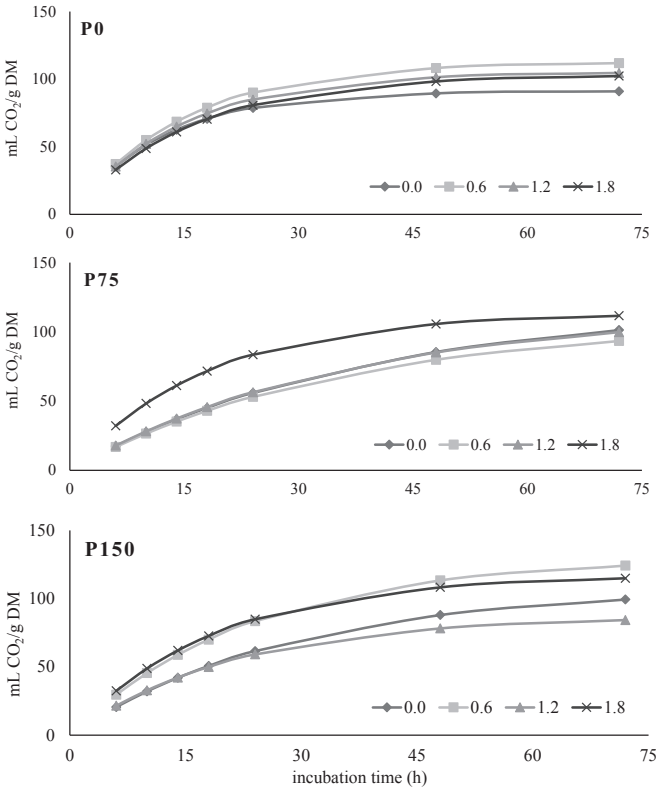

Fig. 3. Effect of experimental diets with different inclusion levels of prickly pear cactus, and *Moringa oleifera* leaf extract, at 0, 0.6, 1.2 and 1.8 mL/g DM, on *in vitro* carbon dioxide production. Experimental diets were characterized by replacing corn grain by 0, 75, and 150 g/kg DM prickly pear cactus for P0, P75, and P150, respectively.

suppressed protozoa populations and a decrease in ruminal methanogenic and gram-positive hydrogen (H_2) producing bacteria. The major type of carbohydrate present in PC (non-fibrous carbohydrates) was expected to shift from short chain fatty acids production to a higher propionic acid concentration (Boadi et al., 2004). Ruminal propionate is a H_2 sink and increased propionate concentration results in reduced supply of H_2 for methane synthesis and subsequently decreasing population of ruminal protozoa (Polyorach et al., 2014). In contrast to the results of this study, Elghandour et al. (2016) observed a higher methane production with increasing amounts of PC in the experimental diets. The reduction in CO_2 production with inclusion of PC might be due to an increase of soluble protein in the experimental diets, since production of H_2 and CO_2 from proteins is lower than from carbohydrates (Singh et al., 2012). The results obtained in this study are contradictory to those obtained recently. It is not clear which factors caused this discrepancy. However, the use of different rumen fluid donors, substrates, and feed additive might have resulted in variable responses.

Addition of MOLE to the experimental diets resulted in a decrease in methane production and an increase in CO_2 production without affecting total GP and the results concur with the studies by Dey et al. (2014) when they used roughage based diet and Soliva et al. (2005) using meadow grass hay based diets. The effects observed on methane production might be because of the presence of secondary metabolites and some active component in the MOLE (Mueller-Harvey, 2006). Plant components such as flavonoids (Broudiscou et al., 2002) and tannins (Carulla et al., 2005) were shown to inhibit the activity of methanogens and subsequently reducing enteric methane emissions. Tannin induced reduction in the activity of methanogens might be due to impaired fiber (Tiemann et al., 2008) and protein digestion (Tavendale et al., 2005) in the rumen.

4.3. Fermentation characteristics

The pH of culture media increased with inclusion of PC accompanied by reduced short-chain fatty acid concentrations, confirming negative correlation between SCFA production and ruminal pH. The decrease in SCFA concentration with increasing PC level in the experimental diet is consistent with observed reduction in ME and OMD values. The observed rise in the value of PF_{24} and decline in OMD with increase in the level of PC may be associated with the reduced conversion of substrate fermented in rumen into microbial biomass (Blümmel et al., 1999). Therefore, replacing corn grain with PC negatively affected fermentability of the experimental diets. The lower ME, SCFA, MCP and GY_{24} production could be attributed to a lower concentration of readily available carbohydrates required for ruminal microbial activity after replacement of corn grain with PC. Dey et al. (2014) observed an increase in *in vitro* OMD, DMD, SCFA, and MCP with inclusion of MOLE contrary to the results of the present study. The discrepancy between both studies might be due to differences in the experimental conditions, the nature of the MOLE, the level of inclusion, the experimental diet used, and the rumen liquor donors.

