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Addressing sustainable ruminal methane and carbon dioxide emissions of soybean hulls by organic acid salts

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ABSTRACT

The current study aimed to study the sustainable mitigation of methane (CH_4) and carbon dioxide (CO_2) emissions as well as ruminal fermentation kinetics by replacing dietary corn grain (CG) with soybean hulls (SH) in the presence of organic acid salts (OAS). Three total mixed rations were prepared where CG was replaced with SH at three levels (/kg DM): 0 g (Control), 75 g (SH75) or 150 g (SH150). The OAS was used at three levels (dose): 0, 5 and 10 mg/g DM of substrates. Increasing SH level increased ($P < 0.05$) the fractional rate of gas production (GP) and lag time. The SH75 and SH150 rations quadratically decreased ($P < 0.001$) the asymptotic CO_2 production and the lag time of CO_2 production. Moreover, the high level of OAS quadratically decreased ($P \leq 0.05$) CO_2 production. The OAS inclusion increased ($P < 0.05$) CH_4 production (expressed as mL/g incubated DM and mL/g degraded DM). Increasing SH in the rations increased ($P < 0.05$) proportional CH_4 production. Inclusion of OAS also increased proportional CH_4 production. Replacing corn grain with soybean hulls could be a valuable means of sustainable mitigation of CH_4 and CO_2 emissions and improvement of the environmental conditions as well as provision of good feedstuff for ruminant livestock due to its *in vitro* fermentation characteristics. The organic acid salts did not affect ruminal gas production but decreased CO_2 emissions; thus its supplementation when soybean hulls replace corn grain is perhaps redundant, though may be considered as environmental friendly way of feeding livestock.

1. Introduction

Agriculture wastes are carbohydrate-rich feeds with a large potential source of dietary energy for ruminants, but in developing countries, they always constitute environmental problems when burnt in the field, and can be used as a cleaner product of animal feed and environment (Kholif et al., 2014). However, intensive ruminant production requires high concentrate diets to assure high productivity and fast growth. Cereals, such as barley, wheat and corn, are commonly used for intensive ruminant production. However, because grain prices are rising worldwide, producers search for alternatives that can partially replace the expensive grains. Apart from being exorbitant, grains are used with some cautions in ruminant diets because they can predispose the animals to acidosis and laminitis at a high level (Owens et al., 1998). Soybean hulls (SH) have been successfully fed as an economic substitute in the diets of ruminants (Costa et al., 2012). Because of the low energy density and fibrous nature of most unconventional ingredients which are majorly agro-industrial by-products, their inclusion in livestock diets requires supplementation with energy feed ingredients (e.g. corn grains (CG)) and additives (e.g. organic acid salts (OAS)). Though SH are readily available and inexpensive, they are fibrous; the composition (g/kg DM) is: crude protein, 116; neutral detergent fiber, 722 and acid detergent fiber, 411 (Costa et al., 2012). Replacement of energy feedstuff such as CG with SH will require some form of supplementation with OAS and acids which are used as energy additives in ruminant diets. Unconventional energy sources such as glycerol, propylene glycol, calcium propionate (Ferraro et al., 2016), or sodium propionate (Bas et al., 2000) or disodium malate or calcium malate (Mungói et al., 2012)

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have been used as ingredients of rations for ruminants. Castillo et al. (2004) suggested that the inclusion OAS can stimulate the production of propionic acid in the rumen with reduced methane (CH_4) emission by acting as a hydrogen (H_2) sink. In their experiment, Newbold et al. (2005) observed that OAS decreased methane emission by between 8 and 17%.

Methane formation from ruminant livestock is one of the sources responsible for greenhouse gas emission causing increasing attention from animal nutritionists (Intergovernmental Panel on Climate Change, 2008). The FAO estimated CH_4 production from livestock to contribute about 18% of all greenhouse gas emissions, while carbon dioxide (CO_2) accounts for about 9% emission. Besides, enteric CH_4 from ruminants as a result of ruminal fermentation of feed in the rumen implies a loss of digested energy (Johnson and Johnson, 1995) depending on diet degradability and chemical composition (Hristov et al., 2013).

