Journal of Cleaner Production 192 (2018) 766–774

Contents lists available at ScienceDirect

Journal of Cleaner Production

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

Influence of *Escherichia coli* inclusion and soybean hulls based diets on ruminal biomethane and carbon dioxide productions in sheep

Mona M.M.Y. Elghandour [a], Xochitl Antolin-Cera [a], Abdelfattah Z.M. Salem [a, *], Alberto Barbabosa-Pliego [a], Benjamin Valladares-Carranza [a], Eziuche Amadike Ugbogu [b]

[a] Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Mexico
[b] Department of Biochemistry, Abia State University, PMB 2000, Uturu, Abia State, Nigeria

ARTICLE INFO

Article history:
Received 3 November 2017
Received in revised form
27 April 2018
Accepted 1 May 2018
Available online 2 May 2018

Keywords:
Carbon dioxide
Corn grains
E. coli
Methane
Sheep
Soybean hulls

ABSTRACT

In livestock production, ruminal fermentation leads to significant loss of digestible feed energy and increase methane (CH_4) and carbon dioxide (CO_2) productions. These gases are the major sources of greenhouse gases that cause environmental degradation and climate change. The present study aimed at investigating the sustainable control of CH_4 and CO_2 production from ruminal fermentation by evaluating ruminal inclusion of *Escherichia coli* (*E. coli*) on diets containing different levels of soybean hulls (SH) replacing corn grains (CG). Three different levels of mixed ration were prepared; CG was replaced with SH at three different levels (per kg dry matter (DM)): 0 g (control), 75 g (SH 75), and 150 g (SH 150). The *E. coli* was used at four doses: 0, 10, 20 and 40 μL/g DM of substrate. The SH rations had decreased linear and quadratic ($P < 0.05$) effects on asymptotic gas production (GP). Interactions occurred between SH ration and *E. coli* doses ($P < 0.05$) on the fractional rate of GP. *E. coli* at all doses did not produce any effect on the CH_4 production parameters. However, the control had the highest CH_4 production at 40 μL/g DM *E. coli* addition compared to other SH rations and their respective *E. coli* doses. SH ration linearly ($P = 0.006$) decreased asymptotic CO_2 production. The study established that SH ration and *E. coli* doses had no effect on the CH_4 production; however, they had a decreased effect on asymptotic GP. This study demonstrated that inclusion of SH 150 ration at different *E. coli* doses reduced asymptotic CO_2 production without effect on CH_4 production and this may be useful for the sustainable mitigation of CO_2 production from livestock production.

1. Introduction

Globally, agricultural waste products are nutrient-rich and can serve as alternative sources of dietary feed for livestock production (Ahmed et al., 2015). In many developing countries, these waste products are burnt in the field thereby leading to pollution, climate change and environmental degradation (Kholif et al., 2014). In this way, agro-based industries contribute significant amounts of greenhouse gases (GHGs) such as carbon dioxide (CO_2) and methane (CH_4) to the atmosphere (Audsley and Wilkinson, 2014). Many researchers (Garnett et al., 2013; Gerber et al., 2013; Herrero et al., 2014) have reported the importance of sustainable intensification in agro-farming and highlighted the need to increase production efficiency and minimize the impact of waste products on the environment.

Currently, agro-food based industries such as livestock production is among the leading contributors of the anthropogenic source of GHGs- CH_4 and CO_2. Slade et al. (2016) in their recent investigation observed that two-third of the direct emissions is due to livestock production. According to the report of the Food and Agriculture Organization (FAO, 2006), animal production is responsible for 18% CH_4 and 9% CO_2 productions of all GHG emissions. Methane has a greater global warming effect (about 23 times) more than CO_2 (Rira et al., 2015) and accounts for 50–60% emitted GHG during ruminant production (Mirzaei-Aghsaghali et al., 2012). Methane production is also responsible for a net loss of 2–12% of total energy of feed in ruminant production (Mirzaei-Aghsaghali et al., 2012; Hristov et al., 2015). In the last decade, animal nutritionists and microbiologists have developed a keen interest in the manipulation of ruminal microbial ecology and its enteric fermentation kinetics. The basic aim of these manipulations is to

* Corresponding author.
E-mail addresses: salem@uaemex.mx, asalem70@yahoo.com (A.Z.M. Salem).

https://doi.org/10.1016/j.jclepro.2018.05.002
0959-6526/

improve animal feed utilization, enhance digestibility of fibrous feeds, reduce protein degradability, increase animal performance, minimize loss of dietary energy during rumen fermentation and also reduce CH_4 and CO_2 productions for eco-friendly animal production (Patra et al., 2006; Benchaar et al., 2007).

The use of agro-based waste products as unconventional feedstuff can be a major breakthrough in livestock production. Not only that they are cheap source of animal feeds, they are available all year round especially during dry season. Thus, they can guarantee continuous supply of animal feeds. They may also help to reduce CH_4 and CO_2 productions from livestock because ruminants depend on diet degradability and chemical composition of their feeds (Hristov et al., 2013; Elghandour et al., 2016a).

Soybean hulls have been used widely as a viable alternative and economic substitute in the ruminant (Costa et al., 2012) and horse (Esquivel-Velázquez et al., 2016) diets. However, due to low energy density and fibrous content of SH, their addition in ruminant diet requires inclusion of high energy feed ingredients such as corn grains (CG) and organic acid salts (Elghandour et al., 2016a). Castillo et al. (2004) reported that the addition of organic acid salts can stimulate propionic acid production in the rumen and reduce CH_4 emission by serving as a hydrogen (H_2) sink. Newbold et al. (2005) also reported that organic acid salts decreased CH_4 production by 8-7%. Elghandour et al. (2016a) investigated the ruminal CH_4 and CO_2 productions of SH by applying organic acid salts as a supplement and observed sustainable mitigation of the gases during livestock production. They also suggested that SH is not only useful as feedstuff for the production of ruminants but can reduce the environmental pollution caused by ruminal gases. However, they noted that supplementing organic acid salts with SH did not influence ruminal gas production but decreased CO_2 production.

In ruminants production, sodium or calcium propionate (Ferraro et al., 2009), organic salts (Elghandour et al., 2017), plant extract (Jiménez-Peralta et al., 2011; Salem et al., 2014a), *Saccharomyces cerevisiae* (Rodriguez et al., 2015) fibrolytic enzymes (Morsy et al., 2016), have been successfully used as rumen modifier. Inclusion of these ingredients as a ration for ruminants diets have shown positive effect on forage quality (Kholif et al., 2017a; b), feed utilization, digestibility, rumen fermentation, and animal performance (Valdes et al., 2015). Recently, it has been hypothesized that ruminal contamination with *E. coli* can affect ruminal microflora and ruminal fermentation thus influence GP, CH_4 and CO_2 productions (Elghandour et al., 2018). This may be due to antagonistic role *E. coli* plays on normal ruminal microflora which often leads to decreased fermentation of the ruminant diets and cause reduced CH_4 and CO_2 productions. Elghandour et al. (2018) reported that inclusion of prickly pear cactus rations with different *E. coli* doses reduced CH_4 and CO_2 productions in ruminants.

This present study aimed to evaluate the level of ruminal inclusion of *E. coli* on the nutritive value and greenhouse gas production of diets containing different levels of soybean hulls replacing corn grains. The reason for using *E. coli* doses with three different SH rations is to establish the optimal precision of the mixture of SH rations and *E. coli* doses that may work best in mitigation of CH_4 and CO_2 productions.

