

# Potential impact of prickly pear cactus flour and *Salix babylonica* extract on cecal fermentation and methane production in horses

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**Abstract** The cecal gas (GP) and methane (CH<sub>4</sub>) production and cecal fermentation kinetics when corn grain (CG) was replaced with prickly cactus (PC) in a horse's diet at different levels of *Salix babylonica* (SB) extract was investigated. Three total mixed rations where CG was replaced with PC at three levels (/kg): 0 g (Control), 75 g (PC75) or 150 g (PC150) were prepared and SB extract added at four levels: 0, 0.6, 1.2 and 1.8 mL/g dry matter (DM) of substrates. No ration type × SB extract dose interaction was observed ( $P > 0.05$ ) for GP kinetics and CH<sub>4</sub> production. Increasing the level of PC in the ration quadratically increased ( $P < 0.01$ ) the asymptotic GP and decreased ( $P < 0.01$ ) the rate and lag time of GP. Increasing the level of PC in the ration, increased GP values ( $P < 0.05$ ). Increasing the level of SB extract linearly decreased ( $P = 0.001$ ) the lag time of GP of

all diets without affecting the asymptotic GP or the rate of GP. Ration type and SB level had no effect ( $P > 0.05$ ) on CH<sub>4</sub> production; however, at 36 h of incubation, SB extract decreased CH<sub>4</sub> production. The rations PC75 and PC150 increased cecal pH compared with the control ration. The PC150 ration had the highest ( $P < 0.05$ ) DM degradability, short chain fatty acids production, and gas yield after 24 h of incubation, with no effect ( $P > 0.05$ ) of SB inclusion on all investigated fermentation kinetic parameters. It is concluded that increasing the level of PC in the diet of horse and replacing CG up to 60%, increased GP and improved cecal fermentation kinetics without affecting CH<sub>4</sub> production. Inclusion of *S. babylonica* extract in the tested rations had weak effects on fermentation kinetics although it decreased the lag time of GP.

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## Introduction

Agricultural byproducts produced worldwide during different agricultural practices are nutrients-rich feed ingredients with a huge potential to be used in ruminant nutrition (Ahmed et al. 2015; Elghandour et al. 2016a). However, in many developing countries,

agriculture byproducts are not adequately utilized cause environmental problems resulting in their burning in the field. Such materials can be used as a cleaner product of animal feed and environmental conservation (Elghandour et al. 2016b, c). The livestock sector is suffering from feed shortages and rising prices of conventional feed such as grains, legumes, etc. for animal production. Moreover, the soaring prices of cereals (e.g., barley, wheat and corn), which are the major energy sources in ruminant diets force nutritionists to search and explore inexpensive alternatives that can partially substitute for the expensive grains. Feeding of unconventional feedstuffs and in some cases agricultural byproducts, which are of no food value to humans, can be one of the solutions to overcome the problem of feed shortages and rising prices.

Cacti (*Opuntia* spp.) has been recognized as one of the most widely used low cost alternative feeds in many parts of the world, especially in semi-arid regions, due to their adaptation to different environmental conditions (Stintzing and Carle 2005; Elghandour et al. 2016c). Cacti has 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 and nutritive value of spineless prickly pear cactus (PC) species differ from region to region depending on many factors including the environment and genotype. The PC is a rich source of non-fibrous carbohydrates (617 g/kg DM), and is an excellent energy source with high dry matter (DM) digestibility (Wanderley et al. 2002). Replacement of energy feedstuff such as corn grain (CG) with PC may require some form of supplementation with other feed additives to improve its fermentation potential and utilization.