5. Conclusion

Based on the results from the present study, inclusion of PC by replacing corn grain cannot be recommended from a nutritional perspective. However, methane production decreased with inclusion of PC (41%) and MOLE (24%) after 24 h of incubation, making

Table 3
In vitro carbon dioxide (CO_2) production in response to experimental diets with three different levels of prickly pear cactus and four different levels *Moringa oleifera* leaf extract (MOLE).

Ration[a]	MOLE	CO_2 production								
		mL/g of DM incubated			mL/g of DM degraded			CO_2 production, % total gas production		
		6 h	24 h	48 h	6 h	24 h	48 h	6 h	24 h	48 h
P0	0	36	79	89	55	122	139	75	53	41
	0.6	37	90	108	58	140	168	68	55	47
	1.2	35	85	101	55	131	156	64	52	44
	1.8	33	81	98	49	119	146	63	52	45
P75	0	17	56	85	27	85	130	46	45	44
	0.6	17	53	80	26	82	123	50	48	46
	1.2	18	56	85	28	88	134	49	48	46
	1.8	32	83	106	49	127	161	75	61	51
P150	0	21	62	88	32	95	135	51	48	46
	0.6	30	84	113	45	126	171	55	51	47
	1.2	22	59	78	33	90	119	69	58	49
	1.8	32	85	108	49	129	164	72	59	49
Pooled SEM[b]		2.2	4.0	4.1	3.2	5.8	5.9	6.7	3.5	1.5
Ration effect										
Linear		<0.001	<0.001	0.0036	<0.001	<0.001	0.003	0.025	0.313	0.032
Quadratic		0.135	0.813	0.285	0.090	0.603	0.434	0.918	0.367	0.033
MOLE effect										
inear		0.008	0.002	0.003	0.001	0.002	0.006	0.043	0.013	0.002
Quadratic		0.039	0.022	0.021	0.051	0.035	0.042	0.560	0.821	0.715
Ration × MOLE		0.006	0.003	0.002	0.003	0.001	0.007	0.103	0.172	0.248

[a] P75, prickly pear cactus was included in the experimental diet at 75 g/kg DM; P150, prickly pear cactus was included in the experimental diet at 150 g/g DM.
[b] SEM standard error of the mean.

Table 4
Ruminal fermentation profile[a] in response to experimental diets with three different levels of prickly pear cactus and four different levels *Moringa oleifera* leaf extract (MOLE).

Ration[b]	MOLE (mg/g DM)	pH	ME	DMD	OMD	SCFA	PF_{24}	MCP	GY_{24}
P0	0	5.82	6.87	641	467	3.20	5.97	515	167
	0.6	5.80	7.23	639	490	3.47	5.87	539	171
	1.2	5.91	7.40	652	504	3.63	5.73	555	174
	1.8	5.96	7.10	666	482	3.40	5.87	532	170
P75	0	6.21	6.80	655	460	3.17	6.07	511	165
	0.6	6.04	6.10	647	418	2.63	6.43	469	156
	1.2	6.10	6.53	640	445	2.93	6.20	495	162
	1.8	6.22	6.60	640	451	3.03	6.13	502	164
P150	0	6.07	6.27	653	426	2.77	6.23	480	160
	0.6	6.50	6.97	661	473	3.37	5.90	528	169
	1.2	6.35	5.67	653	388	2.27	6.73	442	149
	1.8	6.33	6.53	658	445	3.00	6.13	499	163
Pooled SEM[c]		0.150	0.291	6.5	19.2	0.243	0.158	20.1	4.2
Ration effect									
Linear		0.018	0.005	0.986	0.005	0.010	0.005	0.008	0.006
Quadratic		0.003	0.014	0.091	0.013	0.034	0.035	0.033	0.027
MOLE effect									
Linear		0.289	0.677	0.070	0.605	0.618	0.734	0.603	0.584
Quadratic		0.877	0.441	0.185	0.489	0.391	0.178	0.524	0.273
Ration × MOLE		0.606	0.062	0.167	0.071	0.072	0.035	0.082	0.047

[a] Dry matter digestibility (DMD) is the amount of DM degraded in mg/g DM; Metabolizable energy (ME; MJ/kg DM); *In vitro* organic matter digestibility (OMD; mg/g DM); short chain fatty acids (SCFA); Partitioning factor at 24 h post-incubation (PF_{24}); gas yield post 24 h incubation (GY_{24}).
[b] P75, prickly pear cactus was included in the experimental diet at 75 g/kg DM; P150, prickly pear cactus was included in the experimental diet at 150 g/kg DM.
[c] SEM standard error of the mean.

both additives more sustainable alternatives compared to corn grain diets. Hence, inclusion of PC and MOLE in ruminant diet might be an effective strategy to reduce livestock impact on greenhouse gas emissions. However, future research is required not only to validate the findings in this study but also to balance the diets in a way that the nutritional potential of the diets with PC and MOLE is not reduced, and animal performance is not compromised.