The *in vitro* gas production procedure has become a useful tool to study potential rumen degradation of ruminant feeds (Rodriguez et al., 2015; Vallejo et al., 2016). This method allows estimation of how much substrate is used to produce volatile fatty acids and the energetic value of feed as well as to determine the amount of substrate truly fermented which is converted into microbial protein (Elghandour et al., 2015a,b). The current study aimed to investigate the impact of replacing CG of diet with SH in the presence of fermentation modulator containing OAS on the mitigation of the ruminal CH_4 and CO_2 emissions and fermentation kinetics, as a clean product for the environment and animal feed.

2. Materials and methods

2.1. Substrates and treatments

Three total mixed rations were prepared where CG was replaced with SH at three levels (/kg DM): 0 g (Control), 75 g (SH75) or 150 g (SH150). The ingredient and chemical compositions are shown in Table 1. The diets were supplemented with OAS as an additive containing salts of organic acids including monopropylene glycol, calcium propionate, calcium malate and other active compounds (Table 2). The additive was used at three levels: 0, 5 and 10 mg/g DM of substrates.

Table 1
Ingredients and composition of the experimental diets.

	Control	SH75	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
Soybean meal	30	30	20
Molasses	70	80	80
Vitamins/Minerals mixture[a]	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
Nonstructural carbohydrates	454	442	415
Ether extract	24	24	18

[a] 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), 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.

	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-selenomethionene Se	10	0.12	12 ppm
1,25-$(OH)_2$-D_3	10	10	0.1 ppm
E vitamin IU/kg	500,000	1	500 IU/kg

2.2. In vitro fermentation

Rumen inoculum was collected from a Brown Swiss cow (450 kg BW) fitted with a permanent rumen cannula and 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 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 different three 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 produced gases 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. The bottles were then uncapped and the pH was measured using a pH meter (Conductronic pH15, Puebla, Mexico) and the contents of each bottle 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), N (#954.01) and ether extract (#920.39) according to AOAC (1997), while ration's contents for neutral detergent fiber content (NDF) and acid detergent fiber (ADF) 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 (Van Soest et al., 1991).

For estimation of GP, CH_4 and CO_2 kinetics, recorded gas, CH_4 and CO_2 volumes (mL/g DM) were fitted using the NLIN procedure of SAS (2000) according to France et al. (2000) model as:

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

where y is the volume of GP, CH_4 or CO_2 at time t (h); b is the asymptotic GP, the asymptotic CH_4 or the asymptotic CO_2 (mL/g DM); c is the fractional rate of fermentation (/h), and L (h) is the

discrete lag time prior to when any gas, CH_4 or CO_2 is released.

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

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

$$OMD = 148.8 + 8.89\ GP + 4.5\ CP\ (g/kg\ DM) + 0.651\ ash\ (g/kg\ DM)$$

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

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 (2000) 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 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 SH containing rations to increasing addition levels of OAS. Statistical significance was declared at $P < 0.05$.

3. Results

Fig. 1 shows the *in vitro* rumen gas production (mL/g incubated DM) of three different levels of SH as affected by different levels of OAS. Level of SH (ration) and level of OAS (dose) respectively had no effect ($P > 0.05$) on asymptotic GP, but it was affected ($P = 0.027$) by ration × dose interaction. Fractional rate of GP ($P < 0.001$) and lag time ($P < 0.005$) increased linearly with increasing level of SH. Except for *c* which was quadratically ($P < 0.01$) affected, *b* and GP were not affected ($P > 0.05$) by level of OAS (dose) inclusion (Table 3).

