Journal of Cleaner Production 139 (2016) 1362–1369

Contents lists available at ScienceDirect

Journal of Cleaner Production

journal homepage: www.elsevier.com/locate/jclepro

Sustainable anaerobic rumen methane and carbon dioxide productions from prickly pear cactus flour by organic acid salts addition

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ARTICLE INFO

Article history:
Received 17 April 2016
Received in revised form
16 August 2016
Accepted 16 August 2016
Available online 18 August 2016

Keywords:
Fermentation
Gas production
Organic acid salts
Steamed rolled corn
Prickly pear cactus

ABSTRACT

Ruminal fermentation is accompanied by production of methane (CH_4) and carbon dioxide (CO_2) which are greenhouses making the Earth warmer. Therefore, the sustainable production of CH_4 and CO_2 as well as fermentation kinetics when corn grain (CG) was replaced with prickly pear cactus flour (PC) was investigated. Besides, the effect of different levels of organic acid salts (OAS) was studied. Three total mixed rations used as substrates were prepared where CG was replaced with PC at three levels (/kg): 0 g (Control), 75 g (PC75) or 150 g (PC150). The OAS was used at three levels: 0, 5 and 10 mg/g dry matter (DM) of substrates. Asymptotic gas (GP), CH_4, and CO_2 productions as well as lag time had linear responses ($P < 0.05$) as PC level increased in the ration. Fractional rate of GP ($P = 0.007$), GP, organic matter degradability, short chain fatty acid, and microbial biomass production ($P < 0.001$) were increased with increasing level of PC. Fermentation pH ($P < 0.001$), and DM degradability ($P = 0.0448$) were linearly decreased as the PC level increased in the rations. Ration × OAS interaction did not affect fermentation kinetics, GP, CH_4 production and fermentation parameters. Prickly pear cactus flour at 150 g/kg DM inclusion level in ruminant ration has the potential to replace 60% of corn grain with enhanced fermentation and biodegradation efficiency *in vitro*; however, it increased CH_4 production, which cannot be an environmental friendly way of feeding livestock.

1. Introduction

Worldwide, agricultural byproducts produced during different agricultural practices are nutrients-rich feed ingredients with a large potential to be used for ruminants nutrition (Ahmed et al., 2015; Elghandour et al., 2016a); however, in many developing countries, agriculture byproducts always cause environmental problems resulting from their burning in the field. Such feeds can be used as a cleaner product of animal feed and environment (Elghandour et al., 2016b). Moreover, as the global population is increasing, the conventional feed for animal production, such as grains, legumes, etc. is in shortage and highly priced in many parts of the world. The soaring prices of cereals, such as barley, wheat and corn, which are the major energy sources in ruminant diets necessitate search for inexpensive alternatives that can partially substitute for the expensive grains. Feeding of unconventional feedstuffs, which are of no food value to humans, can be one of the solutions. However, apart from being cheap, such unconventional feed must be available all year round, particularly during the critical dry season, to guarantee sustainable continuous supply of feed; but they may affect methane (CH_4) and carbon dioxide (CO_2) productions from livestock as the CO_2 and CH_4 emissions from ruminants depend on diet degradability and chemical composition (Elghandour et al., 2016c). Methane and CO_2 productions from ruminant livestock are one of the sources responsible for greenhouse gas emission (Intergovernmental Panel on Climate Change, 2008) due to ruminal fermentation of feed in the rumen resulting in a loss of digested energy (Johnson and Johnson, 1995). According to the Food and Agriculture Organization of the United Nations (FAO), CH_4 emission from animal production sector is responsible for about 18% of all greenhouse gas emissions, while CO_2 accounts for about 9% (FAO, 2006).

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http://dx.doi.org/10.1016/j.jclepro.2016.08.075

Cacti (*Opuntia* spp.) have been recognized as one of the most widely used low cost alternative feeds in semi-arid regions of the world due to their adaptation to different environmental conditions (Stintzing and Carle, 2005). They have become an important source of green fodder which ensures several livestock species survival in the semi-arid and arid regions of the world with frequent periods of prolonged droughts (Costa et al., 2009). The chemical composition (g/kg dry matter (DM)) of spineless cactus pear species as reported (Costa et al., 2009) is: 78.9 DM, 48.3 crude protein (CP), 10.6 ether extract (EE), 108.7 ash, 290.7 neutral detergent fiber (NDF) and 257.1 acid detergent fiber (ADF). Being rich in non-fibrous carbohydrates (617 g/kg DM), it is an excellent energy source with high DM digestibility coefficient (Wanderley et al., 2002). Replacement of energy feedstuff such as corn grain (CG) with prickly pear cactus (PC) may require some form of supplementation with organic salts and acids which are used as energy additives and rumen modifiers in ruminant diets. Rumen modifiers such as sodium or calcium propionate (Ferraro et al., 2009), disodium malate or calcium malate (Mungói et al., 2012), exogenous fibrolytic enzymes (Morsy et al., 2016) and *Saccharomyces cerevisiae* (Rodriguez et al., 2015) have been used as ingredients of rations for ruminants. However, little is known about the nutritive value of organic acid salts (OAS) in ruminant nutrition.

The *in vitro* gas production (GP) procedure, a useful tool to study potential rumen degradation of ruminant feeds (Getachew et al., 2002; Vallejo et al., 2016), allows estimation of short chain fatty acid (SCFA) from substrate and the energetic value of feed, and the determination of the amount of substrate truly fermented which is used to synthesis microbial protein (Blümmel et al., 2003; Elghandour et al., 2015a,b). The current study aimed to investigate the impact of replacing CG of diet with PC in the presence of a fermentation modulator (i.e., OAS) at different levels on ruminal *in vitro* GP, CH_4 and CO_2 productions as well as fermentation kinetics.