Conflicts of interest

None.

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References

AOAC, 1997. Association of Official Analytical Chemists. Official Methods of Analysis, sixteenth ed. AOAC, Arlington, VA, USA.
Benchaar, C., Calsamiglia, S., Chaves, A.V., Fraser, G.R., Colombatto, D., McAllister, T.A., Beauchemin, K.A., 2008. A review of plant-derived essential oils in ruminant nutrition and production. Anim. Feed Sci. Technol. 145, 209–228.
Blümmel, M., Mgomezulu, R., Chen, X.B., Makkar, H.P., Becker, K., Ørskov, E.R., 1999.

The modification of an *in vitro* gas production test to detect roughage related differences in *in vivo* microbial protein synthesis as estimated by the excretion of purine derivatives. J. Agric. Sci. Cambridge 133, 335–340.
Blümmel, M., Steingass, H., Becker, K., 1997. The relationship between *in vitro* gas production, *in vitro* microbial biomass yield and 15N incorporation and its implications for the prediction of voluntary feed intake of roughages. Br. J. Nutr. 77, 911–921.
Boadi, D., Benchaar, C., Chiquette, J., Massé, D., 2004. Mitigation strategies to reduce enteric methane emissions from dairy cows: update review. Can. J. Anim. Sci. 84 (3), 319–335.
Bodas, R., Prieto, N., García-González, R., Andrés, S., Giráldez, F.J., López, S., 2012. Manipulation of rumen fermentation and methane production with plant secondary metabolites. Anim. Feed Sci. Technol. 176, 78–93.
Broudiscou, L.P., Papon, Y., Broudiscou, A.F., 2002. Effects of dry plant extracts on feed degradation and the production of rumen microbial biomass in a dual outflow fermenter. Anim. Feed Sci. Technol. 101 (1), 183–189.
Carulla, J.E., Kreuzer, M., Machmüller, A., Hess, H.D., 2005. Supplementation of *Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. Crop Pasture Sci. 56 (9), 961–970.
Cedillo, J., Vázquez-Armijo, J.F., González-Reyna, A., Salem, A.Z., Kholif, A.E., Hernández-Meléndez, J., Martínez-González, J.C., Jiménez, R.M.D.O., Rivero, N., López, D., 2014. Effects of different doses of *Salix babylonica* extract on growth performance and diet *in vitro* gas production in Pelibuey growing lambs. Italian J. Anim. Sci. 13 (3), 609–6013.
Costa, R.G., Filho, E.M.B., de Medeiros, A.N., Givisiez, P.E.N., Queirogac, R.C.R.E., Silva Melo, A.A.S., 2009. Effects of increasing levels of cactus pear (*Opuntia ficus*-indica L. Miller) in the diet of dairy goats and its contribution as a source of water. Small Rumin. Res. 82, 62–65.
Dey, A., Paul, S.S., Pandey, P., Rathore, R., 2014. Potential of *Moringa oleifera* leaves in modulating *in vitro* methanogenesis and fermentation of wheat straw in buffalo. Indian J. Anim. Sci. 84 (5), 533–538.
Elghandour, M.M.Y., Kholif, A.E., Bastida, A.Z., Martínez, D.L.P., Salem, A.Z.M., 2015. *In vitro* gas production of five rations of different maize silage and concentrate ratios influenced by increasing levels of chemically characterized extract of *Salix babylonica*. Turk. J. Vet. Anim. Sci. 39 (2), 186–194.
Elghandour, M.M.Y., Kholif, A.E., Salem, A.Z.M., Olafadehan, O.A., Kholif, A.M., 2016. Sustainable anaerobic rumen methane and carbon dioxide productions from prickly pear cactus flour by organic acid salts addition. J. Clean. Prod. 139, 1362–1369.
Elghandour, M.M.Y., Vázquez, J.C., Salem, A.Z.M., Kholif, A.E., Cipriano, M.M., Camacho, L.M., Márquez, O., 2017. *In vitro* gas and methane production of two mixed rations influenced by three different cultures of *Saccharomyces cerevisiae*. J. Appl. Anim. Res. 45 (1), 389–395.
France, J., Dijkstra, J., Dhanoa, M.S., 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.
Getachew, G., Makkar, H.P.S., 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. Cambridge 139, 341–352.