Fig. 2 shows the *in vitro* rumen CH_4 production (mL/g incubated DM) of three different levels of SH as affected by different levels of OAS. Ration type had no effect ($P > 0.05$) on the asymptotic CH_4 production and rate of CH_4 production (Table 3).

Ration type quadratically affected ($P < 0.05$) the asymptotic CO_2 production, the lag time of CO_2 production and the rate of CO_2 production, but the SH75 and SH150 rations decreased (linear effect, $P = 0.011$; quadratic effect, $P = 0.006$) lag time of CO_2 production compared with the control ration. On the other hand, OAS inclusion had no effect ($P > 0.05$) on the asymptotic CO_2 production and the rate of CO_2 production; however, the dose of 10 mg/g DM quadratically increased ($P = 0.004$) the lag time of CO_2 production. The *in vitro* CO_2 production (mL/g incubated DM) of three different levels of SH as affected by different levels of OAS is shown in Fig. 3.

At 6 h of incubation, SH75 and SH150 increased (linear effect, $P = 0.027$) CH_4 production (mL/g incubated DM) compared with the control ration, but there was no effect ($P > 0.05$) of ration type at 24 and 48 h of incubations. However, OAS inclusion at both treatment levels increased (quadratic effect, $P \leq 0.01$) CH_4 production (as mL/g incubated DM) at 24 and 48 h of incubation. Moreover, CH_4 production (as mL/g degraded DM) was increased (linear and quadratic effect, $P \leq 0.015$) with the inclusion of OAS at 5 and 10 mg/g DM at all hours. The proportional CH_4 production at 24 and 48 h of incubation was affected (quadratic effect, $P \leq 0.009$) by the ration type; increasing the percent of SH in the rations resulted in increased proportional CH_4 production. At 6 and 48 h of incubations, the inclusion of OAS at the both treatment levels increased (linear effect, $P \leq 0.017$) proportional CH_4 production but at both 24 and 48 h, both treatment levels had quadratic effect ($P \leq 0.005$) on proportional CH_4 production (Table 4). On the contrary, both of ration type and OAS inclusion had no effect ($P > 0.05$) on CO_2 production (mL/g incubated DM or mL/g degraded DM) and proportional CO_2 production (Table 4).