2. Materials and methods

2.1. Substrates and treatments

Three total mixed rations, used as incubation substrates, were prepared where CG was replaced with PC at three levels (/kg): 0 g (Control), 75 g (PC75) or 150 g (PC150). The chemical composition and ingredients is shown in Table 1. Organic acid salts (Rumalato®, Norel, Mexico), a product containing salts of organic acids including monopropylen glycol, calcium propionate, calcium malate and other active compounds (Table 2), was used at three supplemental levels: 0, 5 and 10 mg/g DM of substrates.

2.2. In vitro fermentation and biodegradation

Rumen inoculum was collected from a Brown Swiss cow (450 kg BW), fitted with a permanent rumen cannula, fed *ad libitum* a formulated total mixed ration of a commercial concentrate (PURINA®, Toluca, Mexico) and alfalfa hay in the ratio of 1:1 DM according to NRC (2001). During collection phase, cow was offered fresh water *ad libitum*. Collected rumen contents were flushed with CO_2, mixed and strained through four layers of cheesecloth into a flask with oxygen (O_2)-free headspace. Samples (0.5 g) of each ration were weighed into 120 mL serum bottles with appropriate addition of OAS dose/g DM. Consequently, 10 mL of particle free rumen fluid was added to each bottle followed by 40 mL of the buffer solution of Goering and Van Soest (1970), with no trypticase added.

Three incubation runs were performed in three different weeks. Eighty one bottles (three bottles for each ration × three levels of OAS × three different runs) plus three bottles as blanks (rumen fluid only) were incubated for 72 h. Once all bottles were filled, they were immediately closed with rubber stoppers, shaken and placed in an incubator at 39 °C. The volume of GP was recorded at 2, 4, 6, 8, 10, 12, 14, 16, 18, 24, 36, 48 and 72 h using the Pressure Transducer Technique (Extech instruments, Waltham, USA) of Theodorou et al. (1994). Both of CH_4 and CO_2 productions were recorded at 2, 6, 12, 18, 24, 36, 48 and 72 h of incubation using Gas-Pro detector (Gas Analyzer CROWCON Model Tetra3, Abingdon, UK). At the end of incubation at 72 h, the fermentation process was stopped by swirling the bottles in ice, then the bottles were uncapped and the pH was measured using a pH meter (Conductronic pH15, Puebla, Mexico) and the contents of each bottle were filtered under vacuum through glass crucibles (coarse porosity no. 1, pore size 100–160 μm; Pyrex, Stone, UK) with a sintered filter to obtain the non-fermented residue for determination of degraded substrate after drying at 65 °C overnight.

2.3. Chemical analyses and calculations

Samples of the rations were analyzed for DM (#934.01), ash (#942.05), nitrogen (#954.01) and EE (#920.39) according to AOAC (1997), while ration's contents for NDF (Van Soest et al., 1991), ADF and lignin (AOAC, 1997; #973.18) analyses were carried out using an ANKOM[200] Fiber Analyzer Unit (ANKOM Technology Corp., Macedon, NY, USA) with the use of an alpha amylase and sodium sulfite.

For estimation of GP, CH_4, and CO_2 kinetic, recorded volumes of each gas (mL/g DM) were fitted using the NLIN procedure of SAS

Table 1
Composition of the experimental total mixed rations.

	Control	PC75	PC150
Ingredients (g/kg DM)			
Oats straw	249	248	248
Steam rolled corn	250	175	100
Soybean hulls	250	250	250
Steam rolled barley	120	110	120
Wheat bran	30	30	30
Corn gluten feed	30	30	20
Prickly pear cactus	0	75	150
Molasses	70	80	80
Vitamins/minerals[a]	1	2	2
Chemical composition (g/kg DM)			
Organic matter	964	940	957
Crude protein	130	119	113
Neutral detergent fiber	356	428	340
Acid detergent fiber	121	130	122
Ether extract	24	22	23
Non-structural carbohydrates	455	371	481

[a] Contained per kilogram: vitamin A (12 000 000 IU), vitamin D_3 (2 500 000 IU), vitamin E (15 000 IU), vitamin K (2.0 g), vitamin B_1 (2.25 g), vitamin B_2 (7.5 g), vitamin B_6 (3.5 g), vitamin B_{12} (20 mg), pantotenic acid (12.5 g), folic acid (1.5 g), biotin (125 mg), niacin (45 g), Fe (50 g), Zn (50 g), Mn (110 g), Cu (12 g), I (0.30 g), Se (200 mg), Co (0.20 g).

Table 2
Composition (g/kg DM) of the rumen fermentative modulator of organic acid salts (Adapted from Elghandour et al., 2016b).

	ppm	Inclusion	Concentration
Monopropylene glycol powder	60	196	118
Calcium propionate	98	393	385
Calcium malate	60	371.9	223
Silicon dioxide	100	20	20
Amino acid-chelate Zn	26	8	2080 ppm
Zinc-L-selenomethionine Se	10	0.12	12 ppm
1,25-$(OH)_2$-D_3	10	10	0.1 ppm
Vitamin E, IU/kg	500,000	1	500 IU/kg

(2002) according to France et al. (2000) model as:

(1) $y = b \times [1 - e^{-c\,(t-L)}]$

where y is the volume of GP at time t (h); b is the asymptotic GP (mL/g DM); c is the fractional rate of fermentation (/h), and L (h) is the discrete lag time prior to any gas is released.