2. Materials and methods

2.1. Substrates and treatments

In this experiment, three different ration mixtures were prepared. In the mixtures, CG was replaced with SH at three different levels per kg dry matter (DM) namely: 0 g (Control), 75 g (SH 75) or 150 g (SH 150). Table 1 shows the ingredients and chemical composition of the experimental diets. *E. coli* was cultivated at the concentration of 1×10^{10} in the laboratory of bacteriology of the Faculty of Veterinary Medicine and Animal Science, Autonomous University of the State of Mexico. The *E. coli* was used at four doses: 0, 10, 20 and 40 μL/g DM of substrate. The treatments were as follows: SH 75 treatment (at 0, 10, 20 and 40 μL/g DM *E. coli* doses) and SH 150 treatment (at 0, 10, 20 and 40 μL/g DM *E. coli* doses). The treatments were tested against the control treatment (without SH).

Table 1
Ingredients and composition of the experimental diets[a].

	Control	SH 75	SH 150
Ingredients (g/kg DM)			
Oats straw	249	248	248
Steam rolled corn	250	175	100
Steam rolled barley	250	250	250
Wheat bran	120	110	120
Corn gluten feed	30	30	30
Soybean meal	30	30	20
Soybean hulls	0	75	150
Molasses	70	80	80
Vitamins/Minerals[b]	1.0	2.0	2.0
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] Adapted from Esquivel-Velázquez et al. (2016).
[b] 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).

2.2. In vitro fermentation

Rumen inoculum was collected from two adult sheep (50 ± 2.5 kg body weight (BW)) fitted with a permanent rumen cannula. These sheep were fed *ad libitum* with a mixed ration of a concentrated commercial formula (PURINA®, Toluca, Mexico) and alfalfa hay in the ratio of 1:1 DM according to National Research Council (NRC, 2001). The collected rumen contents were flushed with CO_2, mixed and filtered through four layers of cheesecloth into a flask with O_2-free headspace. Each SH ration was weighed directly into a 120 mL serum bottles followed by addition of appropriate extract dose per gram DM *E. coli*. Consequently, exactly 10 mL of particle free rumen fluid was included to each of the serum bottles followed by 40 mL of the buffer solution of Goering and Van Soest (1970), without including trypticase.

Three incubation processes were conducted in three weeks. The rumen fluid bottles (as blanks) and the substrates bottles were incubated for 72 h. The rumen fluid bottles (as blanks) and the substrate bottles were incubated for 72 h at 39 °C. The volume of GP were measured every 2 h and after 24 h, two more measurements were taken at 48 h and 72 h with the help of Pressure Transducer Technique (Extech instruments, Waltham, USA) of Theodorou et al. (1994). The CH_4 and CO_2 productions were measured every 4 h and after 24 h two more measurements were taken at 48 and 72 h of incubation using Gas-Pro detector (Gas Analyzer CROWCON Model Tetra 3, Abingdon, UK). The *in vitro* incubation process is summarized in Fig. 1.

At the end of incubation, the fermentation process was stopped by swirling the bottles in ice. The bottles were then uncapped and the pH was determined using a pH meter (Conductronic pH 15, Puebla, Mexico). The contents of each bottle were filtered under vacuum through glass crucibles (coarse porosity no. 1, pore size

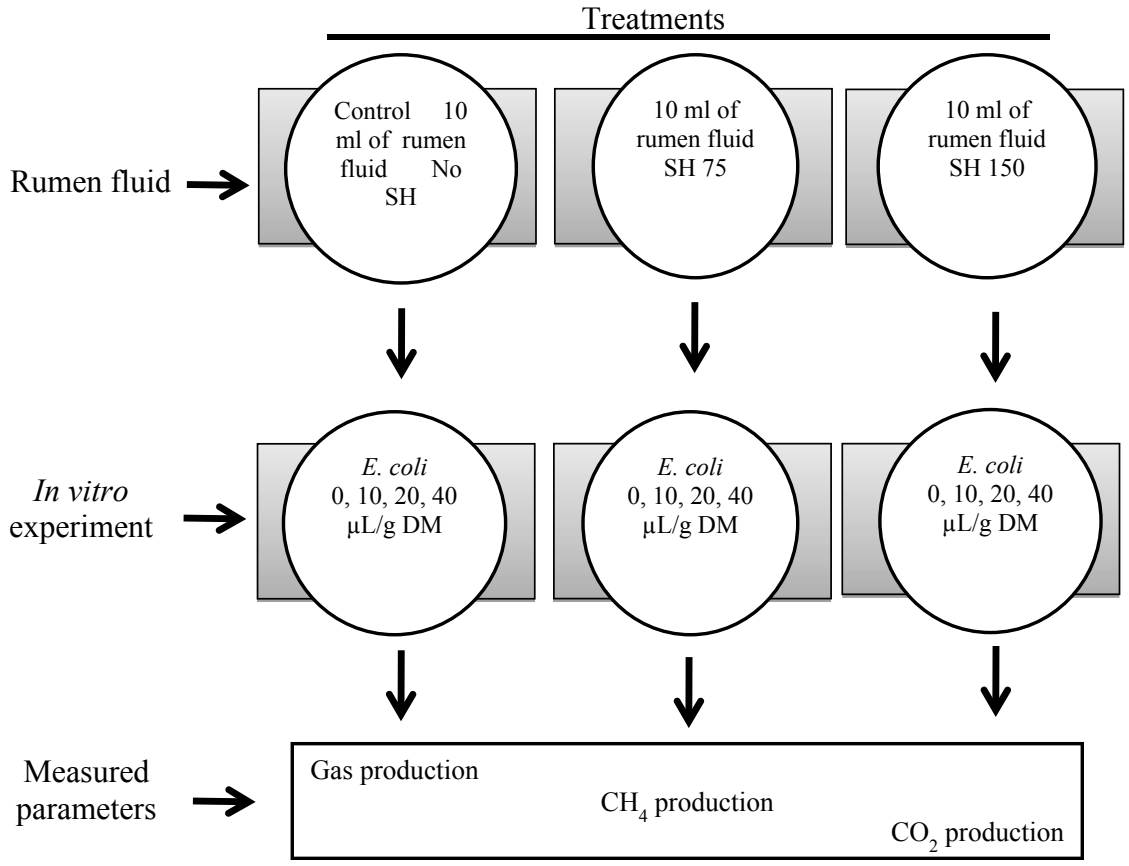

Fig. 1. Flowchart of the *in vitro* incubation process.

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 dry matter (DM) (method ID 934.01), ash (method ID 942.05), Nitrogen (method ID 954.01) and ether extract (method ID 920.39) using Association of Official Analytical Chemists (AOAC, 1997) official methods, Neutral detergent fiber (NDF) analyzed using the method of Van Soest et al. (1991) while acid detergent fiber (ADF) and lignin (AOAC, 1997; method ID 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.

Volumes (mL/g DM) of GP, CH_4 and CO_2 were used to estimate the fermentation kinetics parameters using the nonlinear model procedure of Statistical Analysis System (SAS, 2002) according to France et al. (2000) model as:

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

where, y is the volume (mL/g DM) 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 (1/h), and Lag (h) is the discrete lag time prior to when any gas, CH_4 or CO_2 is released.

2.4. Statistical analyses

Data of each of the three runs within the same sample were averaged prior to statistical analysis. 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 procedure of General Linear Model option of SAS (2002) as:

$$Y_{ijk} = \mu + R_i + D_j + (R \times D)_{ij} + E_{ijk} \quad (2)$$

where: Y_{ijk} is every observation of the ith ration type (R_i) with jth extract dose (D_j); μ is the general mean; $(R \times D)_{ij}$ is the interaction between ration type and extract dose; E_{ijk} is the experimental error. Linear, quadratic and cubic polynomial contrasts were used to examine response of different rations on increasing addition doses per gram DM *E. coli*. Statistical significance was declared at the level of $P < 0.05$.

