Journal of Equine Veterinary Science 53 (2017) 45–54

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

In Vitro Cecal Gas and Methane Production of Soybean Hulls–Containing Diets in the Presence of *Salix babylonica* Extract as a Fermentation Modulator in Horses

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ARTICLE INFO

Article history:
Received 7 October 2016
Received in revised form 4 December 2016
Accepted 8 December 2016
Available online 3 January 2017

Keywords:
Cecal fermentation
Corn grain
Horse nutrition
Salix babylonica extract
Soybean hulls

ABSTRACT

The aim of the present study was to evaluate the cecal gas production (GP) and methane (CH_4) production as well as cecal fermentation kinetics when corn grain (CG) was replaced with soybean hulls (SHs) in horse diets in the presence of different levels of *Salix babylonica* (SB) extract. Corn grains were replaced with SH at different levels (/kg): 0 g (control), 75 g (SH75), or 150 g (SH150), with the inclusion of SB extract at: 0, 0.6, 1.2, and 1.8 mL/g dry matter (DM) of substrates. Ration type × extract dose interactions were observed for GP and CH_4 production at some incubation hours. Diets containing SH, without the inclusion of SB extract, increased the asymptotic GP ($P = .031$) and decreased ($P < .01$) the rate of GP and lag time of GP. The inclusion of SB increased ($P = .009$) the rate of GP, without affecting the asymptotic GP or lag time of GP. Besides, the SH-containing rations decreased ($P < .05$) CH_4 production, with no effect for SB extract dose. The SH75 ration increased ($P < .05$) cecal fermentation pH, metabolizable energy, short chain fatty acids, and gas yield at 24 hours of incubation, but quadratically decreased partitioning factor at 24 hours of incubation ($P = .023$), whereas SB extract dose had no effect. It is concluded that SH-containing rations had higher potential fermentation efficiency and fermentation kinetics superior to that of CG. The level of 75 g SH/kg DM was the best level of inclusion to replace 30% CG in the diets of horses. The inclusion of SB extract did not affect the cecal fermentation kinetics of horse diets containing SH at different levels.

1. Introduction

During agricultural production worldwide, many by-products, which are considered as nutrient-rich feed ingredients that can be used in ruminant nutrition [1–3] as a cleaner product of animal feed and environmental conservation [4–6], are produced. Recently, the increasing prices of the major energy sources in ruminant diets (i.e., cereals) are compelling animal nutritionists to look for inexpensive alternatives to replace the expensive grains. However, some factors, such as good nutritive value and absence of food value to humans, should be considered before utilizing unconventional feedstuffs or agricultural by-products as feed ingredients for livestock.

Soybean hulls (SH) have been successfully used in nutrition of ruminants [7,8] and in horses [9] as an economic substitute for conventional feedstuffs. According to Costa et al [7], SH contained (/kg dry matter [DM]): 116 g crude protein (CP), 722 g neutral detergent fiber (NDF), and 411 g acid detergent fiber (ADF). Higher fiber content makes SH a low energy density and fibrous feed. Therefore,

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http://dx.doi.org/10.1016/j.jevs.2016.12.004

the inclusion of SH in the diet livestock may require supplementation with a rumen fermentative modulator to improve its nutritive value as an energy additive in ruminant diets [4].

Exogenous fibrolytic enzymes [10–12], like *Saccharomyces cerevisiae* [13,14] and *Salix babylonica* (SB) extract [15,16], have been used as feed ingredients for ruminants and horses. Little is known about the nutritive value of SB extract in equine nutrition [17]; however, some information is available on ruminant nutrition [18]. Extract of SB has some antimicrobial effects, can modulate ruminal fermentation, and affect nutrient utilization positively [19], due to its content of a number of secondary plant metabolites such as alkaloids, saponins, and phenolics [20]. Ruminal microorganisms have the ability to utilize plant extracts at low and moderate concentrations without negative effects on rumen fermentation [20,21]. Positive effects, including enhanced feed intake [21], daily gain [22], and milk production [21], were reported with the inclusion of SB in the diets of ruminant animals. Besides, the extract of SB has also been reported to have natural anthelmintic activity [22,23]. To the best of our knowledge, only Parra-García et al [17] reported the effect of SB extract inclusion in diets containing prickly pear cactus as a replacement for corn grain (CG) on cecal fermentation and methane (CH_4) production in horses. Therefore, the aim of the current study was to study the effect of replacing CG in horse diets with SH at different levels in the presence of different levels of SB extract, as a cecal fermentation modulator, on cecal in vitro gas and CH_4 productions and fermentation kinetics.

2. Materials and Methods

2.1. Extract, Substrates, and Treatments

Salix babylonica leaves were collected randomly from several young and mature trees. 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°C to 30°C for 72 hours in jars. After incubation, jars were heated to 39°C for 1 hour and then filtered immediately and the filtrate collected and stored at 4°C for further use.