Rumen and cecal modifiers such as exogenous fibrolytic enzymes (Kholif et al. 2016a; Morsy et al. 2016), *Saccharomyces cerevisiae* (Rodriguez et al. 2015; Salem et al. 2016a) have been used as ration ingredients for ruminants and horses. Little is known about the nutritive value of *Salix babylonica* (SB) extract in equine nutrition; however, some information is available on ruminant nutrition (Rivero et al. 2016). Extracts of SB have been evaluated as feed additives in ruminant nutrition due to its anti-microbial effects and its ability to modulate ruminal fermentation and improve nutrient utilization (Valdes et al. 2015). The

antimicrobial activity of SB extracts has been attributed to its content of a number of plant secondary metabolites such as alkaloids, saponins and phenolics (Cedillo et al. 2014) which rumen microorganisms have the ability to degrade and utilize as an energy source at low and moderate concentrations without negative effects on rumen fermentation (Salem et al. 2014a, 2016b). In ruminants, SB extract enhanced feed intake (Salem et al. 2014b), daily gain (Cedillo et al. 2014), and milk production (Salem et al. 2014b). It has also been reported to have natural anthelmintic activity (Cedillo et al. 2015; Salem et al. 2016c). To the best of our knowledge, this is the first study to include the extract of SB in the diet of horses. The aim of the current study was to investigate the effects of replacing CG in a horse's diet with PC at different levels in the presence of different levels of *S. babylonica* extract on cecal in vitro gas (GP) and methane (CH<sub>4</sub>) production and cecal fermentation kinetics.

## Materials and methods

### Extract, substrates and treatments

Plant leaves of *S. babylonica* were collected randomly from several young and mature trees during summer of 2015. Leaves were freshly chopped into 1–2 cm lengths and immediately extracted at 1 g leaf/8 mL of water. Plant materials were individually soaked and incubated in water in the laboratory at 25–30 °C for 72 h in jar. After incubation, the jar was heated to 39 °C for 1 h and then immediately filtered and the filtrate collected and stored at 4 °C for further use.

Three total mixed rations were prepared where CG was replaced with PC at three levels (/kg): 0 g (Control), 75 g (PC75) or 150 g (PC150). The extract of SB was added at four levels: 0, 0.6, 1.2 and 1.8 mL/g DM of substrates. The chemical composition and ingredients is shown in Table 1.

### In vitro cecal fermentation and biodegradation

Cecal contents (the inoculum source) were collected from 4 Criollo horses (3–4 years of age and weighing 300 ± 15 kg) from the local slaughterhouse of Toluca, Mexico State, Mexico. Horses had about eight hours grazing and were given water twice a day

**Table 1** Composition of the experimental diets<sup>a</sup>. Adapted from Elghandour et al. 2016b, c

	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 <sup>b</sup>	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

<sup>a</sup> 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

<sup>b</sup> Contained: Vitamin A (12,000,000 IU), Vitamin D<sub>3</sub> (2,500,000 IU), Vitamin E (15,000 IU), Vitamin K (2.0 g), Vitamin B<sub>1</sub> (2.25 g), Vitamin B<sub>2</sub> (7.5 g), Vitamin B<sub>6</sub> (3.5 g), Vitamin B<sub>12</sub> (20 mg), Pantothenic 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)

without feed supplementation. The horses had grazed predominantly on pasture containing two native grasses (*Festuca arundinacea* and ryegrass). Individual cecal samples were equally collected from the cecum of each horse and then mixed and homogenized to obtain a homogenized sample of fecal contents which were mixed with the Goering and Van Soest (1970) buffer solution without trypticase in the ratio of 1:4 v/v. The incubation media was subsequently mixed and strained through four layers of cheesecloth into a flask with an O<sub>2</sub>-free headspace, and used to inoculate three identical runs of incubation in 120-mL serum bottles containing 0.5 g DM of substrate in presence of different doses of SB extract.

Bottles with substrates plus three bottles without substrate and SB as blanks were used. After filling all bottles, they were flushed with CO<sub>2</sub> and immediately closed with rubber stoppers, shaken and placed in an incubator set at 39 °C. Gas production was recorded at

2, 4, 6, 8, 10, 12, 14, 24, 36, 48, 54, 60, and 72 h using the Pressure Transducer Technique (Extech instruments, Waltham, USA) of Theodorou et al. (1994). The production of CH<sub>4</sub> was recorded using Gas-Pro detector (Gas Analyzer CROWCON Model Tetra 3, Abingdon, UK) at 2, 6, 10, 14, 24, 36, 48, 54, 60, and 72 h of incubation.

At the end of incubation after 72 h, bottles were uncapped and the pH was measured using a digital pH meter (Conductronic pH15, Puebla, Mexico), and the residual of each bottle was filtered under vacuum through glass crucibles with a sintered filter and the fermentation residues dried at 65 °C for 72 h to estimate DM disappearance (DMD).