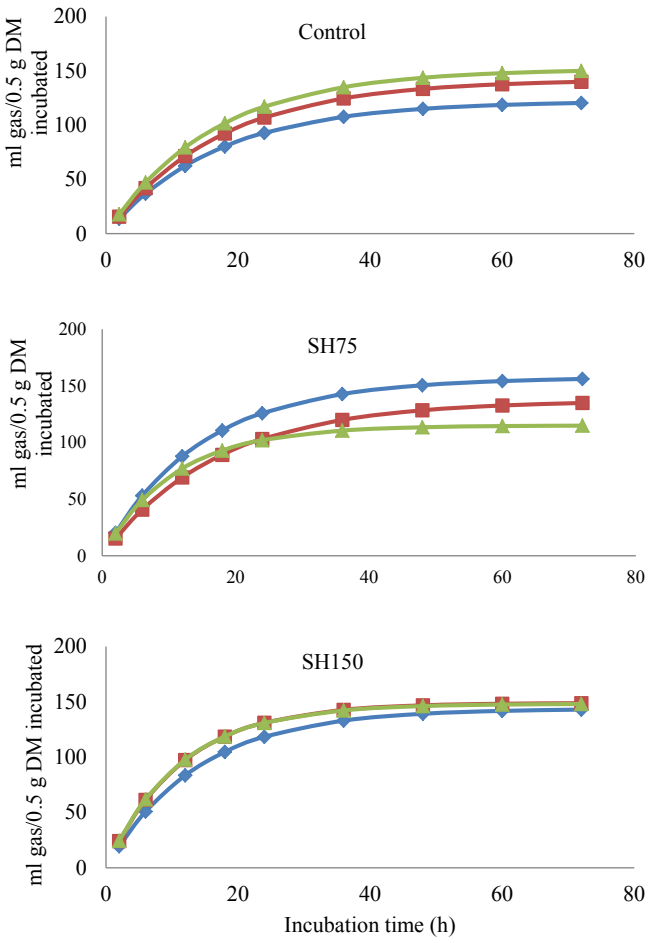

Fig. 1. *In vitro* rumen gas production (mL/0.5 g incubated DM) of three different levels of soybean hulls (SH) as affected by different levels of organic acid salts at 0 (-◆-), 5 (-■-), and 10 (-▲-) mg/g DM of the ration. Control: corn grain was replaced with soybean hulls at 0 g/kg DM; SH75: soybean 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.

Ration effects on pH and DM degradability (DMD; $P < 0.001$), ME, and OMD were linear. Effects of ration and ration × OAS dose interactions were not significant for these parameters (Table 5).

4. Discussion

The *in vitro* GP technique has been used as a measure of ruminal degradation of feeds (Salem et al., 2014). The GP is generally a good indicator of digestibility, fermentability and microbial protein production. Higher gas values indicate a better nutrient availability for rumen micro-organisms (Elghandour et al., 2015a,b). The generally high fractional rate of GP shows that the diets were highly

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

Ration[a]	OAS (mg/g DM)	Gas production (mL/0.5 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	122	0.06	1.35	37.0	0.021	4.38	106.7	0.010	4.45
	5	142	0.06	1.1	66.8	0.028	8.61	78.3	0.015	4.16
	10	152	0.06	1.61	79.9	0.022	9.50	117.5	0.005	5.62
SH75	0	158	0.07	1.84	90.7	0.013	9.62	34.8	0.041	3.22
	5	137	0.06	1.69	80.4	0.020	6.24	31.1	0.026	4.73
	10	115	0.1	1.76	106.5	0.014	6.34	35.1	0.046	6.99
SH150	0	144	0.07	1.69	49.0	0.017	10.05	73.4	0.003	2.02
	5	149	0.09	1.86	114.2	0.012	9.10	96.3	0.001	1.84
	10	148	0.09	1.73	107.9	0.014	8.10	126.5	0.005	2.84
Pooled SEM[e]		9.8	0.007	0.153	17.32	0.0055	1.112	15.51	0.0087	0.746
Ration effect										
Linear		0.317	0.001	0.005	0.054	0.062	0.098	0.872	0.339	0.006
Quadratic		0.390	0.640	0.070	0.189	0.399	0.271	<0.001	<0.001	0.011
Dose effect										
Linear		0.860	0.800	0.540	0.061	0.486	0.970	0.811	0.571	0.581
Quadratic		0.610	0.010	0.310	0.056	0.666	0.979	0.051	0.690	0.004
Ration × Dose		0.027	0.095	0.307	0.344	0.814	0.007	0.334	0.517	0.342

[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/0.5 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.