Three total mixed rations were prepared where CG was replaced with SH at three levels (/kg): 0 g (control), 75 g (SH75), or 150 g (SH150). The extract of SB was added at four levels: 0, 0.6, 1.2, and 1.8 mL/g DM of substrates. The ingredient and chemical composition of the diets is shown in Table 1.

Table 1
Composition of the experimental diets.

	Ration[a]		
	Control	S7H5	SH150
Ingredients (g/kg DM)			
Oats straw	249	248	248
Steam rolled corn	250	175	100
Soybean hulls	0	75	150
Steam rolled barley	250	250	250
Wheat bran	120	110	120
Corn gluten feed	30	30	30
Prickly pear cactus	30	30	20
Molasses	70	80	80
Vitamins/minerals[b]	1	2	2
Chemical composition (g/kg DM)			
Organic matter	964	968	958
Crude protein	130	117	130
Neutral detergent fiber	356	385	395
Acid detergent fiber	121	115	193
Ether extract	24	24	18
Nonstructural carbohydrates	454	442	415

Abbreviation: DM, dry matter.
[a] SH75, soybean hulls were included at 75 g/kg DM of total mixed ration; SH150, soybean hulls were included at 150 g/kg DM of total mixed ration.
[b] Contained: 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), 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), and Co (0.20 g).(Adapted from Elghandour et al [8])

2.2. In Vitro Cecal Fermentation and Biodegradation

Cecal contents (the inoculum source) were collected from four Criollo horses (3–4 years of age and 300 ± 15 kg [body weight]) from the local slaughterhouse at Toluca, Mexico State, Mexico. Horses had about 8 hours grazing and were given water twice a day without feed supplementation. The horses were grazed predominantly on pasture containing two native grasses (*Festuca arundinacea* and ryegrass). Equal amounts of cecal content samples were collected from the cecum of each horse and then mixed to obtain homogenized samples which were also mixed with the Goering and Van Soest [24] buffer solution without trypticase in the ratio of 1:4 vol/vol. The incubation media was subsequently mixed and strained through four layers of cheesecloth into a flask with an O_2-free headspace and used to inoculate three identical runs of incubation in 120-mL serum bottles containing 0.5 g DM of substrate in the 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 carbon dioxide and immediately closed with rubber stoppers, shaken and placed in an incubator set at 39°C. Gas production (GP) was recorded at 2, 4, 6, 8, 10, 12, 14, 24, 36, 48, 54, 60, and 72 hours using the Pressure Transducer Technique (Extech instruments, Waltham) of Theodorou et al [25]. Production of CH_4 was recorded using Gas-Pro detector (Gas Analyzer CROWCON Model Tetra3, Abingdon, UK) at 2, 6, 10, 14, 24, 36, 48, 54, 60, and 72 hours of incubation.

At the end of incubation (i.e., after 72 hours), 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 hours to estimate DM disappearance (DMD).

2.3. Chemical Analyses and Calculations

Samples of the rations were analyzed for DM (#934.01), ash (#942.05), nitrogen (#954.01), and ether extract (#920.39) according to Association of Official Analytical Chemists (AOAC) [26], whereas NDF, ADF, and lignin (#973.18) [26] analyses were carried out using an

ANKOM^{200} Fiber Analyzer Unit (ANKOM Technology Corp, Macedon, NY) 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 [27] according to France et al [28] model:

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

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 (1/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 [29] as:

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

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

The partitioning factor at 24 hours 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 hours (i.e., DMD/total GP [GP_{24}]) according to Blümmel et al [30]. Gas yield (GY_{24}) was calculated as the volume of gas (mL gas/g DM) produced after 24 hours of incubation divided by the amount of DMD (g) as:

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

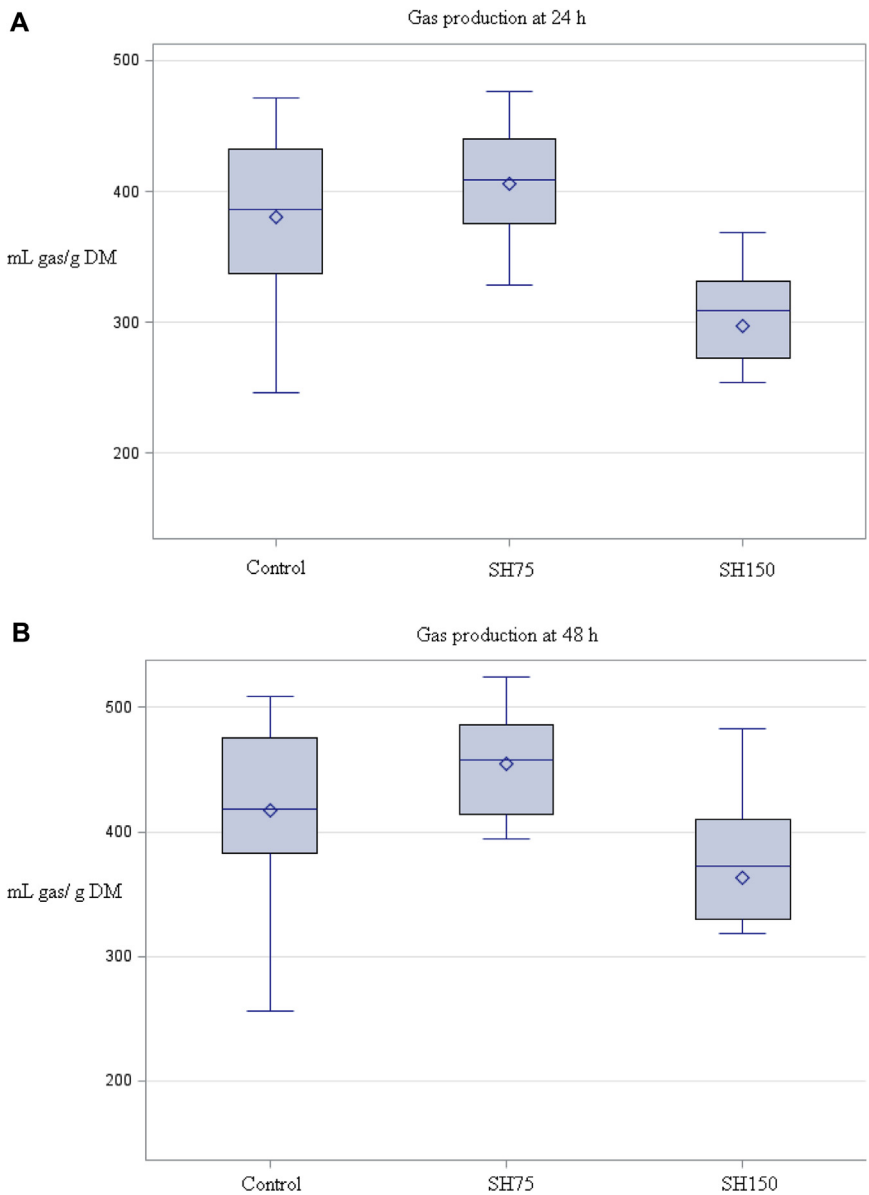

Fig. 1. Mean *in vitro* cecal gas production (mL/g DM) of three levels of soybean hulls (SHs) at 24 (A) and 48 (B) hours of incubation. DM, dry matter.

Short chain fatty acid (SCFA) concentrations were calculated according to Getachew et al [31] as:

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

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

2.4. 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, and 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 [27] as:

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

where Y_{ijk} = is every observation of the ith SH level (R_i) with jth SB extract dose (D_j); μ is the general mean; $(R \times D)_{ij}$ is the interaction between ration type and SB extract dose;