3. Results

3.1. Gas production and fermentation

The levels of SH ration had decreased linear ($P = 0.001$) and quadratic ($P = 0.005$) effects on asymptotic GP. *E. coli* doses had no linear, quadratic and cubic effects on asymptotic GP. No ration and dose interaction were observed on asymptotic GP. However, *E. coli* doses had linear ($P < 0.001$) and cubic ($P = 0.003$) effects on the rate of GP. Inclusion of SH ration and *E. coli* doses had dependent interaction ($P < 0.05$) on the fractional rate of GP. Rational type linearly ($P = 0.006$) and quadratically ($P = 0.013$) affected the initial delay before GP began (Table 2, Fig. 2a–c).

The addition of SH ration had linear and quadratic effects

Table 2
In vitro rumen gas, CH_4 and CO_2 kinetics of three different levels of soybean hulls (SH) as influenced by different doses of *E. coli* (μL/g DM).

Ration[a]	*E. coli*	pH and degradability		Gas production[c]			CH_4 production[d]			CO_2 production[e]		
		pH	DMD [b]	*b*	*c*	*Lag*	*b*	*c*	*Lag*	*b*	*c*	*Lag*
Control	0	5.68	543.2	373.1	0.078	1.11	34.0	0.029	9.91	149.2	0.029	4.08
	10	5.71	548.1	316.4	0.101	1.06	55.1	0.050	13.50	108.0	0.047	3.90
	20	6.04	572.0	366.3	0.093	1.06	50.0	0.032	10.70	219.0	0.052	4.14
	40	6.40	558.0	395.0	0.121	1.17	85.2	0.021	5.91	191.1	0.056	4.11
SH75	0	6.46	498.3	268.1	0.075	1.40	35.1	0.028	14.30	118.2	0.045	4.23
	10	6.40	537.4	229.3	0.092	1.62	19.0	0.043	21.20	135.3	0.034	3.33
	20	6.47	536.2	288.0	0.079	1.50	34.0	0.019	7.82	129.2	0.018	2.44
	40	6.49	497.1	230.2	0.122	1.30	22.2	0.113	10.30	97.4	0.040	3.51
SH150	0	6.65	507.4	305.1	0.085	1.03	45.3	0.027	15.30	165.0	0.035	3.39
	10	6.59	530.0	235.3	0.215	1.66	54.4	0.033	9.11	92.3	0.074	4.78
	20	5.98	524.3	221.1	0.092	1.64	55.3	0.032	12.10	98.0	0.052	3.62
	40	6.10	529.0	230.0	0.252	1.37	38.0	0.036	8.53	81.1	0.061	4.08
Pooled SEM[f]		0.212	15.90	27.40	0.0191	0.118	16.70	0.015	4.814	24.41	0.014	0.784
Ration effect												
Linear		0.020	0.007	<0.001	<0.001	0.006	0.523	0.925	0.719	0.006	0.349	0.874
Quadratic		0.024	0.032	0.005	0.003	0.013	0.036	0.072	0.363	0.257	0.079	0.212
***E. coli* effect**												
Linear		0.677	0.525	0.489	<0.001	0.691	0.458	0.057	0.140	0.619	0.304	0.881
Quadratic		0.415	0.039	0.201	0.333	0.012	0.784	0.421	0.853	0.954	0.857	0.534
Cubic		0.725	0.834	0.055	0.003	0.211	0.984	0.138	0.421	0.058	0.196	0.436
Ration × Dose		0.058	0.689	0.320	0.005	0.063	0.507	0.039	0.699	0.070	0.518	0.742

[a] SH 75, soybean hulls were included at 75 g/kg DM of total mixed ration; SH 150, soybean hulls were included at 150 g/kg DM of total mixed ration.
[b] DMD is the dry matter degradability (g degraded/g incubated).
[c] *b* is the asymptotic gas production (mL/g DM); *c* is the rate of gas production (h^{-1}); *Lag* is the initial delay before gas production begins (h).
[d] *b* is the asymptotic methane production (mL/g DM); *c* is the rate of methane production (h^{-1}); *L* is the initial delay before methane production begins (h).
[e] *b* is the asymptotic carbon dioxide production (mL/g DM); *c* is the rate of carbon dioxide production (h^{-1}); *L* is the initial delay before carbon dioxide production begins (h).
[f] SEM standard error of the mean.

($P = 0.02$) on pH but *E. coli* inclusion at different doses produced no effects on pH. The control had the highest dry matter degradability (DMD) of 572 mg/g DM at 40 μL/g DM *E. coli* while the lowest DMD of 497 mg/g DM was recorded at SH 75 ration. The SH ration had decreased linear and quadratic effects ($P < 0.05$) on DMD. *E. coli* showed a quadratic effect ($P < 0.05$) on DMD, however no ration and dose dependent effects were observed on DMD (Table 2).

3.2. CH_4 and CO_2 productions

The SH ration had quadratic effects ($P = 0.04$) on asymptotic CH_4 production. *E. coli* at different doses had no linear, quadratic or cubic effects on asymptotic CH_4 production. Interactions between SH ration and *E. coli* doses were observed ($P < 0.04$). No linear or quadratic effects were observed with the inclusion of SH ration and *E. coli* doses on the rate or initial delay before CH_4 productions. The SH ration had quadratic effects ($P < 0.05$) for 24 h and 48 h incubation time on mL/g incubated DM and mL/g degraded DM of CH_4 production (Fig. 3a–c). The SH ration had linear effects at 24 h ($P = 0.018$) and 48 h ($P = 0.027$) of incubation during proportional CH_4 production. SH ration and *E. coli* dose interaction occurred at 8 h incubation ($P = 0.010$) (Table 3).

The SH ration produced linear effects ($P = 0.006$) on asymptotic CO_2 production. The control had the highest asymptotic CO_2 production of 219 mL/g DM at 20 μL/g DM *E. coli* inclusion while SH 150 had the lowest asymptotic CO_2 production of 81 mL/g DM at 40 μL/g DM *E. coli* inclusion. Addition of SH ration had no linear or quadratic effects on the rate of CO_2 production. No effect of SH rations and *E. coli* doses interactions was observed on the rate of CO_2 production and initial delay before CO_2 production began. However, in the CO_2 production of mL/g incubated DM, linear and quadratic effects were observed ($P < 0.05$) on the addition of SH rations at 8 h, 24 h and 48 h incubation time (Fig. 4a–c). The SH ration had quadratic effect ($P < 0.05$) at all incubation time but had linear effects ($P < 0.05$) at 24 and 48 h incubation time of mL/g degraded DM of CO_2 production. No linear, quadratic or cubic effects were noted on the inclusion of different *E. coli* doses for mL/g incubated DM, mL/g degraded DM and proportional CO_2 production (Table 4).

4. Discussion

Worldwide, agricultural by-product is one of the major contributors of GHGs such as CH_4, CO_2, and NO_2, of which livestock production accounts for the two-thirds of the direct emissions (Slade et al., 2016). Enteric fermentation from ruminants digestive system produces CH_4 and CO_2 that contribute significantly to environmental degradation and pollution attributed to human activity. One of the greatest challenges that animal nutritionists are facing today is how to reduce greenhouse gas production in livestock farming for cleaner environmental production through feed formulation. Mitigating these gases (CH_4 and CO_2) from ruminant production will not only decrease greenhouse gases from agricultural by-products but also will significantly reduce the net feed energy loss and improve animal production (Eckard et al., 2010; Hernández et al., 2017). This present study used *in vitro* GP technique a simple, sensitive and powerful method to evaluate *in vitro* gas production, rumen fermentation kinetics (Salem et al., 2014a; b) and efficacy of feed utilization.