digestible because the rate at which a feed or its chemical constituents are digested in the rumen is as important as the extent of digestion. The values obtained were within the range of 0.056–0.17 mg/g DM reported for CG and canola (Getachew et al., 2004). The increasing fractional rate of GP with increased level of replacement of CG with SH is indicative of enhanced degradability or fermentability of the diets. The rate at which different chemical constituents are fermented is a reflection of microbial growth and accessibility of the feed to microbial enzymes (Getachew et al., 2004). It appears that partial replacement of CG with SH has the propensity of improve feed intake and consequently the performance of ruminants. This conjecture is supported by the assertion of Khazaal et al. (1996) who stated that the intake of a feed is mostly explained by the fractional rate of GP which affects the rate of passage of the feed through the rumen. Fractional rate of GP reduced from 0 to 5 mg OAS/g DM supplementation and increased as the level of OAS was increased to 10 mg/g DM. Higher fractional rate of GP at 10 mg OAS/g DM supplementation depicts enhanced fermentation and degradation. Using three energy additives, glycerol, propylene glycol and molasses, Ferraro et al. (2016) attributed higher fractional rate of GP of molasses to its greater fermentability. As earlier opined, supplementation with 10 mg OAS/g DM has the tendency to enhance voluntary feed intake and performance. The values obtained for the rate of GP are similar to those reported by Ferraro et al. (2016). The delay in the onset of GP and thus a longer lag time as SH replaced CG suggests that feeding of SH delayed microbial adaptation to the diets. According to Ferraro et al. (2016) continuous feeding of an energy additive in the sheep diet induces microbial adaptation and shortens the lag phase. Similarly, lower lag phase of the control diet is suggestive of its availability to provide a greater proportion of nutrients. The increasing GP with increased level of replacement of CG with SH indicates that SH contained a significant amount of rapidly fermentable carbohydrate. Rapidly fermentable carbohydrate has been reported to increase GP (Elghandour et al., 2015a,b). Furthermore, increased GP with CG replacement likely promoted higher nutrient availability to rumen micro-organisms, especially available N (Guerrero et al., 2012). Ration × dose interaction indicates a synergy between levels of replacement of CG with SH and rate of OAS on asymptotic GP.

During ruminal fermentation process, many gases are produced within the rumen mainly consisting of H_2, CO_2, and CH_4. In the present study, replacing CG with SH decreased CO_2 production, which is very important from environmental view, as the CO_2 and CH_4 have direct global warming effects. Increasing fibers and decreasing nonstructural carbohydrates in the rations containing SH may be the main reason. Increasing cell wall content may reduce the microbial activities, causing a lowered CO_2 production and decreasing lag time of CO_2 production. Almost no published reports are available in the literature on the effect of replacing CG with SH in diets on CO_2 production; therefore, the present results could not be compared.

Ration type had no effect on CH_4 production, the rate of production and lag time of production. Similarly, the ration type × OAS dose interaction had no effect on CO_2 production. The reason for lack of effect of level of SH and OAS on mitigation of CH_4 emission is unknown because it was expected that the pronounced increase in GP with SH inclusion should be accompanied by marked changes in both CH_4 mitigation and CO_2 emissions. Lack of response of CH_4 mitigation to both OAS dose and ration type × OAS dose interaction may be attributed to non-significant effects of these two factors on GP. However, at some hours of incubation, OAS increased CH_4 production. The present results of CH_4 production are in contrast to most of published reports. In their review, Sahoo and Jena (2014) reported that OAS has the ability to decrease methanogenesis by 'sinking' H_2 during their conversion to propionate (Newbold and Rode, 2006). Increasing H_2 removal stimulates cellulolytic bacteria resulting in increased fibers digestion. The different responses reported in the present study and the previous ones may be due to the level and nature of the OAS (i.e., the concentration of each component of the product). The OAS preparation, in the current study, included monopropylene glycol, calcium propionate, calcium malate and other active compounds. The response to the OAS is specific for each acid (Strauss and Hayler, 2001); for example, Gram positive bacteria are sensitive to long chain acids, whereas Gram negative bacteria are only sensitive to acids with less than eight carbon atoms (Partanen, 2001).

The OAS at the low level increased the lag time of CH_4 production while the high level decreased it implying that the