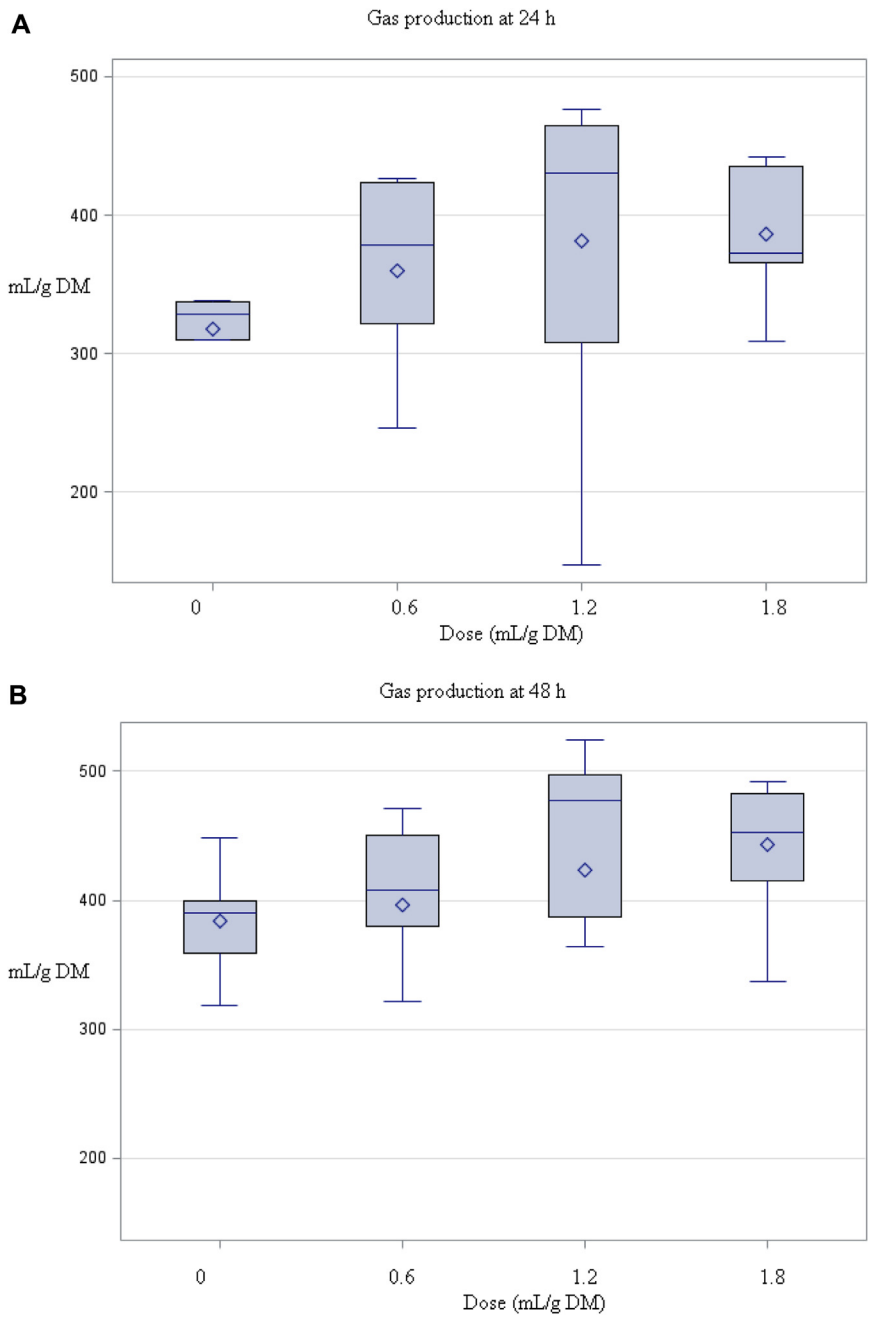

Fig. 2. Mean *in vitro* cecal gas production (mL/g DM) of four levels of *Salix babylonica* doses at 24 (A) and 48 (B) hours of incubation. DM, dry matter.

E_{ijk} is the experimental error. Linear and quadratic polynomial contrasts were used to examine responses of different SH levels (rations) to increasing addition levels of SB extract. Statistical significance was declared at $P < .05$.

3. Results

3.1. Gas Production Kinetics

Figs. 1 and 2 show the mean in vitro cecal GP (mL/g DM) of three levels of SH and four levels of SB doses, respectively at 24 hours and 48 hours of incubation. Ration type × extract dose interactions were observed for GP from 6 to 14 hours of incubation (Table 2). Replacing CG with SH, without the inclusion of SB extract, increased the asymptotic GP (quadratic effect, $P = .031$) and decreased both of the rate of GP (linear effect, $P = .007$) and lag time of GP (linear effect, $P = .003$; quadratic effect, $P < .001$). Gas production, at all incubation hours, differed among the different rations. The inclusion of SB extract did not affect ($P > .05$) the asymptotic GP or lag time of GP from all rations; however, it increased (quadratic effect, $P = .009$) the rate of GP compared with those with no SB extract inclusion. During the incubation hours from 2 to 4 hours of incubation, inclusion of SB extract linearly increased ($P < .05$) GP from all rations compared with the rations without SB extract.

3.2. Methane Production

Figs. 3 and 4 show the mean in vitro cecal CH_4 (mL/g DM) of three levels of SH and four levels of SB doses, respectively at 24 hours and 48 hours of incubation. Interactions between ration type and SB extract dose were observed ($P < .05$) at 24, 36, and 48 hours of incubation (Table 3). No CH_4 was produced during the incubation hours from 2 to 14 hours. With no SB extract inclusion, replacing CG with SH decreased (linear and quadratic effects, $P < .05$) CH_4 production at incubation hours from 48 to 72 hours of incubation. Inclusion of SB extract did not affect CH_4 production during all incubation hours, with exception of CH_4 production at 36 hours of incubation (linear and quadratic effect, $P < .05$).

3.3. Cecal Fermentation Kinetics

Although there were no interactions ($P > .05$) between ration type and SB extract dose for ME, DMD, SCFA, PF_{24}, and GY_{24}, interaction was observed for cecal fermentation pH ($P = .002$) (Table 4). Ration effects on fermentation kinetic parameters were significant with SH75 ration having increased cecal fermentation pH (linear effect, $P = .004$), ME (quadratic effect, $P = .003$), SCFA (quadratic effect, $P = .003$), and GY_{24} (quadratic effect, $P = .016$) and quadratically decreased PF_{24} ($P = .023$). Inclusion of SB extract did not affect ($P > .05$) the fermentation kinetic parameters.