4.1. Gas production and fermentation

Generally, the level of digestibility and fermentation of ruminant feeds are measured by gas released in *in vitro* rumen fermentation kinetics. Higher GP indicates higher digestibility, better fermentability and increase in microbial protein production which result in better accessibility of nutrients to rumen microorganisms (Getachew et al., 2004). Inclusion of SH as a replacement for CG, linearly and quadratically decreased asymptotic GP, indicating decreased fermentability and digestibility of the diets (Fig. 2a–c). This finding is not in consonance with the report of

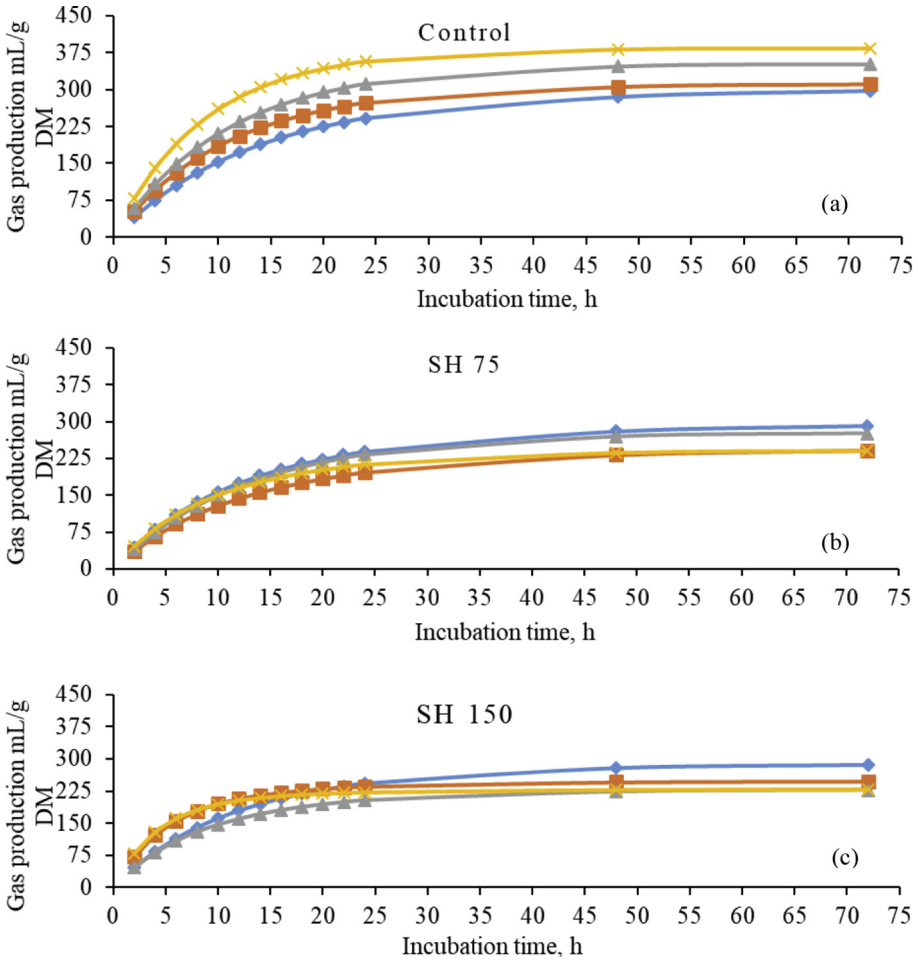

Fig. 2. a-c. *In vitro* rumen gas production (mL/g incubated DM) of three different levels of soybean hulls as affected by different levels of *E. coli* at 0 (-◆-), 10 (-■-), 20 (-▲-) and 40 (-X-) μL/g DM. Control: corn grain was replaced by soybean hulls at 0 g/kg DM; SH 75: soybean hulls were included at 75 g/kg DM of total mixed ration; SH 150: soybean hulls were included at 150 g/kg DM of total mixed ration.

(Elghandour et al., 2015, 2016b), who observed significant increase in asymptotic GP with increasing level of prickly pear cactus flour replacement with corn grain. They reported that the observed increase in GP may be due to the availability of fermentable carbohydrate which has the tendency to promote microbial growth (Forsberg et al., 2000). Guerrero et al. (2012) opined that increase in GP improves availability of nitrogen and promote higher nutrients to rumen microbes. *E. coli* inclusion did not affect asymptotic GP. The inability of *E. coli* doses to positively influence the asymptotic GP suggests a decreased availability of carbohydrate for the microbial growth. This result agrees with the findings of Elghandour et al. (2016a) who reported that inclusion of organic acid salt on prickly pear cactus flour had no effect on the asymptotic GP. Ruiz et al. (2013) reported that inclusion of fibrolytic enzymes did not affect GP. This result suggests that replacement of SH with CG lack the propensity to improve ruminant feed intake and performance. The control rations had a lower lag phase when compared to the SH ration. This agrees with the report of (Ferraro et al., 2016) suggesting a faster adaptation of the microbes to the CG ration indicating its availability to provide more proportion of nutrient to the ruminant animals than the SH. However, the observed higher lag phase of 10 and 20 μL/g DM *E. coli* doses indicate delay in microbial degradation and adaptation of the rumen microbes to the diets. Ferraro et al. (2016) reported that consistent feeding of sheep with diets supplemented with energy additives improves microbial adaptability and reduces lag phase. This is in agreement with the report of Elghandour et al. (2016a) but in contrast to the findings of Rodriguez et al. (2015) that *Saccharomyces cerevisiae* reduced lag time. Elghandour et al. (2015) reported that fibrolytic enzymes have no influence on lag time of GP. Furthermore, the decrease in GP when SH replaced CG did not enhance nutrient availability to the rumen microbes. No ration and dose dependent interactions occurred indicating that there was no observable synergy between CG replacements with SH and *E. coli* doses on asymptotic GP.

4.2. CH_4 and CO_2 productions

The SH ration had quadratic effects on asymptotic CH_4. *E. coli* at different doses had no effect on asymptotic CH_4 production as shown in Fig. 3a–c. No ration and dose dependent interactions occurred on asymptotic CH_4 production. However, the control had the highest asymptotic CH_4 production of 85 mL/g DM *E. coli* dose of 40 μL/g DM. The control also had the highest mL/g incubated DM and mL/g degraded DM of CH_4 at the same *E. coli* dose. The least asymptotic CH_4 production, mL/g incubated DM and mL/g degraded DM was observed at the dose of 10 μL/g DM of SH 75 ration. This result is similar to the findings of Elghandour et al. (2016a) who reported that replacing CG with SH did not affect CH_4 production. In their work, Elghandour et al. (2016a) reported that addition of organic acid salts doses had no effect on mitigation