#### Chemical analyses and calculations

Samples of the rations were analyzed for DM (#934.01), ash (#942.05), N (#954.01) and ether extract (#920.39) according to AOAC (1997) and the ration's contents for neutral detergent fiber content (NDF, Van Soest et al. 1991), acid detergent fiber (ADF) and lignin (AOAC 1997; #973.18) analyses were carried out using an ANKOM<sup>200</sup> Fiber Analyzer Unit (ANKOM Technology Corp., Macedon, NY, USA) with the use of an alpha amylase and sodium sulfite.

For estimation of GP kinetic, recorded gas volumes (mL/g DM) were fitted using the NLIN procedure of SAS (2002) according to France et al. (2000) model as:

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

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  $Lag$  (h) is the discrete lag time prior to any gas release.

Metabolizable energy (ME, MJ/kg DM) was estimated according to Menke et al. (1979) as:

$$ME = 2.20 + 0.136 \text{ GP (mL/0.5 g DM)} + 0.057 \text{ CP (g/kg DM)}$$

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<sub>24</sub>; 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 (GP<sub>24</sub>)] according to Blümmel et al. (1997). Gas yield (GY<sub>24</sub>) 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:

$GY_{24} = \text{mL gas/g DM/g DMD}$

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

$SCFA(\text{mmol}/200 \text{ mg DM}) = 0.0222 GP - 0.00425$

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

### Statistical analyses

Data from 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 cecal 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  $i$ th ration type ( $R_i$ ) with  $j$ th SB extract dose ( $D_j$ );  $\mu$  is the general mean;  $(R \times D)_{ij}$  is the interaction between ration type and SB extract dose;  $E_{ijk}$  is the experimental error. Linear and quadratic polynomial contrasts were used to examine responses of different PC rations (levels) to increasing addition levels of SB extract. Statistical significance was declared at  $P < 0.05$ .

## Results

### Gas production kinetics

No ration type  $\times$  SB extract dose interaction was observed ( $P > 0.05$ ) for all investigated parameters of GP kinetics (Table 2). Ration type affected ( $P \leq 0.001$ ) the asymptotic GP, the rate of GP and the lag time of GP. Ignoring the effect of SB addition (i.e. 0 mL SB/g DM), increasing the level of PC in the ration increased the asymptotic GP (quadratic effect,  $P = 0.001$ ), decreased both of the rate of GP (linear and quadratic effect,  $P < 0.001$ ) and the lag time of GP (quadratic effect,  $P < 0.001$ ). Besides, GP at different incubation hours were affected ( $P < 0.05$ ) by different rations i.e. increasing the level of PC in the ration increased GP values.

The level of SB extract did not affect the asymptotic GP or the rate of GP. Increasing the level of SB extract linearly decreased ( $P = 0.001$ ) the lag time of GP of all diets. However, increasing level of SB extract tended to decreased (quadratic effect,  $P = 0.07$ ) the

rate of gas production. The extract of SB linearly affected ( $P < 0.05$ ) GP at some incubation hours.

### Methane production

No interaction was observed ( $P > 0.05$ ) between ration type and SB extract level for  $CH_4$  production. No  $CH_4$  was released before 24 h of incubation (Table 3). With the exception of  $CH_4$  at 36 h of incubation, ration type had no effect ( $P > 0.05$ ) on  $CH_4$  production. The ration with PC75 had the highest ( $P = 0.022$ )  $CH_4$  production at 36 h of incubation compared with the other rations.

No effect was observed ( $P > 0.05$ ) on  $CH_4$  production with the inclusion of SB extract in the rations at all incubation hours, with the exception of  $CH_4$  at 36 h of incubation; where SB extract addition decreased  $CH_4$  (linear effect,  $P = 0.006$ ; quadratic effect,  $P = 0.001$ ) compared with rations without SB inclusion.

