Small Ruminant Research 115 (2013) 15–20

Contents lists available at ScienceDirect

Small Ruminant Research

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

Long-term effects of ensiled cornstalk diet on methane emission, rumen fermentation, methanogenesis and weight gain in sheep

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ARTICLE INFO

Article history:
Received 30 January 2013
Received in revised form 15 July 2013
Accepted 22 July 2013
Available online 3 August 2013

Keywords:
Sheep
Ensiled cornstalk
Methane
VFA
Methanogen abundance

ABSTRACT

The objective of this study was to compare the long-term effects of diets rich in either ensiled or dry cornstalk at different concentrate-to-forage ratios on the methane production from sheep. A total of fifty-nine sheep (male, 25.06 ± 0.39 kg live weight at the beginning) were randomly divided into four groups with four diet treatments as follows: ensiled cornstalk diet at 40% (CSH) and 20% (CSL) concentrate level, dry cornstalk diet at 40% (DSH) and 20% (DSL) concentrate level. At different concentrate levels, the sheep fed ensiled cornstalk diet produced a lower methane output (L/d or L/kg DM) than the sheep fed dry cornstalk diet, and the difference was significant when 20% concentrate was added in the diet ($P < 0.05$). For the rumen fluid analysis, the VFAs concentration showed no difference between four groups, but the acetate: propionate ratio tended to decrease in the ensiled cornstalk-fed sheep in comparison to the dry cornstalk-fed sheep, especially significantly different in stage 1 and 3 ($P < 0.05$). The methanogen abundance in the ensiled cornstalk-fed sheep was shown to be significantly lower than the dry cornstalk-fed sheep at 20% concentrate level ($P < 0.05$). During the whole period, the weight gain was not significantly influenced ($P > 0.05$). This result showed that ensiled cornstalk as roughage added to their diet had the effect of reducing the methane emission from sheep when fed 20% concentrate, and the decrease in acetate: propionate ratio might cause the suppression of methanogenesis by depriving of the hydrogen utilized by methanogens to produce methane.

1. Introduction

With regard to the gradual increase in greenhouse gases (GHGs) and the effects on global warming, the methane produced by domesticated ruminants has been generally acknowledged to be one of largest sources of GHGs. It is reported that the annual global methane emission is approximately 500 Tg of which the discharge by ruminants is 65–85 Tg, accounting for 15–25% of the total emissions (Smith et al., 2007). The methane emissions from ruminants account for nearly 97% of the methane emissions from livestock worldwide (Johnson et al., 1991). Furthermore, approximately 2–15% of the gross energy is converted into methane, which reduces the efficiency of diet utilization (Johnson and Johnson, 1995; Van Nevel and Demeyer, 1996). Therefore, developing effective strategies to reduce methane emission and enhance the digestibility of the diets would improve the development of livestock husbandry.

Methane is produced by the methanogens that inhabit the rumen, and the emission volume is mainly influenced by the diet type, intake level, environmental temperature, chyme outflow rate and animal genotype (Johnson and Johnson, 1995). Decreasing methane emission from

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http://dx.doi.org/10.1016/j.smallrumres.2013.07.011

ruminants without altering animal production is desirable both as a strategy to reduce global greenhouse gas emissions and as a means of improving feed conversion efficiency (Martin et al., 2010). To date, many efforts have been made to control methane synthesis by means of additives, vaccines, microbial techniques or animal breeding (Boadi et al., 2004; Wright et al., 2004; Beauchemin et al., 2007; Tiemann et al., 2008; Morgavi et al., 2008; Wood et al., 2009). Besides, animal breeding and genotype selection are also proposed to be associated with animal methane mitigation (Alford et al., 2006; Chagunda et al., 2009; Amlan, 2011). Although these new procedures highlight the perspective to mitigate the emission of methane by ruminants, most of the techniques are not currently availableand are still in the investigative phase.

Ensiled diet is enriched with abundant nutrients, easy to be digested and efficient for methane reduction for ruminants. Increasing concentrate proportion has the same effect, but excessive addition can cause acidosis and more cost (Commun et al., 2009). The regulation of concentrate-to-forage ratio and roughage selection is both important for assessing nutrient conditions and controlling the output of methane. Thus, the optimal and effective utilization of crop stalk for feed is important to solve the problem of enteric methane production and increase digestibility. In this study, we performed a long-term investigation to compare the effect of concentrate-to-forage ratio and roughage types on the methane production, fermentation, methanogen population and live weight gain in sheep, with the goal of providing basic information for the further mitigation of methane from ruminants.