Metabolizable energy (ME, MJ/kg DM) and *in vitro* organic matter digestibility (OMD, g/kg DM) were estimated according to Menke et al. (1979) as:

(2) ME = 2.20 + 0.136 GP (mL/0.5 g DM) + 0.057 CP (g/kg DM)
(3) OMD = 148.8 + 8.89 GP + 4.5 CP (g/kg DM) + 0.651 ash (g/kg DM)

where: GP is net GP in mL from 200 mg of dry sample after 24 h of incubation.

The partitioning factor at 24 h of incubation (PF_{24}; a measure of fermentation efficiency) was calculated as the ratio of DM degradability *in vitro* (mg) to the volume (mL) of GP at 24 h (*i.e.*, DMD/total GP at 24 h of incubation (GP_{24})) according to Blümmel et al. (1997). Gas yield (GY_{24}) was calculated as the volume of gas (mL gas/g DM) produced after 24 h of incubation divided by the amount of DMD (g) as:

(4) GY_{24} = mL gas/g DM/g DMD

Short chain fatty acid concentrations were calculated according to Getachew et al. (2002) as:

(5) SCFA (mmol/200 mg DM) = 0.0222 GP - 0.00425

where: GP is the 24 h net GP (mL/200 mg DM).

Microbial biomass production (MCP) was calculated (Blümmel et al., 1997) as:

(6) MCP (mg/g DM) = milligrams DMD - (milliliter gas × 2.2 mg/mL)

where the 2.2 mg/mL is a stoichiometric factor that expresses mg of carbon, hydrogen (H_2), and O_2 required for the SCFA gas associated with production of 1 mL of gas (Blümmel et al., 1997).

2.4. Statistical analyses

Data of each of the three runs within the same sample of each of the three individual samples of rations were averaged prior to statistical analysis, then mean values of each individual sample were used as the experimental unit. Results of *in vitro* GP and rumen fermentation parameters were analyzed as a factorial experiment using the PROC GLM option of SAS (2002) as:

$$Y_{ijk} = \mu + R_i + D_j + (R \times D)_{ij} + E_{ijk}$$

where: Y_{ijk} is every observation of the ith ration type (R_i) with jth OAS dose (D_j); μ is the general mean; $(R \times D)_{ij}$ is the interaction between ration type and OAS dose; E_{ijk} is the experimental error. Linear and quadratic polynomial contrasts were used to examine responses of different rations (substrates) to increasing addition levels of OAS. Statistical significance was declared at $P < 0.05$.

3. Results

Fig. 1 shows the *in vitro* rumen GP (mL/g incubated DM) of three different levels of PC as affected by different levels of OAS.

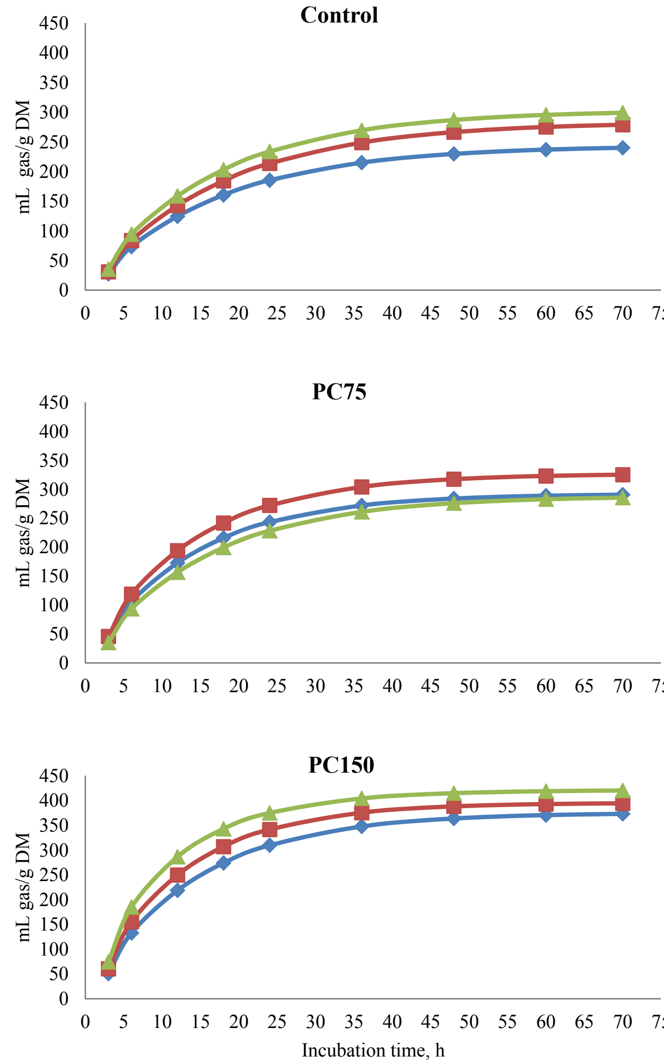

Fig. 1. *In vitro* rumen gas production (mL/g incubated DM) of three different levels of prickly pear cactus flour as affected by different levels of organic acid salts at 0 (-◆-), 5 (-■-), and 10 (-▲-) mg/g DM of the ration. Control: corn grain was replaced with prickly pear cactus flour at 0 g/kg DM; PC75: prickly pear cactus flour was included at 75 g/kg DM of total mixed ration; PC150: prickly pear cactus flour was included at 150 g/kg DM of total mixed ration.