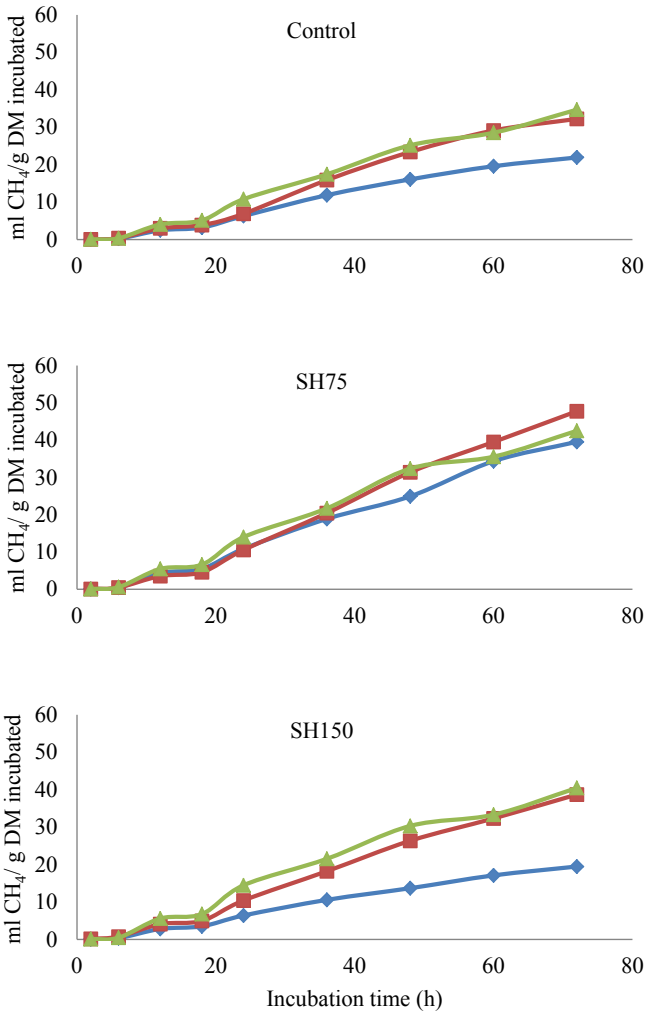

Fig. 2. *In vitro* methane production (mL/g incubated DM) of three different levels of soybean hulls (SH) as affected by different levels of organic acid salts at 0 (-♦-), 5 (-■-), and 10 (-▲-) mg/g DM of the ration. Control: corn grain was replaced with soybean hulls at 0 g/kg DM; SH75: soybean hulls 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.