4. Discussion

4.1. Gas Production

The occurrence of ration type × extract dose interaction effect on GP from 2 to 14 hours of incubation hours reveals that the effect of SB extract dose on GP at these hours depended on ration type (i.e., SH level in the diet). On the other hand, the absence of interaction between these two factors on GP for the rest of the incubation hours (i.e., from

Table 2
In vitro cecal gas kinetics of three levels of soybean hulls (SHs) at different levels (mg/g DM) of *Salix babylonica* (SB) extract inclusion (mL/g DM).

Ration[a]	SB Extract	Gas Production Parameters[b]			Gas Production (mL/g DM) at												
		b	*c*	*Lag*	2 hr	4 hr	6 hr	8 hr	10 hr	12 hr	14 hr	24 hr	36 hr	48 hr	54 hr	60 hr	72 hr
Control	0	381	0.083	8.85	58	107	149	185	214	240	261	329	362	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	471	471	472
	1.8	463	0.093	7.02	79	144	198	243	280	311	337	412	446	457	459	461	462
SH75	0	429	0.070	6.57	56	105	147	184	216	244	268	349	394	414	419	423	426
	0.6	454	0.104	5.60	85	154	210	255	292	322	346	415	442	450	452	453	453
	1.2	505	0.102	5.99	93	168	230	280	321	355	382	460	491	500	502	503	504
	1.8	462	0.086	5.34	73	133	184	227	263	293	319	398	437	452	456	458	461
SH150	0	406	0.048	4.01	37	70	101	128	154	176	197	275	331	363	374	381	392
	0.6	381	0.074	5.17	52	97	136	170	199	224	245	316	354	370	374	376	379
	1.2	315	0.097	3.99	45	82	113	139	161	180	197	252	285	300	305	308	312
	1.8	446	0.072	4.72	56	104	146	183	214	242	266	347	396	420	427	432	438
Pooled SEM		39.1	0.0126	0.774	5.0	8.8	11.9	14.4	16.6	18.5	20.2	26.8	32.0	35.1	36.1	36.9	37.8
P value																	
Ration effect																	
Linear		.151	.248	.003	.957	.817	.690	.578	.485	.409	.350	.205	.156	.148	.146	.146	.147
Quadratic		.031	.007	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	.003	.003	.005	.008	.015
Dose effect																	
Linear		.121	.131	.229	.001	.001	.001	.002	.003	.004	.006	.005	.022	.049	.063	.075	.093
Quadratic		.984	.009	.562	.002	.004	.007	.002	.003	.006	.012	.133	.445	.695	.779	.841	.919
Ration × dose		.190	.762	.618	.070	.054	.045	.040	.037	.037	.038	.061	.107	.147	.161	.171	.183

Abbreviations: DM, dry matter; SEM, standard error of the mean.
[a] SH75, soybean hulls were included at 75 g/kg DM of total mixed ration; SH150, soybean hulls were 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 (1/h); *Lag* is the initial delay before gas production begins (h).