### Cecal fermentation kinetics

Interactions were observed between ration type and SB level for cecal pH ( $P < 0.001$ ) and DMD ( $P = 0.01$ ) (Table 4). At the level of 0 mL SB/g DM, the PC75 and PC150 rations increased cecal pH compared with the control ration. The PC150 ration had the highest ( $P < 0.05$ ) DMD, SCFA, and  $GY_{24}$ . The inclusion of SB extract had no effect ( $P > 0.05$ ) on all investigated fermentation kinetic parameters.

## Discussion

### Gas production

No ration type  $\times$  SB extract dose interaction was observed ( $P > 0.05$ ) for GP kinetics. Therefore, the effect of ration type and SB extract dose will be individually discussed. Gas production is generally a good indicator of digestibility, fermentability and rumen microbial protein production (Rodriguez et al. 2015; Vallejo et al. 2016). Gas production is dependent on nutrient availability for rumen micro-organisms (Elghandour et al. 2015a, b). The quadratic increases in asymptotic GP and lower rate and lag time of GP with increasing level of PC replacement for CG reveals increased fermentation of the insoluble but

**Table 2** In vitro ceal gas kinetics of three levels of prickly pear cactus (PC) at different levels (mg/g DM) of *Salix babylonica* (SB) extract inclusion

Ration <sup>a</sup>	SB extract	Gas production parameters <sup>b</sup>		Gas production (mL/g DM) at:													
		<i>b</i>	<i>c</i>	<i>Lag</i>	2 h	4 h	6 h	8 h	10 h	12 h	14 h	24 h	36 h	48 h	54 h	60 h	72 h
Control	0	381	0.083	8.85	58	107	149	185	214	240	261	329	361	374	377	379	380
	0.6	369	0.125	7.45	80	143	192	231	261	284	303	350	365	368	369	369	369
	1.2	473	0.104	7.32	88	160	218	266	304	335	361	432	461	469	472	471	472
	1.8	463	0.093	7.02	79	144	198	243	280	311	337	412	446	457	459	461	462
PC75	0	414	0.059	8.35	46	86	122	154	183	208	230	310	361	387	394	400	407
	0.6	421	0.072	7.71	54	102	142	177	208	235	258	336	381	402	408	412	417
	1.2	437	0.067	7.34	54	100	141	177	208	235	259	341	391	414	421	425	431
	1.8	472	0.059	3.21	52	98	139	176	208	237	263	355	414	442	451	457	465
PC150	0	548	0.052	3.92	53	102	145	184	219	250	279	384	457	497	509	519	531
	0.6	458	0.059	4.48	51	96	136	172	203	232	257	345	402	430	438	444	451
	1.2	502	0.055	3.34	51	98	139	176	209	239	266	363	428	463	473	481	490
	1.8	538	0.051	2.98	52	99	141	179	213	244	272	376	449	488	501	510	523
Pooled SEM		32.6	0.0067	0.837	5.2	9.3	12.5	15.0	17.0	18.6	19.9	23.9	26.8	28.8	29.6	30.2	31.1
<b>Ration effect</b>																	
	Linear	0.536	<0.001	0.103	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.014	0.265	0.787	0.991	0.863	0.692
	Quadratic	0.001	<0.001	<0.001	0.001	0.003	0.007	0.017	0.044	0.101	0.210	0.537	0.036	0.005	0.002	0.002	0.001
<b>Dose effect</b>																	
	Linear	0.118	0.643	0.001	0.061	0.057	0.054	0.052	0.049	0.049	0.048	0.050	0.062	0.076	0.083	0.089	0.098
	Quadratic	0.968	0.070	0.644	0.045	0.054	0.065	0.080	0.097	0.117	0.140	0.305	0.538	0.714	0.774	0.820	0.881
	Ration × dose	0.4479	0.448	0.284	0.129	0.132	0.147	0.160	0.171	0.180	0.189	0.196	0.238	0.303	0.363	0.386	0.404

SEM standard error of the mean

<sup>a</sup> 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

<sup>b</sup> *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)

**Table 3** Proportional in vitro methane (CH<sub>4</sub>) productions of three levels of prickly pear cactus (PC) at different levels (mg/g DM) of *Salix babylonica* (SB) extract inclusion