Asymptotic GP was increased linearly ($P < 0.001$) and quadratically ($P = 0.044$) by the level of PC, but it was not influenced by inclusion of OAS (dose) and ration × OAS dose interaction, respectively. Fractional rate of GP was increased ($P = 0.007$) linearly with increasing level of PC, while effects of ration, and ration × dose interaction were not pronounced ($P > 0.05$) (Table 3). Ration effect on lag time showed both linear ($P = 0.022$) and quadratic ($P = 0.036$) trends. Dose effect on lag time was quadratic ($P = 0.022$) but ration × OAS dose interaction did not influence lag time ($P = 0.748$). Both dose and ration × OAS dose interaction had no effect ($P > 0.05$) on GP at all hours of incubation (Table 4).

Methane production (mL/g incubated DM) is shown in Fig 2. Increasing PC level in the ration linearly increased ($P < 0.001$) the asymptotic CH_4 production (Table 3). The ration PC75 had the highest ($P < 0.05$) CH_4 production (as mL/g DM incubated and mL/g DM incubated) at 24 and 48 h of incubation (Table 4). Level of OAS affected the asymptotic CH_4 production, with the highest ($P < 0.05$) values being observed with the doses 5 and 10 mg/g DM of the PC150 ration. Both PC levels and OAS doses had no effect ($P > 0.05$)

Table 3
In vitro rumen gas, methane (CH_4) and carbon dioxide (CO_2) kinetics of three different levels of prickly pear cactus as affected by different levels of organic acid salts (OAS).

Ration[a]	OAS (mg/g DM)	Gas production (mL/g DM)[b]			CH_4 production (mL/g DM)[c]			CO_2 production (mL/g DM)[d]		
		b	*C*	*L*	*b*	*c*	*L*	*b*	*c*	*L*
Control	0	244	0.060	1.35	57.9	0.021	14.38	177	0.007	5.56
	5	284	0.058	1.10	63.1	0.033	17.12	210	0.010	4.82
	10	304	0.062	1.61	67.6	0.038	16.58	208	0.005	6.39
PC75	0	293	0.075	1.79	89.0	0.032	13.65	174	0.012	5.34
	5	328	0.074	1.81	63.6	0.040	14.82	250	0.015	5.11
	10	289	0.065	1.95	81.8	0.031	13.78	198	0.010	6.80
PC150	0	376	0.073	1.53	98.9	0.021	15.70	238	0.006	4.73
	5	396	0.083	1.64	107.1	0.020	14.26	249	0.005	6.15
	10	422	0.095	2.05	125.6	0.023	14.06	230	0.248	4.43
Pooled SEM[e]		22.7	0.0071	0.188	6.07	0.0102	1.932	22.1	0.0640	1.258
Ration effect										
Linear		<0.001	0.007	0.022	<0.001	0.294	0.403	0.035	0.148	0.641
Quadratic		0.044	0.974	0.036	0.061	0.273	0.368	0.484	0.458	0.657
Dose effect										
Linear		0.106	0.679	0.809	0.430	0.440	0.608	0.040	0.971	0.883
Quadratic		0.271	0.517	0.022	0.014	0.683	0.896	0.798	0.101	0.520
Ration × Dose		0.534	0.283	0.748	0.440	0.870	0.826	0.571	0.207	0.659

[a] Control: corn grain was replaced with prickly pear cactus flour at 0 g/kg DM; PC75, prickly pear cactus was included at 75 g/kg DM of total mixed ration; PC150, prickly pear cactus was included at 150 g/kg DM of total mixed ration.
[b] *b* is the asymptotic gas production (mL/g DM); *c* is the rate of gas production (/h); *L* is the initial delay before gas production begins (h).
[c] *b* is the asymptotic methane production (mL/g DM); *c* is the rate of methane production (/h); *L* is the initial delay before methane production begins (h).
[d] *b* is the asymptotic carbon dioxide production (mL/g DM); *c* is the rate of carbon dioxide production (/h); *L* is the initial delay before carbon dioxide production begins (h).
[e] SEM standard error of the mean.

on lag time and rate of CH_4 production (Table 3).

Fig. 3 shows the *in vitro* rumen CO_2 (mL/g incubated DM) of three different levels of PC as affected by different levels of OAS. Interaction between ration × OAS dose was observed for CO_2 production expressed as mL/g incubated DM. With no effect ($P > 0.05$) on rate of CO_2 and lag time of CO_2, the PC150 had the highest (linear effect, $P = 0.035$) asymptotic CO_2 production (Table 3). However, ration type and OAS levels did not affect ($P > 0.05$) CO_2 production (mL/g incubated DM, mL/g degraded DM and proportional CO_2 production) at 6, 24 and 48 h of incubation (Table 4).

Both dose and ration × OAS dose interactions had no ($P > 0.05$) effect on the *in vitro* fermentation profiles but ration effect was pronounced for all the fermentation parameters. Whereas pH and PF_{24} ($P < 0.001$), and DMD ($P = 0.0448$) were decreased with increasing level of PC, ME, OMD, SCFA, MCP and GY_{24} was increased ($P < 0.001$) with increasing level of PC in the ration (Table 5).