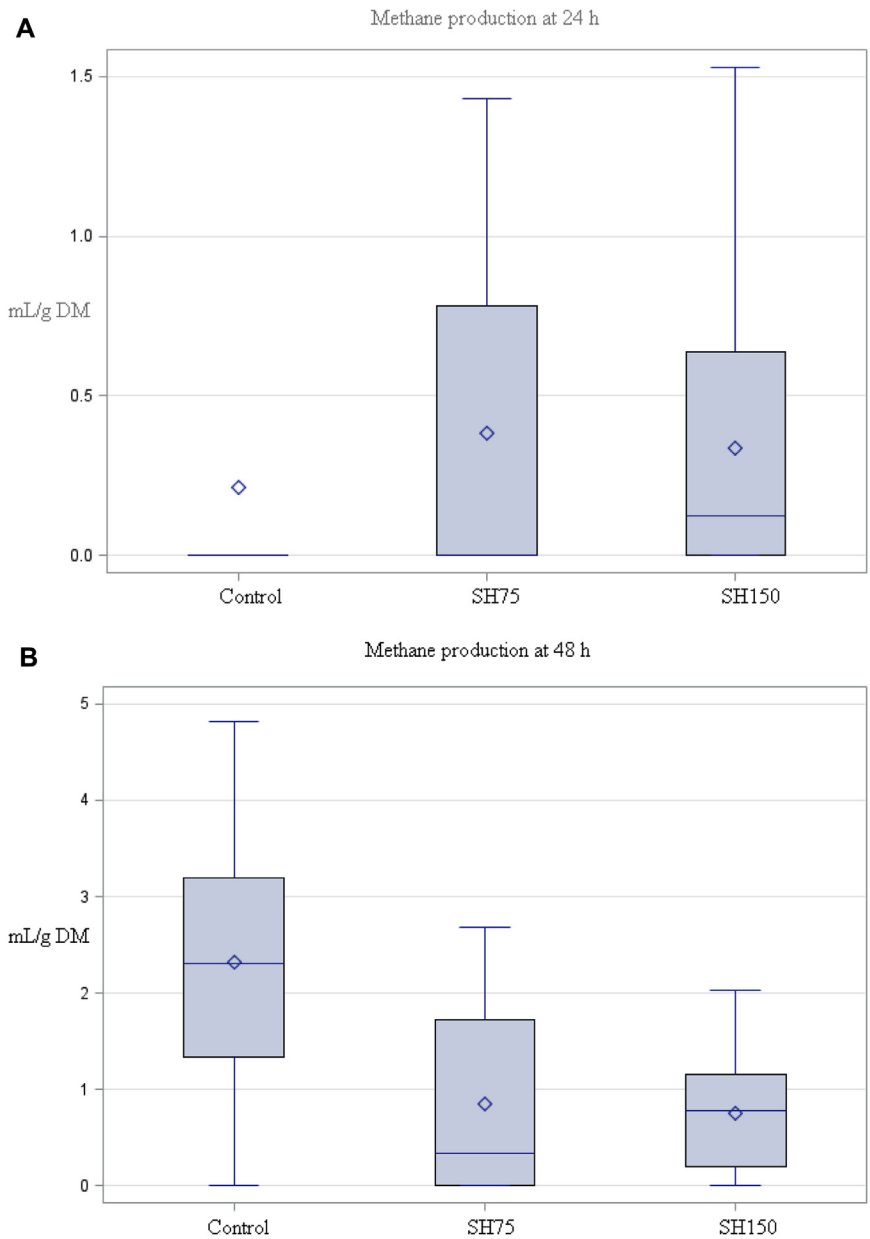

Fig. 3. Mean *in vitro* cecal methane production (mL/g DM) of three levels of soybean hulls (SH) at 24 (A) hours and 48 (B) hours of incubation. DM, dry matter.

24 to 72 hours) indicates that beyond 14 hours of incubation, both ration type and SB extract dose were independent of each and thus did not have synergistic effect on GP. Similarly, lack of ration type × SB extract dose interaction effect on GP parameters is an indication of independency of the two factors. Therefore, it would be better to discuss the effect of ration type and SB extract dose separately. The rations SH75 and SH150 had higher GP with lower rate and lag time of GP compared with the control ration. Increased GP is a good indicator of improved nutrient digestibility, fermentability, and rumen microbial protein production [32,33]. Higher GP has also been reported to indicate higher nutrient availability for ruminal microorganisms [34]. In corroboration of previous studies, Velázquez et al [9] observed unaffected fecal horse GP parameters when CG was replaced with SH at the same levels used in the present experiment. Similarly, Elghandour et al [8], using rumen liquor as a bacterial inoculum, obtained unaffected GP parameters when CG replaced SH.

The decreased rate and lag time of GP with replacing CG with SH is an indicator of increased fermentation of the insoluble but degradable fraction in the incubated substrates [8]. These results suggest a steady increase in availability of the carbohydrate fractions to the microbial population for growth and other activities and are in agreement with previous studies [33,34]. According to