Ration <sup>a</sup>	SB extract	Methane production <sup>b</sup> (ml/g DM) at:						
		24 h	36 h	48 h	54 h	60 h	72 h	
Control	0	ND	3.29	3.40	4.65	8.28	8.31	
	0.6	0.84	0.88	0.89	3.08	3.08	4.41	
	1.2	ND	ND	2.03	6.18	6.19	8.04	
	1.8	ND	ND	2.99	4.41	4.99	7.22	
PC75	0	0.82	4.21	4.86	5.65	5.73	6.48	
	0.6	ND	0.53	0.57	1.16	1.17	1.18	
	1.2	ND	0.52	1.15	2.51	2.53	2.56	
	1.8	0.82	4.16	5.83	5.95	7.50	8.17	
PC150	0	1.04	2.76	5.61	5.76	8.54	8.72	
	0.6	1.13	1.29	1.91	4.15	4.19	4.25	
	1.2	0.59	1.16	5.77	5.90	8.82	8.98	
	1.8	ND	0.58	0.64	1.31	1.34	1.38	
Pooled SEM		0.430	0.754	1.346	1.454	2.002	2.162	
Ration effect								
	Linear		0.518	0.022	0.422	0.467	0.332	0.130
	Quadratic		0.161	0.590	0.362	0.928	0.526	0.980
Dose effect								
	Linear		0.332	0.006	0.193	0.230	0.088	0.215
	Quadratic		0.422	0.001	0.350	0.815	0.882	0.902
	Ration × dose		0.892	0.925	0.050	0.057	0.129	0.144

SEM standard error of the mean, ND not detected (i.e. 0 mL CH<sub>4</sub>/g DM)

<sup>a</sup> 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

<sup>b</sup> No CH<sub>4</sub> was produced at 2, 6, 10, 14 h of incubation

degradable fraction (Elghandour et al. 2016b). These results suggest a steady increasing availability of carbohydrate fractions to the microbial population growth and activity, in agreement with previous studies (Elghandour et al. 2015a, b). Blümmel and Ørskov (1993) showed that the asymptotic GP can be used to predict feed intake because 88% of variance in intake was due to GP implying the PC75 and PC150 rations had the propensity to induce feed intake and growth rate in cattle (Blümmel and Ørskov 1993). The decreased rate with increasing the level of PC in the ration indicate that the time necessary for degradation was longer than for PC rations, thus less gas was produced in the short term. Microbial growth and accessibility of microbial enzymes to feed particles are reflected by the rate at which different chemical constituents are degraded. Since fractional rate of GP was 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 decreased with the PC75 and PC150 ration suggesting faster microbial adaptation to the ration, in agreement with previous reports (Elghandour et al. 2015a, b, 2016c).

The inclusion of SB extract did not affect the asymptotic GP or the rate of GP; however, it decreased the lag time of GP. This effect indicates positive effects on ruminal fermentation (Salem et al. 2014a; Elghandour et al. 2015b), and ruminal microorganisms activity possibly due to the ability of rumen microorganisms to degrade secondary metabolites in SB extract and utilize them as an energy source (Hart et al. 2008).

**Table 4** Degradation and in vitro cecal fermentation profile of three levels of prickly pear cactus (PC) at different levels (mg/g DM) of *Salix babylonica* (SB) extract inclusion

Ration <sup>a</sup>	SB	pH	ME	DMD	SCFA	PF <sub>24</sub>	GY <sub>24</sub>
Control	0	5.43	13.1	861	8.3	4.81	208
	0.6	5.10	12.9	930	8.2	4.86	206
	1.2	6.55	15.6	885	10.4	4.66	215
	1.8	6.57	15.3	879	10.1	4.67	214
PC75	0	7.00	13.4	849	8.6	4.79	209
	0.6	6.96	13.8	848	8.9	4.76	210
	1.2	7.01	14.1	861	9.2	4.75	211
	1.8	6.86	14.9	857	9.8	4.70	213
PC150	0	7.07	16.4	877	11.0	4.63	216
	0.6	6.09	14.6	862	9.5	4.72	212
	1.2	6.32	15.4	861	10.2	4.67	214
	1.8	6.19	16.1	859	10.8	4.64	216
Pooled SEM		0.187	0.78	10.5	0.64	0.054	2.3
Ration							
Linear		<0.001	0.776	<0.001	0.784	0.949	0.984
Quadratic		0.894	0.005	0.316	0.005	0.014	0.010
Dose							
Linear		0.796	0.076	0.764	0.076	0.128	0.116
Quadratic		0.415	0.714	0.480	0.714	0.742	0.746
Ration × dose		<0.001	0.363	0.010	0.364	0.486	0.456