4. Discussion

The extent of feed fermentation and digestibility is reflected by gas produced in *in vitro* fermentation. The linear and quadratic increases in asymptotic GP with increasing level of PC replacement for CG indicate an increasing fermentation of the insoluble but degradable fraction. The result suggests a steady increasing availability of carbohydrate fractions to the microbial population, in consonance with previous studies (Elghandour et al., 2015a,b;

Table 4
Proportional *in vitro* methane (CH_4) and carbon dioxide (CO_2) productions as a percent of total gas production of three different levels prickly pear cactus as affected by different levels of organic acid salts (OAS).

Ration[a]	OAS (mg/g DM)	CH_4 production									CO_2 production								
		mL/g incubated DM			mL/g degraded DM			Proportional CH_4 production			mL/g incubated DM			mL/g degraded DM			Proportional CO_2 production		
		6 h	24 h	48 h	6 h	24 h	48 h	6 h	24 h	48 h	6 h	24 h	48 h	6 h	24 h	48 h	6 h	24 h	48 h
Control	0	6.7	22.5	36.2	8.2	27.5	44.2	9.30	12.2	15.8	7.3	26.1	45.5	9.4	33.6	58.6	10.1	13.9	19.3
	5	10.8	31.7	45.1	14.3	41.9	59.6	14.1	15.9	17.8	14.0	48.7	81.8	18.8	65.4	109.8	14.9	20.6	28.1
	10	13.5	36.5	49.8	18.5	50.5	67.9	14.9	15.7	17.3	6.1	23.1	43.4	8.1	31.0	58.2	6.1	9.4	14.6
PC75	0	15.5	47.2	69.0	21.3	65.2	95.4	14.8	19.4	24.3	10.6	36.8	62.7	14.7	51.2	87.1	9.9	15.2	22.2
	5	12.9	34.6	47.1	17.0	45.8	62.7	12.6	13.9	15.7	21.0	73.8	125.8	28.3	99.8	170.0	18.4	27.7	40.0
	10	13.6	40.0	57.9	17.5	51.7	74.8	14.9	18.0	21.4	12.5	41.7	66.9	16.2	54.0	86.7	12.2	16.8	22.5
PC150	0	12.0	39.9	63.4	16.0	52.9	84.2	9.4	13.1	17.6	8.1	30.2	55.0	10.7	39.9	72.7	6.5	10.1	15.4
	5	12.2	41.0	66.3	17.2	58.0	93.7	7.8	11.9	17.0	8.0	28.7	49.9	11.2	40.2	69.8	5.0	8.1	12.2
	10	16.4	52.9	81.8	21.7	70.0	108.4	10.2	15.0	20.4	16.3	57.9	99.2	21.6	76.7	132.4	8.8	15.4	24.0
Pooled SEM[b]		3.69	8.46	9.41	4.89	11.11	12.18	4.42	3.93	3.47	7.00	6.72	3.45	6.41	5.27	14.47	1.13	1.90	9.58
Ration effect																			
Linear		0.302	0.050	0.003	0.263	0.037	0.001	0.326	0.703	0.627	0.058	0.056	0.138	0.059	0.054	0.135	0.172	0.390	0.660
Quadratic		0.449	0.602	0.894	0.460	0.600	0.878	0.328	0.281	0.270	0.426	0.828	0.770	0.434	0.854	0.739	0.679	0.683	0.403
Dose effect																			
Linear		0.854	0.915	0.666	0.806	0.998	0.798	0.926	0.753	0.410	0.757	0.386	0.265	0.747	0.364	0.244	0.669	0.393	0.333
Quadratic		0.299	0.258	0.208	0.316	0.284	0.243	0.533	0.517	0.500	0.380	0.057	0.207	0.401	0.064	0.237	0.078	0.221	0.519
Ration × Dose		0.792	0.633	0.418	0.676	0.467	0.236	0.927	0.840	0.618	0.042	0.017	0.049	0.043	0.018	0.051	0.049	0.045	0.106

[a] Control: corn grain was replaced with prickly pear cactus flour at 0 g/kg DM; PC75, prickly pear cactus was included at 75 g/kg DM of total mixed ration; PC150, prickly pear cactus were included at 150 g/kg DM of total mixed ration.
[b] SEM standard error of the mean.