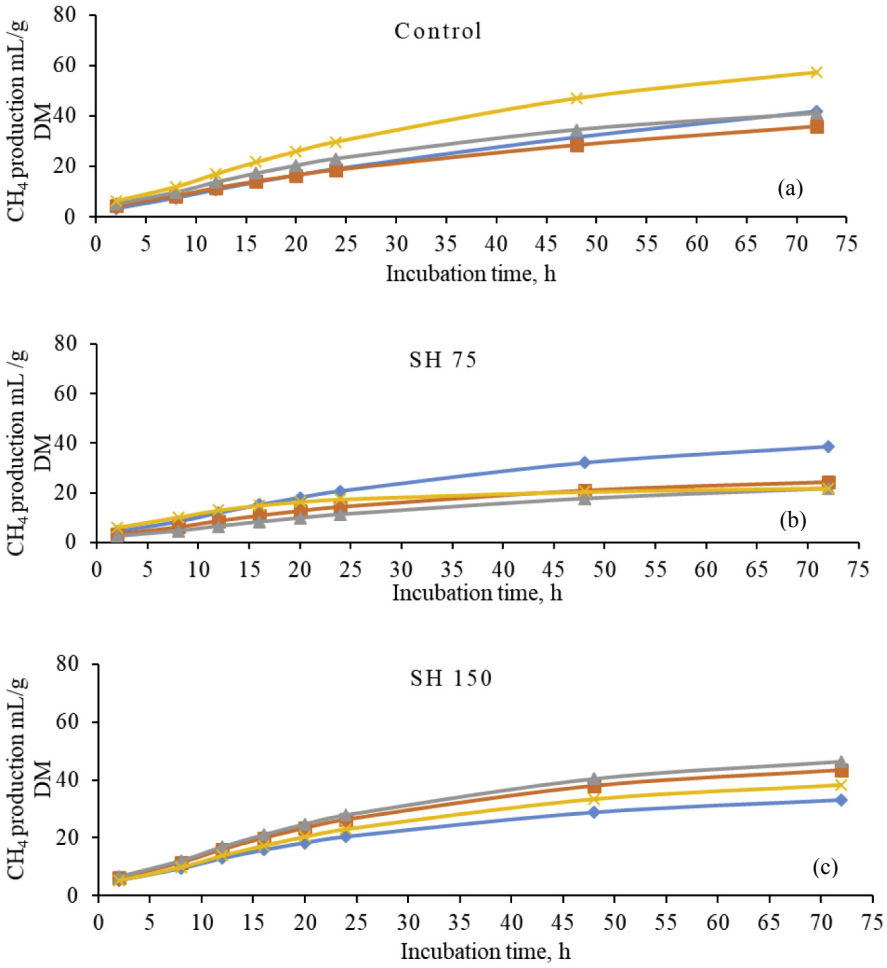

Fig. 3. a-c. *In vitro* CH_4 production (mL/g incubated DM) of three different levels of soybean hulls (SH) as influenced by different doses of *E. coli* at 0 (-◆-), 10 (-■-), 20 (-▲-) and 40 (-X-) μL/g DM. Control: corn grain was replaced with soybean hulls at 0 g/kg DM; SH 75: soybean hulls were included at 75 g/kg DM of total mixed ration; SH 150: soybean hulls were included at 150 g/kg DM of total mixed ration.

Table 3
Proportional *in vitro* CH_4 production as a percent of total gas production of three different levels of soybean hulls (SH) as influenced by different doses of *E. coli* (μL/g DM).

Ration[a]	*E. coli*	CH_4 production								
		mL/g incubated DM			mL/g degraded DM			Proportional CH_4 production		
		8 h	24 h	48 h	8 h	24 h	48 h	8 h	24 h	48 h
Control	0	7.23	16.50	23.29	13.60	30.66	43.27	3.99	5.09	6.35
	10	8.57	18.41	26.99	15.50	33.15	48.37	5.04	7.05	9.97
	20	10.60	25.35	37.55	18.79	44.87	66.39	5.63	7.62	9.97
	40	12.91	33.13	53.29	23.02	59.06	95.00	5.13	8.61	13.11
SH75	0	6.60	16.19	24.72	13.64	33.55	51.44	5.84	8.09	10.92
	10	5.13	11.14	15.23	9.60	20.85	28.53	5.32	5.82	6.40
	20	3.70	9.62	15.92	6.92	17.97	29.68	2.72	3.87	5.55
	40	13.10	20.58	21.95	24.52	38.51	41.09	12.01	10.97	10.46
SH150	0	9.01	21.91	33.02	17.62	42.82	64.56	6.26	8.35	10.84
	10	12.46	29.36	42.65	24.41	57.55	83.59	6.81	13.45	19.44
	20	12.22	28.62	41.64	23.26	54.48	79.27	9.35	13.92	19.18
	40	8.77	20.66	30.06	17.22	40.59	59.08	4.28	8.68	12.55
Pooled SEM[b]		2.932	6.411	9.502	5.310	11.721	17.230	1.381	2.063	3.161
Ration effect										
Linear		0.713	0.700	0.821	0.451	0.419	0.506	0.103	0.018	0.027
Quadratic		0.103	0.027	0.015	0.114	0.030	0.016	0.451	0.158	0.045
***E. coli* effect**										
Linear		0.112	0.210	0.270	0.143	0.271	0.361	0.127	0.260	0.409
Quadratic		0.779	0.945	0.975	0.679	0.830	0.904	0.756	0.714	0.534
Cubic		0.762	0.998	0.868	0.740	0.980	0.874	0.866	0.573	0.610
Ration × Dose		0.397	0.496	0.410	0.379	0.469	0.376	0.010	0.129	0.254

[a] SH 75, soybean hulls were included at 75 g/kg DM of total mixed ration; SH 150, soybean hulls were included at 150 g/kg DM of total mixed ration.
[b] SEM standard error of the mean.