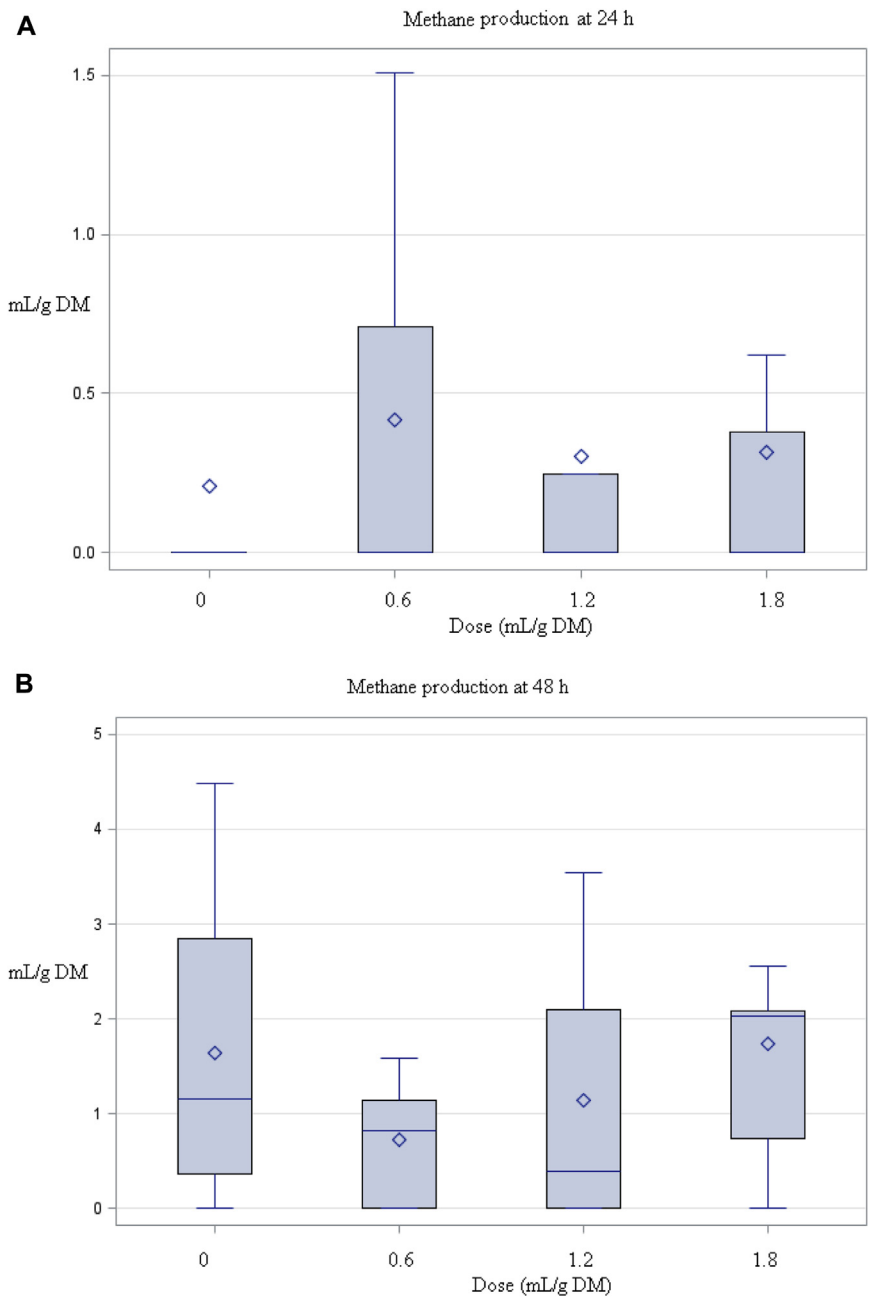

Fig. 4. Mean *in vitro* cecal methane production (mL/g DM) of four levels of *Salix babylonica* doses at 24 (A) and 48 (B) hours of incubation. DM, dry matter.

Blümmel and Ørskov [35], the asymptotic GP could be used to predict feed intake because 88% of variance in intake is accounted for by GP. This implies that the SH75 and SH150 rations had the tendency to induce feed intake and growth rate in ruminants [35] and may be in horses, since fractional rate of GP was correlated with feed intake [36]. This is because performance is largely a function of feed intake, which is a better indicator of nutritive value of feed than apparent digestibility [37]. The decreased discrete lag time prior to GP with the SH75 and SH150 rations suggests faster microbial adaptation to the ration, in agreement with previous reports [33,34].

Without affecting the asymptotic GP or the rate of GP, increased lag time of GP was observed with the inclusion of SB extract. This may be due to the negative effects of its secondary metabolites on the cecal microorganisms' activities; however, an ability of rumen microorganisms to degrade secondary metabolites in phytogenic extract and utilize them as an energy source was reported [38]. Negative effects of SB extract on ruminal fermentation and microorganisms have been attributed to deleterious impact of the secondary metabolites in the extract [18–21]. Parra-García et al [17], using horse cecal contents as an inoculum source, observed an unaffected asymptotic GP or rate of GP, and a

Table 3
In vitro methane (CH_4) productions of three levels of soybean hulls (SHs) at different levels (mg/g DM) of *Salix babylonica* (SB) extract inclusion (mL/g DM).

Ration[a]	SB Extract	CH_4 Production[b] at					
		24 hr	36 hr	48 hr	54 hr	60 hr	72 hr
Control	0	ND[c]	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
SH75	0	0.41	0.56	1.01	1.30	1.31	1.45
	0.6	0.17	0.18	0.25	0.25	0.25	0.31
	1.2	0.83	1.06	1.25	1.93	1.94	2.07
	1.8	0.13	0.54	0.88	1.01	1.18	1.48
SH150	0	0.22	0.40	0.52	0.53	0.57	0.74
	0.6	0.24	0.90	1.04	1.07	1.08	1.29
	1.2	0.08	0.10	0.13	0.13	0.13	0.16
	1.8	0.81	0.97	1.33	1.43	1.45	1.50
Pooled SEM		0.26	0.37	0.61	0.86	0.91	1.22
P value							
Ration effect							
Linear		.367	.089	.002	<.001	<.001	<.001
Quadratic		.800	.328	.036	.001	.001	.001
Dose effect							
Linear		.631	.005	.853	.864	.266	.923
Quadratic		.824	.035	.211	.395	.752	.975
Ration × dose		.043	.043	.001	.252	.408	.079

Abbreviations: DM, dry matter; SEM, standard error of the mean.
[a] SH75, soybean hulls were included at 75 g/kg DM of total mixed ration; SH150, soybean hulls were included at 150 g/kg DM of total mixed ration.
[b] No CH_4 was produced at 2, 6, 10, and 14 hours of incubation.
[c] ND means not detected (i.e., 0 mL CH_4/g DM).