Goering, M.K., Van Soest, P.J., 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures and Some Applications). Agriculture Handbook, No 379. Agricultural Research Service, USDA, Washington, DC.
Hernández, A., Kholif, A.E., Lugo-Coyote, R., Elghandour, M.M.Y., Cipriano, M., Rodríguez, G.B., Odongo, N.E., Salem, A.Z.M., 2017. The effect of garlic oil, xylanase enzyme and yeast on biomethane and carbon dioxide production from 60-d old Holstein dairy calves fed a high concentrate diet. J. Clean. Prod. 142, 2384–2392.
Johnson, K.A., Johnson, D.E., 1995. Methane emissions from cattle. J. Anim. Sci. 73, 2483–2492.
Kholif, A.E., Gouda, G.A., Morsy, T.A., Salem, A.Z.M., Lopez, S., Kholif, A.M., 2015. *Moringa oleifera* leaf meal as a protein source in lactating goat's diets: feed intake, digestibility, ruminal fermentation, milk yield and composition, and its fatty acids profile. Small Rumin. Res. 129, 129–137.
Makkar, H.P.S., Becker, K., 1995. Nutritional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. Anim. Feed Sci. Technol. 63, 211–228.
Makkar, H.P.S., 2003. Effects and fat of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Rumin. Res. 49, 241–256.
McGinn, S.M., Beauchemin, K.A., Coates, T., Colombatto, D., 2004. Methane emissions from beef cattle: effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. J. Anim. Sci. 82, 3346–3356.
Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D., Schneider, W., 1979. 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. Cambridge 93, 217–222.
Mueller-Harvey, I., 2006. Review, unraveling the conundrum of tannins in animal nutrition and health. J. Sci. Food Agric. 86, 2010–2037.
Polyorach, S., Wanapat, M., Cherdthong, A., 2014. Influence of yeast fermented cassava chip protein (YEFECAP) and roughage to concentrate ratio on ruminal fermentation and microorganisms using *in vitro* gas production technique. Asian-Australas. J. Anim. Sci. 27, 36–45.
Salem, A.Z., Kholif, A.E., Olivares, M., Elghandour, M.M., Mellado, M., Arece, J., 2014. Influence of *S. babylonica* extract on feed intake, growth performance and diet *in vitro* gas production profile in young lambs. Trop. Anim. Health Prod. 46 (1), 213–219.
SAS, 2002. Statistical Analysis System. User's Guide: Statistics. Ver 9.0. SAS Institute, Cary, NC.
Singh, S., Kushwaha, B.P., Nag, S.K., Mishra, A.K., Singh, A., Anele, U.Y., 2012. *In vitro* ruminal fermentation, protein and carbohydrate fractionation, methane production and prediction of twelve commonly used Indian green forages. Anim. Feed Sci. Technol. 178, 2–11.
Soliva, C.R., Kreuzer, M., Foidl, N., Foidl, G., Machmuller, A., Hess, H.D., 2005. Feeding value of whole and extracted *Moringa oleifera* leaves for ruminants and their effects on ruminal fermentation *in vitro*. Anim. Feed Sci. Technol. 118, 47–62.
Tavendale, M.H., Meagher, L.P., Pacheco, D., Walker, N., Attwood, G.T., 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, 403–419.
Theodorou, M.K., Williams, B.A., Dhanoa, M.S., McAllan, A.B., France, J., 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim. Feed Sci. Technol. 48, 185–197.
Tiemann, T.T., Lascano, C.E., Wettstein, H.-R., Mayer, A.C., Kreuzer, M., Hess, H.D., 2008. Effect of the tropical tannin-rich shrub legumes *Calliandra calothyrsus* and *Flemingia macrophylla* on methane emission and nitrogen and energy balance in growing lambs. Animal 2, 790–799.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fibre, neutral detergent fibre, and non-starch carbohydrates in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.
Wanderley, W.L., Ferreira, M.A., Andrade, D.K.B., Véras, A.S.C., Farias, I., Lima, L.E., Dias, A.M.A., 2002. Replacement of forage cactus (*Opuntia fícus* indica Mill) for sorghum silage (*Sorghum bicolor* (L.) oench) in the dairy cows feeding. Rev. Bras. Zootec. 31, 273–281.