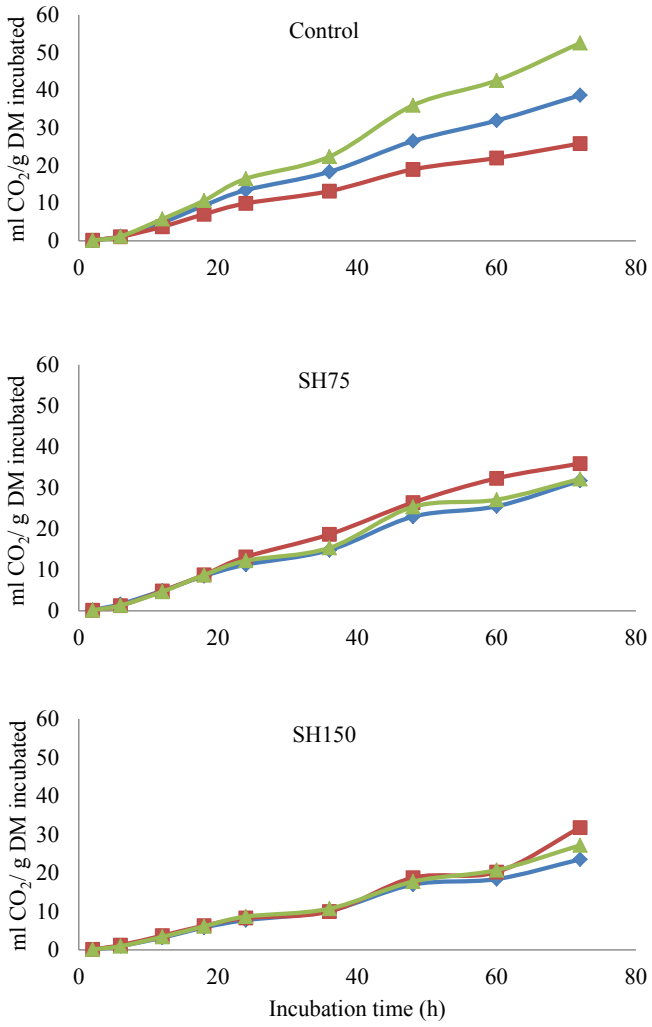

Fig. 3. *In vitro* carbon dioxide production (mL/g incubated DM) of three different levels of soybean hulls (SH) as affected by different levels of organic acid salts at 0 (-♦-), 5 (-■-), and 10 (-▲-) mg/g DM of the ration. Control: corn grain was replaced with soybean hulls at 0 g/kg DM; SH75: soybean 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.

response of lag time of CH_4 production to the inclusion of OAS is dose dependent. The delay in the onset of CH_4 production and thus a longer lag time with the inclusion of OAS suggests delayed adaptation of methanogenic archaea and bacteria to the OAS.

The decline in ruminal pH and rising of OMD with increasing level of SH confirm the earlier assertion that replacement of CG with SH diets improved the availability of readily fermentable carbohydrate which was rapidly degraded to produce volatile fatty acids. Positive correlations between readily fermentable carbohydrate and pH (Walsh et al., 2009) and pH and volatile fatty acids (Ramos et al., 2009) have been documented. Generally, dietary carbohydrates are fermented to short chain fatty acids and gases mainly CO_2 and CH_4 (Blümmel and Ørskov, 1993). It has been suggested that GP at 24 h is proportional to the amount of actually digested carbohydrates at maintenance ME intake, and is highly correlated to the ME content of feedstuffs (Giger-Riverdan et al., 2000). In the current study, ME is positively correlated to gas yield, which equally increased as the level of CG replacement with SH increased. Increased replacement level of SH for CG resulted in increased ME values. This is possibly due to increased OMD and availability of fermentable carbohydrate and N. Differences in ME among feeds have been ascribed to variation in fermentable carbohydrates and available N among them (Guerrero et al., 2012). Linear increase in OMD with increasing replacement of CG by SH suggests that more nutrients, particularly N, were made available to facilitate microbial degradation (Akinfemi et al., 2009). The combination of GP measurements with the concomitant quantification of the truly degraded substrate provides important information about partitioning of fermentation products. The result suggests greater fermentability of SH relative to CG which resulted in increased nutrient availability for microbial biomass production. Similarly, increasing ME with increasing SH level must have made more energy available for microbial protein production. Linear decline in *in vitro* DMD with increasing SH level needs further investigation because *in vitro* DMD was expected to increase with SH level, since SH appears to be more degraded than CG. This is based on increasing rate of GP and GP as the level of replacement of CG with SH level increased in the diets. *In vitro* DMD has reported to be positively correlated with rate of GP and GP (Akinfemi et al., 2009).