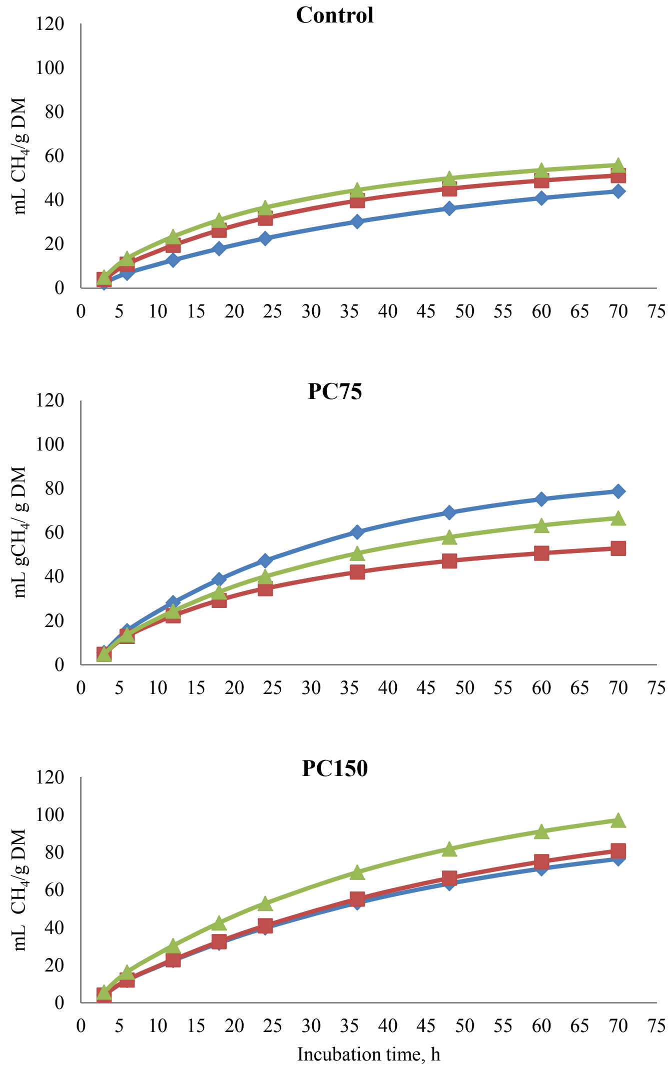

Fig. 2. *In vitro* methane (CH_4) production (mL/g incubated DM) of three different levels of prickly pear cactus flour as affected by different levels of organic acid salts at 0 (-◆-), 5 (-■-), and 10 (-▲-) mg/g DM of the ration. Control: corn grain was replaced with prickly pear cactus flour at 0 g/kg DM; PC75: prickly pear cactus flour was included at 75 g/kg DM of total mixed ration; PC150: prickly pear cactus flour was included at 150 g/kg DM of total mixed ration.

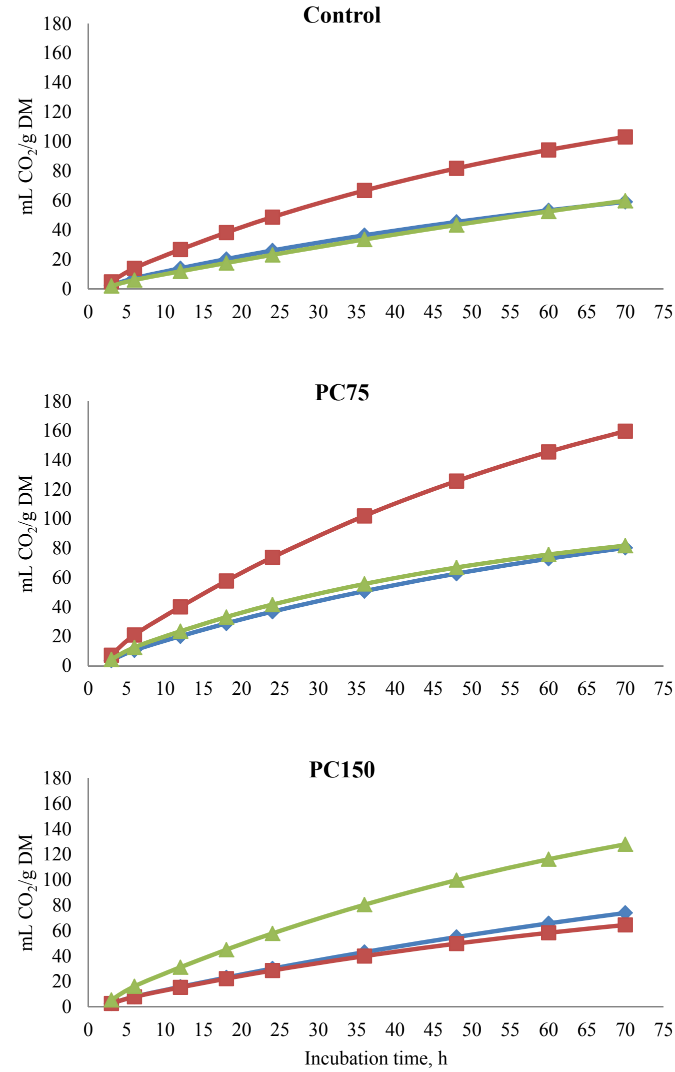

Fig. 3. *In vitro* carbon dioxide (CO_2) production (mL/g incubated DM) of three different levels of prickly pear cactus flour as affected by different levels of organic acid salts at 0 (-◆-), 5 (-■-), and 10 (-▲-) mg/g DM of the ration. Control: corn grain was replaced with prickly pear cactus flour at 0 g/kg DM; PC75: prickly pear cactus flour was included at 75 g/kg DM of total mixed ration; PC150: prickly pear cactus flour was included at 150 g/kg DM of total mixed ration.

Rodriguez et al., 2015; Vallejo et al., 2016). Since the asymptotic GP can be used to predict feed intake because 88% of variance in intake is due to GP (Blümmel and Ørskov, 1993), PC150 ration has the propensity to induce feed intake. Lack of OAS dose effect on asymptotic GP shows that the rumen modulator did not enhance the availability of carbohydrate. The linear increase in fractional rate of GP with increased level of replacement of CG with PC is indicative of enhanced degradability or fermentability of the rations. Thus, PC promotes microbial growth, colonization and degradation more than CG. Microbial growth and accessibility of the feed to microbial enzymes are reflected by the rate at which different chemical constituents are degraded. Ferraro et al. (2016) attributed higher fractional rate of GP of molasses to its greater fermentability relative to glycerol and propylene glycol. Since fractional rate of GP is positively correlated with feed intake (Khazaal et al., 1995), PC150 ration would likely enhance feed intake and performance of ruminants. This is because performance is largely a function of feed intake, which is a better indicator of nutritive value of feed than apparent digestibility (Okunade et al., 2014). The discrete lag time prior to GP was increased from the control ration to the PC75 ration and declined as the level of PC was further increased to PC150 ration. Lower lag phase of the control ration relative to the PC based rations suggests faster microbial adaptation to the ration, and is in agreement with previous reports (Elghandour et al., 2015a,b; Ferraro et al., 2016). The same holds true for lower lag time of the ration PC150 compared to the PC75 ration. Higher lag time of 10 mg OAS dose is an indication of delayed microbial degradation. It seems that rumen modulator like OAS delays the adaptation of rumen microbes to the substrate, in contrast to reports where other rumen enhancers such as *S. cerevisiae* shortens lag time (Rodriguez et al., 2015) or exogenous fibrolytic enzymes have no effect on lag time (Elghandour et al., 2015a). This variability in results, among others, may be due to factors such as the species and physiological status of rumen fluid donor, characteristics of the substrate used, composition and quantity of rumen modulator applied and application methods (Gallardo et al., 2010). Substrate degradation or fermentation rate has been reported to be directly proportional to GP (Dhanoa et al.,

