Journal of Cleaner Production 135 (2016) 194–200

Contents lists available at ScienceDirect

Journal of Cleaner Production

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

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

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ARTICLE INFO

Article history:
Received 20 April 2016
Received in revised form
11 June 2016
Accepted 15 June 2016
Available online 17 June 2016

Keywords:
Corn grain
Greenhouse gases emission
Organic acid salts
Rumen fermentation
Soybean hulls

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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http://dx.doi.org/10.1016/j.jclepro.2016.06.081
0959-6526/

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