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