Table 5
In vitro rumen fermentation profile[a] of three different levels of prickly pear cactus as affected by different levels of organic acid salts (OAS).

Ration[b]	OAS (mg/g DM)	pH	ME	DMD	OMD	SCFA	PF_{24}	MCP	GY_{24}
Control	0	6.27	5.41	824	373	2.04	7.06	450	142
	5	6.53	5.80	755	398	2.35	6.71	476	150
	10	6.14	6.07	742	416	2.58	6.48	495	155
PC75	0	5.62	6.20	723	424	2.68	6.34	504	158
	5	5.96	6.59	744	450	3.00	6.17	531	163
	10	5.60	5.99	774	411	2.52	6.50	490	154
PC150	0	5.37	7.08	750	481	3.42	5.86	566	171
	5	5.31	7.52	706	510	3.77	5.69	596	176
	10	5.44	7.97	754	540	4.14	5.62	627	179
SEM[c]		0.204	0.359	21.0	235.0	0.293	0.191	24.7	4.9
Ration effect									
Linear		<0.001	<0.001	0.045	<0.001	<0.001	<0.001	<0.001	<0.001
Quadratic		0.417	0.150	0.575	0.153	0.144	0.455	0.144	0.290
Dose effect									
Linear		0.301	0.185	0.089	0.185	0.185	0.166	0.185	0.164
Quadratic		0.430	0.346	0.681	0.346	0.346	0.438	0.346	0.431
Ration × Dose		0.704	0.520	0.048	0.520	0.520	0.400	0.520	0.476

[a] DMD is the DM degraded substrate (mg/g DM); ME is the metabolizable energy (MJ/kg DM); OMD is the *in vitro* organic matter digestibility (mg/g DM); SCFA, short chain fatty acids; PF_{24}, partitioning factor at 24 h of incubation; GY_{24}, gas yield at 24 h.
[b] Control: corn grain was replaced with prickly pear cactus flour at 0 g/kg DM; PC75, prickly pear cactus was included at 75 g/kg DM of total mixed ration; PC150, prickly pear cactus were included at 150 g/kg DM of total mixed ration.
[c] SEM standard error of the mean.

2000). The higher the GP, the higher the potential degradability of the substrate. Higher GP of PC based rations compared to CG ration (control ration) indicates a higher content of highly fermentable constituents of PC than CG. The steady increase in GP with the replacement of CG by PC could be due to additional availability of the fermentable carbohydrates which possibly promoted microbial growth (Forsberg et al., 2000; Vallejo et al., 2016) and also enhanced the incubation environment. It can therefore be inferred that PC will supply more fermentable carbohydrates, promote degradability, digestibility and microbial protein synthesis relative to CG. *In vitro* gas volume has been shown to be a good predictor of digestibility, fermentation of end-products and microbial protein synthesis by rumen microbes (Sommart et al., 2000). Gas production is dependent on nutrient availability for rumen microorganisms (Elghandour et al., 2015a,b). Nutrients availability for rumen micro-organisms will promote the fermentability of different feed constituents and increased availability of energy for enhanced microbial growth with consequential increased ruminal degradation, reduction in digesta retention time and consumption of more feed. Gas volumes have been reported to have a close relationship with feed intake (Blümmel and Becker, 1997) and growth rate in cattle (Blümmel and Ørskov, 1993). Level of OAS did not affect total GP, that is, total fermentable material was not increased. This suggests that the rations were nutritionally adequate to meet the requirements of rumen microbes and promoted ruminal fermentation, and that supplementation with OAS conferred no additional nutritive value or benefits (Elghandour et al., 2016b). Some studies also reported situations where other rumen modulators, in particular fibrolytic enzyme, did not alter GP (Ruiz et al., 2013). Elghandour et al. (2016b) observed that inclusion of OAS (the same preparation used in the present study) did not affect fermentation of rations containing soybean hulls instead of CG at 7.5% and 15%. Lack of ration × OAS dose effect on fermentation kinetics and GP shows that addition of OAS did not alter fermentation efficiency, nutrient availability, enhance incubation environment and microbial growth. Based on these findings in the current study, addition of OAS to rations in which CG is replaced by PC is redundant and will only amount to increased cost of feed preparation. However, further studies involving *in vivo* trials should be conducted for more elaborate results.