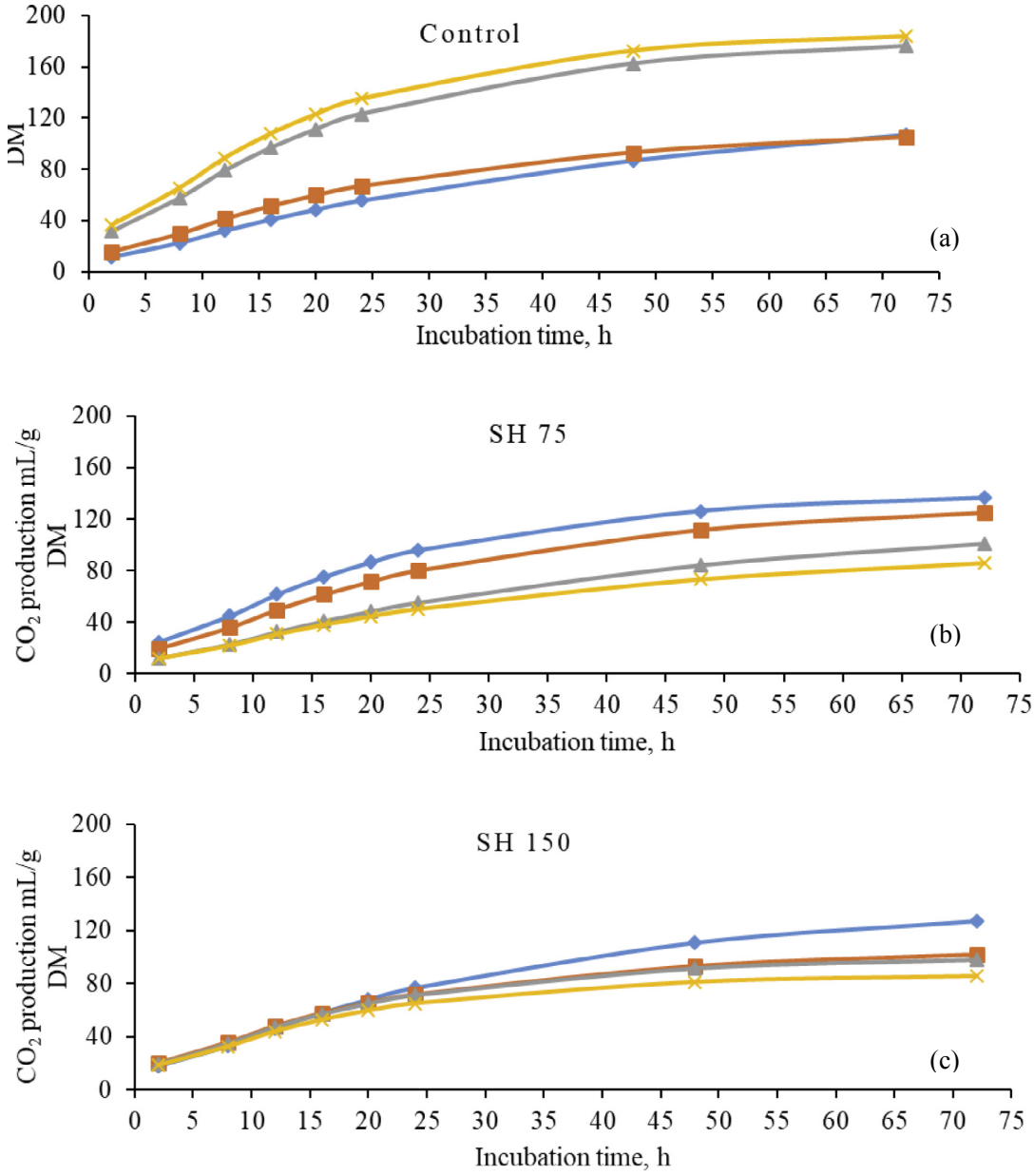

Fig. 4. a-c. *In vitro* CO_2 production (mL/g incubated DM) of three different levels of soybean hulls as influenced by different doses of *E. coli* at 0 (-◆-), 10 (-■-), 20 (-▲-) and 40 (-X-) μL/g DM. Control: corn grain was replaced with soybean hulls at 0 g/kg DM; SH 75: soybean hulls were included at 75 g/kg DM of total mixed ration; SH 150: soybean hulls were included at 150 g/kg DM of total mixed ration.

of CH_4. Elghandour et al. (2016b) reported that replacement of CG with prickly pear cactus flour, SH ration and organic acid salts dose dependent interaction did not influence CH_4 production. Colombatto et al. (2003) observed no influence of enzymes supplementation on CH_4 production while McGinn et al. (2004) reported similar findings in steers fed with barley silage-based diets supplemented with various feed additives. In contrast, Kholif et al. (2016) stated that the supplementation of exogenous enzymes at different doses decreased CH_4 production in ruminant diets. Polyorach et al. (2014) reported decreased in CH_4 production when ruminant animals were fed with *Saccharomyces cerevisiae* fermented cassava chip protein rather than soybean meal. They attributed the reduction in the formation of CH_4 to the ability of *Saccharomyces cerevisiae* to influence H_2 metabolism in the rumen by altering the *in vitro* fermentation. Sahoo and Jena (2014) opined that organic acid salts have the capacity to reduce methanogenesis via sinking of H_2 during propionate formation. This resulted in high fiber digestion due to stimulated cellulolytic bacteria. Santoso and Hariadi (2009) stated that the slower fermentation of carbohydrates ruminant feedstuffs may be associated with increase *in vitro* CH_4 production. Singh et al. (2012) reported that low protein content of feed diets may contribute to higher CH_4 production because of minimal H_2 and CO_2 production of protein. Moreover, they suggested that protein rich diets have limited potential for methanogenic when compared to carbohydrate diets.

In this present study, replacement of CG with SH has linear effects on asymptotic CO_2 production. No effects or ration and dose interactions were noted at different doses of *E. coli*. The SH 150 had the least asymptotic CO_2 production of 81 mL/g DM at 40 μL/g DM *E. coli* inclusion. This decrease in asymptotic CO_2 production may be

Table 4
Proportional *in vitro* CO_2 production as a percent of total gas production of three different levels of soybean hulls (SH) as influenced by different doses of *E. coli* (mg/g DM).

Ration[a]	*E. coli*	CO_2 production								
		mL/g incubated DM			mL/g degraded DM			Proportional CO_2 production		
		8 h	24 h	48 h	8 h	24 h	48 h	8 h	24 h	48 h
Control	0	29.67	71.19	106.41	55.13	132.29	197.74	16.27	22.07	29.14
	10	33.65	72.64	96.19	60.55	130.70	173.06	20.17	27.89	34.70
	20	71.56	151.25	197.18	127.38	268.83	349.95	38.06	45.66	52.62
	40	69.24	140.94	177.53	123.76	252.17	317.92	27.78	37.11	44.42
SH75	0	34.46	76.08	103.10	69.71	153.68	207.94	30.92	36.21	40.80
	10	31.03	72.42	104.75	58.10	135.63	196.26	32.60	37.90	43.89
	20	16.33	42.62	70.31	30.36	79.30	130.94	12.43	17.44	24.59
	40	21.55	47.74	67.60	40.28	89.34	126.69	19.79	25.48	31.40
SH150	0	38.66	89.93	129.54	75.70	176.14	253.83	27.25	33.82	40.95
	10	37.61	69.26	84.12	73.93	135.94	164.90	20.68	31.71	38.34
	20	34.20	70.81	90.26	65.06	134.72	171.73	25.18	32.68	38.88
	40	31.79	62.09	76.01	61.77	120.76	147.95	16.55	28.12	34.14
Pooled SEM[b]		9.502	16.923	20.114	17.031	29.224	33.913	5.404	5.721	5.633
Ration effect										
Linear		0.040	0.011	0.005	0.085	0.022	0.009	0.425	0.697	0.601
Quadratic		0.011	0.011	0.019	0.012	0.010	0.018	0.986	0.387	0.272
***E. coli* effect**										
Linear		0.328	0.540	0.963	0.448	0.742	0.743	0.439	0.833	0.858
Quadratic		0.798	0.870	0.958	0.958	0.908	0.766	0.629	0.643	0.572
Cubic		0.574	0.297	0.149	0.588	0.278	0.121	0.798	0.852	0.877
Ration × Dose		0.051	0.025	0.020	0.044	0.017	0.012	0.035	0.043	0.050

[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.

associated with reduced cell wall content which may lead to reduced microbial activities. The control produced more CO_2 in all the incubation time than any of the SH rations and this result is in agreement with the findings of Kholif et al. (2016), and similar finding was observed by Elghandour et al. (2016a). In their experiment, they reported a decrease in CO_2 production when CG was replaced with SH. In contrast, Elghandour et al. (2016b) reported that replacing CG with prickly pear cactus flour increased CO_2 production. Although both experiments used the same organic acid salts addition, the observed different effects may be due to ration dependent.

E. coli at different levels did not have any effect on the rate of CO_2 and initial delay before CO_2 productions began. This result is similar to the work of Elghandour et al. (2018) who reported that inclusion of prickly pear cactus flour rations and *E. coli* doses had no much influence on the rate of CO_2 and initial delay before CO_2 productions. However, SH ration affected the mL/g incubated DM and mL/g degraded DM of CO_2 production at all incubation time. The control had the highest mL/g incubated DM and mL/g degraded DM CO_2 production when compared with SH rations and *E. coli* doses, thus revealing the ability of SH ration and *E. coli* doses to decrease CO_2 production.

5. Conclusions

Ruminal fermentation is accompanied by large amount of CH_4 and CO_2 productions. Mitigation of these gases will significantly reduce net loss of feed energy and can lead to cleaner environmental production of livestock. This study demonstrated that SH ration and *E. coli* doses had no effect on the CH_4 production and therefore redundant in mitigation of CH_4, however, they have a decreasing effect on asymptotic GP. This study also established that inclusion of SH 150 ration at different *E. coli* doses reduced asymptotic CO_2 production which can be an environmental friendly way of feeding livestock. Further studies are required to evaluate the effectiveness of SH ration along with *E. coli* doses to improve the ruminal fermentation parameters.

References

Ahmed, M.H., Elghandour, M.M.Y., Salem, A.Z.M., Zeweil, H.S., Kholif, A.E., Klieve, A.V., Abdelrassol, A.M.A., 2015. Influence of *Trichoderma reesei* or *Saccharomyces cerevisiae* on performance, ruminal fermentation, carcass characteristics and blood biochemistry of lambs fed *Atriplex nummularia* and *Acacia saligna* mixture. Livest. Sci. 180, 90–97.