Table 4
Degradation and in vitro cecal fermentation profile[a] of three levels of soybean hulls (SHs) at different levels (mg/g DM) of *Salix babylonica* (SB) extract inclusion (mL/g DM).

Ration[b]	SB	pH	ME	DMD	SCFA	PF_{24}	GY_{24}
Control	0	5.43	13.06	861	8.28	4.81	208
	0.6	5.10	12.91	930	8.16	4.86	206
	1.2	6.55	15.64	885	10.39	4.66	215
	1.8	6.57	15.32	879	10.12	4.67	214
SH75	0	6.64	14.13	827	9.17	4.74	211
	0.6	6.63	15.11	812	9.97	4.69	213
	1.2	6.57	16.48	858	11.09	4.62	216
	1.8	5.88	15.16	860	10.01	4.68	214
SH150	0	5.86	12.82	796	8.05	4.84	207
	0.6	6.27	13.00	811	8.19	4.83	207
	1.2	5.58	11.11	828	6.65	5.17	195
	1.8	6.26	14.37	838	9.31	4.74	211
Pooled SEM		0.233	0.955	26.7	0.779	0.120	4.4
P value							
Ration effect							
Linear		.004	.157	.015	.148	.419	.342
Quadratic		.226	.003	.010	.003	.023	.016
Dose effect							
Linear		.186	.050	.164	.051	.340	.257
Quadratic		.439	.695	.486	.693	.404	.525
Ration × dose		.002	.148	.576	.147	.265	.229

Abbreviations: DM, dry matter; SEM, standard error of the mean.
[a] DMD is the in vitro dry matter digestibility (mg/g DM); GY_{24} is the gas yield at 24 hours (mL gas/g DMD); ME is the metabolizable energy (MJ/kg DM); PF_{24} is the partitioning factor at 24 hours of incubation (mg DMD/mL gas); pH is the fermentation pH; SCFA is the short-chain fatty acids (mmol/g DM).
[b] SH75, soybean hulls were included at 75 g/kg DM of total mixed ration; SH150, soybean hulls were included at 150 g/kg DM of total mixed ration.

decreased lag time of GP with the inclusion of SB extract in rations where CG was replaced with SHs. Variations in the results of effect of additives on lag time of GP have been adduced to differences in the incubated substrates [8].

4.2. Methane Production

The occurrence of ration type × SB extract dose interaction on CH_4 production reveals that CH_4 production was SB extract dose and ration dependent. Both SH75 and SH150 rations decreased CH_4 production. Methane production from ruminants depends mainly on the degradability and chemical composition of diets [5,8,39]. Livestock sector is one of the sources responsible for about 18% of greenhouse gas emission [40], as a result of ruminal fermentation of fed diets in the rumen causing a loss of digested energy [41]. Using the same rations used in the present experiment, Elghandour et al [8] observed that replacing CG with SHs did not affect CH_4 production. Differences in response between the present experiment and that of Elghandour et al [8] may be due to different inoculum source (rumen contents vs. cecal contents).

The inclusion of SB extract did not affect CH_4 production. Parra-García et al [17] obtained unaffected CH_4 production with the inclusion of SB extract to rations where CG was replaced with SHs.

4.3. Fermentation Kinetics

The SH75 ration increased cecal fermentation pH, revealing better fermentation conditions for cecal microbial activities for fiber degradation [42]. Also, the ration increased ME, SCFA, and GY_{24}. Dhanoa et al [43] reported that feed degradation and fermentation rate are directly proportional to GP. Improved fermentation kinetics is the main reason for improved GP. Increased SCFA production and ME are associated with high activities of microbes in the rumen. Higher SCFA is important in terms of enhanced lactose production, milk volume, and overall energy balance in ruminants [44,45]. Besides, the improved fermentation parameters when CG was replaced with SH could be due to additional availability of the fermentable carbohydrates which possibly promoted microbial growth [46] and also improved the incubation environment. These results reveal more fermentable carbohydrates availability, enhanced degradability, and improved microbial protein synthesis of SH relative to CG.

Addition of SB extract to the ration did not affect the cecal fermentation parameters probably due to its inefficiency in improving fermentation efficiency, fermentation kinetics, and GP [17]. Parra-García et al [17] observed unaffected fermentation kinetics with the addition of SB extract to rations containing different levels of prickly pear cactus as a replacement of CG.

5. Conclusions

Results of GP and fermentation kinetics showed that SH has a potential fermentation efficiency and fermentation

kinetics superior to that of CG and that SH can replace CG in the diet of horses. The level of 75 g SHs/kg DM is the best level of inclusion (replacement of CG at 30%). Further *in vivo* trials with different levels of SH replacing CG in the presence or absence of SB extract inclusion should be conducted to validate current findings.

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