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 of soybean hulls (SH) 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			mL/g incubated DM			mL/g degraded DM		
		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	0.23	6.3	16.1	4.30	14.4	23.1	0.64	6.9	14.1	1.24	13.47	26.5	1.57	17.1	33.4	3.30	14.42	22.8
	5	0.36	6.9	23.4	8.00	25.4	39.3	0.86	6.5	17.7	1.09	9.95	19.0	1.46	22.2	25.3	2.5	9.23	14.3
	10	0.34	10.8	25.2	8.77	29.2	46.9	0.68	9.1	17.7	1.18	16.50	36.0	1.60	13.3	48.4	2.41	13.14	24.1
SH75	0	0.43	10.9	24.9	5.93	21.0	36.1	0.78	8.9	16.8	1.65	11.27	23.0	2.33	15.9	32.3	2.97	8.82	15.3
	5	0.49	10.6	31.4	8.57	28.9	46.8	1.20	10.5	24.4	1.28	13.15	26.4	1.74	18.1	36.4	3.04	12.38	20.0
	10	0.59	14.0	32.4	8.13	28.5	48.5	1.22	13.6	28.2	1.32	12.2	25.4	1.85	17.2	35.5	2.66	11.29	21.6
SH150	0	0.34	6.4	13.7	4.37	14.8	24.4	0.68	5.5	10.0	1.00	7.74	17.0	1.48	11.4	25.0	1.98	6.48	12.1
	5	0.74	10.4	26.3	5.87	20.4	34.6	1.20	7.8	18.1	1.23	8.29	18.7	1.80	12.0	27.3	1.99	6.33	12.4
	10	0.46	14.5	30.3	8.03	28.3	48.6	0.78	10.9	20.8	0.97	8.66	17.8	1.45	12.9	26.4	1.61	6.67	12.2
Pooled SEM[b]		0.103	2.09	3.25	1.183	3.54	5.48	0.180	1.57	2.17	0.422	4.708	7.52	0.605	6.53	10.57	0.786	3.960	4.90
Ration effect																			
Linear		0.027	0.171	0.483	0.347	0.534	0.899	0.288	0.658	0.909	0.771	0.203	0.146	0.949	0.325	0.289	0.181	0.091	0.055
Quadratic		0.232	0.094	0.007	0.252	0.124	0.063	0.058	0.009	0.001	0.340	0.669	0.655	0.348	0.634	0.619	0.312	0.610	0.455
Dose effect																			
Linear		0.031	0.409	0.004	0.015	0.011	0.013	0.017	0.371	0.002	0.788	0.926	0.902	0.804	0.949	0.951	0.733	0.856	0.777
Quadratic		0.660	0.007	0.010	0.020	0.006	0.002	1.000	0.005	0.002	0.768	0.592	0.398	0.824	0.555	0.374	0.469	0.791	0.372
Ration × Dose		0.356	0.772	0.670	0.734	0.724	0.724	0.665	0.855	0.428	0.963	0.940	0.746	0.955	0.954	0.782	0.985	0.865	0.676

[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] SEM standard error of the mean.

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

Ration[b]	OAS (mg/g DM)	pH	ME	DMD	OMD
Control	0	6.27	5.41	824.2	372.5
	5	6.53	5.80	755.3	397.7
	10	6.14	6.07	741.5	415.7
SH75	0	5.97	6.32	712	432.2
	5	6.06	5.71	741.5	391.8
	10	6.01	5.68	717.1	390
SH150	0	5.39	6.12	679	419.1
	5	5.34	6.47	692.1	441.7
	10	5.54	6.47	676.9	441.4
Pooled SEM[c]		0.162	0.279	22.18	18.23
Ration effect					
Linear		<0.001	0.018	<0.001	0.018
Quadratic		0.226	0.443	0.771	0.447
Dose effect					
Linear		0.465	0.870	0.633	0.871
Quadratic		0.800	0.622	0.173	0.622
Ration × Dose		0.528	0.186	0.181	0.185

[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).
[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.
[c] SEM standard error of the mean.

5. Conclusions

The organic acid salts had no effect on ruminal gases and thus its supplementation when soybean hulls replace corn grain may be redundant. Lack of soybean hulls × organic acid salts interaction on the ruminal fermentation indicates absence of synergy between the two factors in enhancing ruminal fermentation. Replacing dietary corn grain with soybean hulls could be a valuable means of sustainable mitigation of animal ruminal gases (i.e., CH_4 and CO_2) production and improvement of the environmental conditions. Corn grain can therefore be replaced with soybean hulls up to 150 g/kg DM without adversely affecting rumen fermentation but preventing environmental degradation due to reduction in CH_4 and CO_2 emissions. Soybean hulls can be used as a good cleaner product for the environment and feedstuff for ruminant livestock to control the environmental contamination by ruminal gases due to its anaerobic *in vitro* fermentation characteristics. Further research should, however, be conducted to establish the efficacy of replacement of corn grain with soybean hulls in *in vivo* trials.

Conflict of interest

None.

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