AOAC, 1997. Association of Official Analytical Chemists. Official Methods of Analysis, sixteenth ed. AOAC, Arlington, VA, USA.

Audsley, E., Wilkinson, M., 2014. What is the potential for reducing national greenhouse gas emissions from crop and livestock production systems? J. Clean. Prod. 73, 263–268.

Benchaar, C., Petit, H.V., Berthiaume, R., Ouellet, D.R., Chiquette, J., Chouinard, P.Y., 2007. Effects of essential oils on digestion, ruminal fermentation, rumen microbial populations, milk production, and milk composition in dairy cows fed alfalfa silage or corn silage. J. Dairy Sci. 90, 886–897.

Castillo, C., Benedito, J.L., Méndez, J., Pereira, V., López-Alonso, M., Miranda, M., Hernández, J., 2004. Organic acids as a substitute for monensin in diets for beef cattle. Anim. Feed Sci. Technol. 115, 101–116.

Colombatto, D., Hervás, G., Yang, W.Z., Beauchemin, K.A., 2003. Effects of enzyme supplementation of a total mixed ration on microbial fermentation in continuous culture, maintained at high and low pH. J. Anim. Sci. 81, 2617–2627.

Costa, S.B.M., Ferreira, M.A., Pessoa, R.A.S., Batista, A.M.V., Ramos, A.O., da Conceição, M.G., Gomes, L.H.S., 2012. Tifton hay, soybean hulls, and whole cottonseed as fiber source in spineless cactus diets for sheep. Trop. Anim. Health Prod. 44, 1993–2000.

Eckard, R.J., Grainger, C., De Klein, C.A.M., 2010. Options for the abatement of methane and nitrous oxide from ruminant production. Livest. Sci. 130, 47–56.

Elghandour, M.M.M.Y., Kholif, A.E., Marquez-Molina, O., Vazquez-Armijo, J.F., Puniya, A.K., Salem, A.Z.M., 2015. Influence of individual or mixed cellulase and xylanase mixture on *in vitro* rumen gas production kinetics of total mixed rations with different maize silage and concentrate ratios. Turk. J. Vet. Anim. Sci. 39 (4), 435–442.

Elghandour, M.M.Y., Kholif, A.E., Salem, A.Z.M., de Oca, R.M., Barbabosa, A., Mariezcurrena, M., Olafadehan, O.A., 2016a. Addressing sustainable ruminal methane and carbon dioxide emissions of soybean hulls by organic acid salts. J. Clean. Prod. 135, 194–200.

Elghandour, M.M.Y., Kholif, A.E., Salem, A.Z.M., Olafadehan, O.A., Kholif, A.M., 2016b. Sustainable anaerobic rumen methane and carbon dioxide productions from prickly pear cactus flour by organic acid salts addition. J. Clean. Prod. 139, 1362–1369.

Elghandour, M.M.Y., Kholif, A.E., Hernandez, A., Salem, A.Z.M., Mellado, M., Odongo, N.E., 2017. Effects of organic acid salts on ruminal biogas production and fermentation kinetics of total mixed rations with different maize silage to concentrate ratios. J. Clean. Prod. 147, 523–530.

Elghandour, M.M.Y., Khusro, A., Salem, A.Z.M., Mariezcurrena-Berasain, M.A., Díaz, L.M.C., Cipriano-Salazar, M., 2018. Role of dose dependent *Escherichia coli* as ruminal anti-microflora agent to mitigate biogases production in prickly pear cactus flour based diet. Microb. Pathog. 115, 208–215.

Esquivel-Velázquez, A., Kholif, A.E., Elghandour, M.M.Y., Salem, A.Z.M., Jiménez, R.M., Barbabosa-Pliego, A., Odongo, N., Bórquez, J.L., Cipriano, M., Olivares, J., 2016. Effect of partial replacement of steam rolled corn with soybean hulls or prickly pear cactus in the horse's diet in the presence of live *Saccharomyces cerevisiae* on *in vitro* fecal gas production. J. Equine Vet. Sci. 42, 94–101.

FAO, 2006. Livestock's Long Shadow, Environmental Issues and Options, Rome, Italy.

Ferraro, S., Mendoza, M., Miranda, L., Gutierrez, C.G., 2009. *In vitro* gas production and ruminal fermentation of glycerol, propylene glycol and molasses. Anim. Feed Sci. Technol. 154, 112–118.

Ferraro, S.M., Mendoza, G.D., Miranda, L.A., Gutiérrez, C.G., 2016. *In vitro* ruminal fermentation of glycerol, propylene glycol and molasses combined with forages and their effect on glucose and insulin blood plasma concentrations after an oral drench in sheep. Anim. Feed Sci. Technol. 123, 74–80.

Forsberg, C., Forano, E., Chesson, A., 2000. Microbial adherence to the plant cell wall and enzymatic hydrolysis. In: Cronje, P.B. (Ed.), Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction. CABI Publishing, Wallingford, UK, pp. 79–97.

France, J., Dijkstra, J., Dhanoa, M.S., Lopez, S., Bannink, A., 2000. Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed *in vitro*: derivation of models and other mathematical considerations. Br. J. Nutr. 83, 143–150.

Garnett, T., Appleby, M.C., Balmford, A., Bateman, I.J., Benton, T.G., Bloomer, P., Burlingame, B., Dawkins, M., Dolan, L., Fraser, D., Herrero, M., Hoffmann, I., Smith, P., Thornton, P.K., Toulmin, C., Vermeulen, S.J., Godfray, H.C.J., 2013. Sustainable intensification in agriculture: premises and policies. Science 341, 33–34.

Gerber, P.J., Steinfeld, H., Henderson, B., Mottet, A., Opio, C., Dijkman, J., Falcucci, A., Tempio, G., 2013. Tackling Climate Change through Livestock e a Global Assessment of Emissions and Mitigation Opportunities. FAO, Rome.

Getachew, G., DePeters, E.J., Robinson, P.H., 2004. *In vitro* gas production provides effective method for assessing ruminant feeds. Calif. Agric. 58, 1–12.

Goering, M.K., Van Soest, P.J., 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures and Some Applications). Agriculture Handbook, No 379. Agricultural Research Service, USDA, Washington, DC.

Guerrero, M., Cerrillo-Soto, M.A., Ramírez, R.G., Salem, A.Z.M., Gonzalez, H., Juarez-Reyes, A.S., 2012. Influence of polyethylene glycol on *in vitro* gas production profiles and microbial protein synthesis of some shrub species. Anim. Feed Sci. Technol. 176, 32–39.

Hernández, A., Kholif, A.E., Elghandour, M.M.M.Y., Camacho, L.M., Cipriano, M.M., Salem, A.Z.M., Cruz, H., Ugbogu, E.A., 2017. Effectiveness of xylanase and *Saccharomyces cerevisiae* as feed additives on gas emissions from agricultural calf farms. J. Clean. Prod. 148, 616–623.

Herrero, M., Thornton, P.K., Bernues, A., Baltenweck, I., Vervoort, J., Steeg, J., Makokha, S., Wijk, M.T., Karanja, S., Rufino, M.C., Staal, S.J., 2014. Exploring future changes in smallholder farming systems by linking socio-economic scenarios with regional and household models. Global Environ. Change 24, 165–182.

Hristov, A.N., Oh, J., Firkins, J.L., Dijkstra, J., Kebreab, E., Waghorn, G., Makkar, H.P.S., Adesogan, A., Yang, W., Lee, C., Gerber, P.J., Henderson, B., Tricarico, J., 2013. Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options. J. Anim. Sci. 91, 5045–5069.