Increasing PC level in the ration linearly increased the asymptotic CH_4 production. It was expected that the pronounced increase in GP with PC inclusion should be accompanied by marked changes in both CH_4 mitigation and CO_2 emissions. Elghandour et al. (2016b) observed that replacing CG with soybean hulls did not affect CH_4 production. The different response between soybean hulls and PC may be due to different chemical composition. The highest CH_4 production was observed with the doses 5 and 10 mg OAS/g of PC15 ration with lack of effect on lag time and rate of CH_4 production. However, Sahoo and Jena (2014) reported that OAS has the ability to decrease methanogenesis by sinking H_2 during propionate formation resulting in increased fiber digestion due to stimulated cellulolytic bacteria. The different responses between that reported in the current study and the assumption of Sahoo and Jena (2014) may be due to the level and nature of the OAS (i.e., the concentration of each component of the product). The used OAS preparation in the present experiment contained monopropylene glycol, calcium propionate, calcium malate and other active compounds, with a different and specific effect for each component (Strauss and Hayler, 2001). Increasing fiber and decreasing protein contents in the rations containing PC may be the main reason (Elghandour et al., 2016c). Nature of diets affects CH_4 production during fermentation with indirect effect through production of SCFA (Johnson and Johnson, 1995). Ruminal fermentation of cell wall content produces mainly H_2 and CO_2 along with the acetate and butyrate. Thus, feeds with high fiber content produce a higher acetate resulting in a higher proportion of H_2 produced by cellulolytic microorganisms and a higher CH_4 production (Blümmel et al., 2005). Santoso and Hariadi (2009) reported that the slowly fermentable carbohydrates of feedstuffs are associated with higher *in vitro* CH_4 production. The relatively low protein content in feeds can contribute to increased CH_4 output due to the lower H_2 and CO_2 production of protein than carbohydrates that suggested a relatively limited methanogenic potential of protein compared to carbohydrates (Singh et al., 2012). In their experiment, Elghandour et al. (2016b) observed a lack of effect of OAS on mitigation of CH_4. The response differed between the two experiments; however, both of them used the same OAS preparation revealing that the effect is ration dependent.

Ration type and OAS did not affect CO_2 production at different hours of incubation. The cause of lack of ration and OAS effects on CO_2 production, despite its pronounced effects on GP and fermentation kinetics, is unknown and needs further investigation.

Elghandour et al. (2016b) observed that rations containing soybean hulls instead of CG, and OAS at different levels of inclusion had no effect on CO_2 production.

The declining pH with increasing level of PC is obviously due to rapid fermentation of the readily available carbohydrate contents of PC to SCFA. A negative correlation has been established between SCFA and ruminal pH (Elghandour et al., 2013). Fermentation of dietary carbohydrates to SCFA produces gases in the rumen, which mainly constitutes H_2, CO_2 and CH_4 (Rodriguez et al., 2015). Increasing SCFA with increased PC level is consistent with the increased OMD and ME, in agreement with earlier reports (Elghandour et al., 2013). Increased SCFA is important in terms of enhanced lactose production, milk volume and overall energy balance (Kholif et al., 2015, 2016). With the exception of DMD and PF_{24}, the linear increases of fermentation profiles with increasing level of PC may be due to increased fiber digestion and enhanced ruminal fermentation (Nsereko et al., 2002) and improved attachment and colonization of PC rations by ruminal micro-organisms (Nsereko et al., 2002; Elghandour et al., 2013). Replacement of CG with PC decreased PF_{24} values. A decreased PF_{24} with increasing PC would reflect lower conversion of degraded substrate into microbial biomass and *vice versa*. The decline in PF_{24} can be explained by decreasing DMD with increasing GP as the PC level increased. This is confirmed by the negative relationships between GP, and DMD and PF_{24} respectively in the current study. In confirmation of highly positive correlation between ME and GP at 24 h (Menke et al., 1979), both ME and GY_{24} increased linearly with increasing PC level in the ration indicating an improved incubation environment and thus fermentability. The rising MCP with increasing PC level is obviously due to improved fermentability of the PC rations, nutrient and energy availability, and enhanced ruminal environment for microbial proliferation. The linear decline in DMD with increasing GP as the level of PC increased was unexpected because increased DMD or substrate fermentability ought to be accompanied by increased GP. However, these observations may be explained by the submissions of Posada and Noguera (2005), who observed no causal relationship between GP and *in vitro* apparent DMD, and that higher GP does not necessarily imply greater efficiency of substrate utilization by ruminal micro-organisms. Based on this, PF_{24}, which is the relationship between DMD and volume of gas produced, indicates variations in the microbial biomass production. The PF_{24} decreased as the incubation hour progressed, so that GP and microbial biomass per unit of degraded substrate varied resulting in an inverse relationship between the two, in tandem with previous reports (Blümmel et al., 1999). The OAS addition to the ration was not effective in improving all the ruminal fermentation parameters probably due to its inefficiency in improving fermentation efficiency, fermentation kinetics and GP.

5. Conclusion

This study suggests that PC has a potential fermentation efficiency and fermentation profile superior to that of CG, and therefore could be included in concentrate ration to replace conventional energy sources (e.g., CG, barley and sorghum) in ruminant diets. Dietary inclusion of 150 g PC/kg DM (replacement of CG at 60%) may not require supplementation with rumen modifier such as OAS. Increasing gas production was paralleled by increasing CH_4 production which cannot be an environmental friendly way of feeding livestock. Further research in which CG is replaced with PC flour with or without OAS supplementation should be conducted in *in vivo* trials to establish current findings with more efforts to study this effect on CH_4 and CO_2 productions.

Conflict of interest

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

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