DMD, the in vitro dry matter digestibility (mg/g DM); GY<sub>24</sub>, gas yield at 24 h (mL gas/g DMD); ME is the metabolizable energy (MJ/kg DM); PF<sub>24</sub>, partitioning factor at 24 h of incubation (mg DMD/mL gas); pH, fermentation pH; SCFA, short-chain fatty acids (mmol/g DM)

SEM standard error of the mean

<sup>a</sup> 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

## Methane production

Without the occurrence of ration type × SB extract dose interaction, ration type and SB level did not affect CH<sub>4</sub> production. Methane emission from ruminants depends on diet degradability and chemical composition (Elghandour et al. 2016c, d). Ruminant livestock is one of the sources responsible for greenhouse gas (e.g. CH<sub>4</sub> emission from animal production sector is responsible for about 18% of all greenhouse gas emissions) emission (Intergovernmental Panel on Climate Change 2008). Methane is produced as a result of ruminal fermentation of feed in the rumen causing a loss of digested energy (Johnson and Johnson 1995). With the same rations used in the present experiment, Elghandour et al. (2016c) observed that increasing PC level in the ration linearly

increased the asymptotic CH<sub>4</sub> production; however, in another experiment, the same research team (Elghandour et al. 2016b) observed that replacing CG with soybean hulls did not affect CH<sub>4</sub> production. The different response between soybean hulls and PC may be due to different chemical composition. Besides, the different inoculum source (rumen contents vs cecal contents) can be another reason.

## Fermentation kinetics

The rations PC75 and PC150 increased cecal pH. However, Elghandour et al. (2016c) observed a declining pH with increasing level of PC. The difference maybe related with the inoculum source. In their experiment, Elghandour et al. (2016c) used rumen liquor compared with inoculum from cecal

contents in the present experiment. Higher pH is required for the activity of microorganisms presented in the cecum of horses, for their activity and ability to degrade fiber of the diet. The PC150 ration increased DMD, SCFA, and  $GY_{24}$ . Feed degradation and fermentation rate has been reported to be directly proportional to GP (Dhanoa et al. 2000). The higher GP is a good indicator of the higher potential degradability of the substrate (i.e. PC150 ration). Higher GP with PC based rations compared to CG ration (control ration) indicates a higher content of highly fermentable constituents of PC than CG. The increased DMD with increasing GP as the level of PC increased confirms the hypothesis that increasing DMD or substrate fermentability ought to be accompanied by increased GP. The improvements of fermentation parameters observed 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) and also enhanced the incubation environment. It has been shown that availability of nutrients for rumen microorganisms will stimulate the degradability of different nutrients (Paya et al. 2007). Besides, increased SCFA production and ME are associated with high activities of microbes in the rumen. It can therefore be inferred that PC will supply more fermentable carbohydrates, promote degradability, digestibility and microbial protein synthesis relative to CG. Increasing SCFA with increased PC level was 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, 2016b). The 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). In confirmation of highly positive correlation between ME and GP at 24 h (Menke et al. 1979), both ME and  $GY_{24}$  increased with increasing PC level in the ration indicating an improved incubation environment and thus fermentability. The SB extract 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.

Results reported in the present experiment suggest that prickly pear cactus flour has a potential fermentation efficiency and fermentation profile superior to that of corn grain. It can therefore be used to replace corn grain in concentrate ration to replace conventional energy sources (e.g., maize, barley and sorghum) in ruminant diets. The inclusion of *S. babylonica* extract in the tested rations had a weak effect on their fermentation. The best level of dietary inclusion of prickly pear cactus was 150 g PC/kg DM (replacement of CG at 60%). Further research in which CG is replaced with PC flour with or without SB extract inclusion should be conducted in in vivo trials to validate current findings.

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#### Compliance with ethical standards

**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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