Hristov, A.N., Oh, J., Giallongo, F., Frederick, T.W., Harper, M.T., Weeks, H.L., Branco, A.F., Moate, P.J., Deighton, M.H., Williams, S.R.O., Kindermann, M., Duval, S., 2015. An inhibitor persistently decreased enteric methane emission from dairy cows with no negative effect on milk production. Proc. Nat. Acad. Sci. USA 112 (34), 10663–10668.

Jiménez-Peralta, F.S., Salem, A.Z.M., Mejía-Hernández, P., González-Ronquillo, M., Albarrán-Portillo, B., Rojo-Rubio, R., Tinoco-Jaramillo, J.L., 2011. Influence of individual and mixed extracts of two tree species on *in vitro* gas production kinetics of high-concentrate diet fed to growing lambs. Livest. Sci. 136, 192–200.

Kholif, A.E., Khattab, H.M., El-Shewy, A.A., Salem, A.Z.M., Kholif, A.M., El-Sayed, M.M., Gado, H.M., Mariezcurrena, M.D., 2014. Nutrient digestibility, ruminal fermentation activities, serum parameters and milk production and composition of lactating goats fed diets containing rice straw treated with *Pleurotus ostreatus*. Asian-Australas. J. Anim. Sci. 27 (3), 357–364.

Kholif, A.E., Baza-García, L.A., Elghandour, M.M.Y., Salem, A.Z.M., Barbabosa, A., Dominguez-Vara, I.A., Sanchez-Torres, J.E., 2016. *In vitro* assessment of fecal inocula from horses fed on high-fiber diets with fibrolytic enzymes addition on gas, methane, and carbon dioxide productions as indicators of hindgut activity. J. Equine Vet. Sci. 39, 44–50.

Kholif, A.E., Elghandour, M.M.Y., Rodríguez, G.B., Olafadehan, O.A., Salem, A.Z.M., 2017a. Anaerobic ensiling of raw agricultural waste with a fibrolytic enzyme cocktail as a cleaner and sustainable biological product. J. Clean. Prod. 142 (1), 2649–2655.

Kholif, A., Elghandour, M., Salem, A., Barbabosa, A., Márquez, O., Odongo, N., 2017b. The effects of three total mixed rations with different concentrate to maize silage ratios and different levels of microalgae *Chlorella vulgaris* on *in vitro* total gas, methane and carbon dioxide production. J. Agric. Sci. 155 (3), 494–507.

McGinn, S.M., Beauchemin, K.A., Coates, T., Colombatto, D., 2004. Methane emissions from beef cattle: effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. J. Anim. Sci. 82, 3346–3356.

Mirzaei-Aghsaghali, A., Syadati, S.A., Fathi, H., Rasouli, S., Sadaghian, M., Tarahomi, M., 2012. Garlic in ruminants feeding. Asian J. Bio. Sci. 5, 328–340.

Morsy, T.A., Kholif, A.E., Kholif, S.M., Kholif, A.M., Sun, X., Salem, A.Z.M., 2016. Effects of two enzyme feed additives on digestion and milk production in lactating Egyptian buffaloes. Ann. Anim. Sci. 16, 209–222.

Newbold, C.J., Lopez, S., Nelson, N., Ouda, J.O., Wallace, R.J., Moss, A.R., 2005. Propionate precursors and other metabolic intermediates as possible alternative electron acceptors to methanogenesis in ruminal fermentation *in vitro*. Br. J. Nutr. 94, 27–35.

NRC, 2001. Nutrient Requirement of Dairy Cattle, 7th rev. ed. National Research Council, National Academy Press, Washington, DC, USA.

Patra, A.K., Kamra, D.N., Agarwal, N., 2006. Effects of plant extracts on *in vitro* methanogenesis, enzyme activities and fermentation of feed in rumen liquor of buffalo. Anim. Feed Sci. Technol. 128, 276–291.

Polyorach, S., Wanapat, M., Cherdthong, A., 2014. Influence of yeast fermented cassava chip protein (YEFECAP) and roughage to concentrate ratio on ruminal fermentation and microorganisms using *in vitro* gas production technique. Asian-Australas. J. Anim. Sci. 27, 36–45.

Rira, M., Chentli, A., Boufenera, S., Bousseboua, H., 2015. Effects of plants containing secondary metabolites on ruminal methanogenesis of sheep *in vitro*. Energy Procedia 74, 15–24.

Rodriguez, M.P., Mariezcurrena, M.D., Mariezcurrena, M.A., Lagunas, B.C., Elghandour, M.M.Y., Kholif, A.M., Kholif, A.E., Almaraz, E.M., Salem, A.Z.M., 2015. Influence of live cells or cells extract of *Saccharomyces cerevisiae* on *in vitro* gas production of a total mixed ration. Ital. J. Anim. Sci. 14, 590–595.

Ruiz, P.J.A., Moreno, A.J., Salem, A.Z.M., Castelán Ortega, O., González-Ronquillo, M., 2013. Chemical composition and *in vitro* gas production from different varieties of native and hybrid maize silage with the addition of acetic acid or enzymes. Anim. Nutr. Feed Technol. 13, 593–599.

Sahoo, A., Jena, B., 2014. Organic acids as rumen modifiers. Int. J. Sci. Res. 3 (11), 2262–2266.

Salem, A.Z.M., Kholif, A.E., Elghandour, M.M.Y., Hernandez, S.R., Domínguez-Vara, I.A., Mellado, M., 2014a. Effect of increasing levels of seven tree species extracts added to a high concentrate diet on *in vitro* rumen gas output. Anim. Sci. J. 85 (9), 853–860.

Salem, A.Z.M., Ryena, A.C., Elghandour, M.M.Y., Camacho, L.M., Kholif, A.E., Salazar, M.C., Domínguez, I.A., Jiménez, R.M., Almaraz, E.M., Martínez, A.G., Mariezcurrena, M.A., 2014b. Influence of *Salix babylonica* extract in combination or not with increasing levels of minerals mixture on *in vitro* rumen gas production kinetics of a total mixed ration. Ital. J. Anim. Sci. 13, 873–879.

Santoso, B., Hariadi, B.T., 2009. Evaluation of nutritive value and *in vitro* methane production of feedstuffs from agricultural and food industry by-products. J. Indones. Trop. Anim. Agric. 34, 189–195.

SAS, 2002. Statistical Analysis System. User's Guide: Statistics. SAS Institute, Cary, NC, Ver 9.0.

Singh, S., Kushwaha, B.P., Nag, S.K., Mishra, A.K., Singh, A., Anele, U.Y., 2012. *In vitro* ruminal fermentation, protein and carbohydrate fractionation, methane production and prediction of twelve commonly used Indian green forages. Anim. Feed Sci. Technol. 178, 2–11.

Slade, E.M., Riutta, T., Roslin, T., Tuomisto, H.L., 2016. The role of dung beetles in reducing greenhouse gas emissions from cattle farming. Sci. Rep. 6, 18140. https://doi.org/10.1038/srep18140.

Theodorou, M.K., Williams, B.A., Dhanoa, M.S., McAllan, A.B., France, J., 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim. Feed Sci. Technol. 48, 185–197.

Valdes, K.I., Salem, A.Z.M., López, S., Alonso, M.U., Rivero, N., Elghandour, M.M.Y., Domínguez, I.A., Ronquillo, M.G., Kholif, A.E., 2015. Influence of exogenous enzymes in presence of *Salix babylonica* extract on digestibility, microbial protein synthesis and performance of lambs fed maize silage. J. Agric. Sci. 153 (04), 732–742.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fibre, neutral detergent fibre, and non-starch carbohydrates in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.