2. Materials and methods

2.1. Animals, diets and management

This study took place over a 6-month period from October 2010 to April 2011. Fifty-nine healthy sheep (males, six month old), with an average weight of 25.06 ± 0.39 kg were selected as the experimental animals. All of the sheep were randomly allocated to four groups and fed cornstalk silage or dry cornstalk of different concentrate-to-forage ratios for six months. Each sheep was housed in a single pen and DM intake was calculated for each individual. This study was divided into three stages, and each stage lasted for about 20 days. It took 12 days for the animals to be adapted, and 7 days to collect experimental data. As shown in Table 1, the diet treatments were as follows: ensiled cornstalk diet at 40% (CSH) and 20% (CSL) concentrate level, dry cornstalk diet at 40%(DSH) and 20% (DSL) concentrate level. The ensiled cornstalk was placed in a baler and wrapped into round bales. The plastic membrane prohibited the cornstalk from the air and maintained anaerobic environment. All the round bales were placed in a workplace installed with air conditionings for long-term storage. All of the sheep had free access to food and water.

2.2. Gas collection and methane measurement

The SF_6 tracer technique was used for the methane emission measurements, according to the method described in previous reports (Boadi et al., 2002; Lassey, 2008). One week before the first methane gas collection, brass permeation tube containing at1.2 g of SF_6 and with calculated release rate (approx. 2.3 mg per day) were placed in the rumen. Each animal was simultaneously trained to adapt to the gas collection device affixed to its back after the SF_6 permeation tube was placed in its rumen. The gas collection was conducted after the tracer gas had equilibrated in the rumen for one week. The exhaled gas from the nose and mouth was drawn into pre-evacuated (−0.1 MPa) stainless steel collection canisters (2.5 L) through 1.2 m capillary tubing with a filter and flexible nosepiece fitted to a halter (Boadi et al., 2002).

Table 1
Ingredients and composition of the experimental diets (DM basis).

Item	Treatment			
	CSH	CSL	DSH	DSL
Ingredients,%				
Ensiledcornstalk	42.0	50.8		
Dry cornstalk			33.0	40.0
Alfalfa	18.0	29.2	27.0	40.0
Premix	0.5	0.2	0.5	0.2
Concentrate, %				
Corn grain	22.9	11.5	22.9	11.5
Wheat bran	8.0	4.0	8.0	4.0
Sunflower seed	4.1	2.0	4.1	2.0
Cotton seed	4.1	2.1	4.1	2.1
Salts	0.5	0.2	0.5	0.2
Composition, %				
DM	47.8	45.9	82.0	81.3
NDF	67.3	62.5	68.0	62.2
ADF	30.0	34.4	30.7	32.2
EE	1.8	1.7	1.4	1.5
CP	10.9	9.7	11.3	11.1
ME (Mcal/kg)	7.13	7.07	6.06	6.04

[a]Premix supplied per kilogram of supplement: 15,000 IU of vitamin A; 5000 IU of vitamin D_2; 50 mg of vitamin E; 9 mg of Fe/kg; 12.5 mg of Cu/kg; 130 mg of Mn/kg; 100 mg of Zn/kg; 0.3 mg of Se/kg; 1.5 mg of I/kg; and 0.5 mg of Co/kg.
[b]CSH, ensiled cornstalk diet composed of 40% concentrate; CSL, ensiled cornstalk diet composed of 20% concentrate; DSH, dry cornstalk diet composed of 40% concentrate; DSL, dry cornstalk diet composed of 20% concentrate.

Over the course of the feeding experiment, the gas samples were collected on seven consecutive days. Background air samples were also collected on each day using the same collection apparatus. After a 24 h period of collection, the pressure in the collection devices was tested to identify the efficiency of the gas collection, and the collection devices were then immediately pressurized to 110 kPa with pure nitrogen (99.9999%). When the gases were completely mixed in the collection devices, the gases were transferred to collecting bags for further analysis. All of the gas samples were analyzed in less than three days.

The daily methane production from each animal was calculated according to a described method (Johnson and Johnson, 1995) using the known permeation rate of SF_6 and the concentrations of SF_6 and methane in the breath samples, as follows:CH_4(L/d) = SF_6 permeation rate (L/d) × [CH_4]/[SF_6], L/d meant emission volume per day. The concentrations of the gas samples were determined using the Shimadzu GC-14B gas chromatograph instrument (Shimadzu, Japan), with an electron capture detector (ECD) for the SF_6 concentration and a flame ionization detector (FID) for the methane concentration under the following chromatographic conditions: oven temperature of 80 °C, column temperature of 100 °C, detector temperature of 200 °C, air flow rate of 50 mL/s, hydrogen flow rate of 60 mL/s, nitrogen flow rate of 270 mL/s and sample volume of 1 mL. The concentration of standard SF_6 gas was 10.4×10^{-12} (V/V). The standard concentration of methane gas was 24.9×10^{-6} (V/V) (National Standard Material Center, Beijing, China).

2.3. Ruminal fluid sampling and fermentation parameters

Approximately 100 mL of fluid was aspirated using a flexible plastic tube, with the first 50 mL of the fluid discarded to avoid the contamination of saliva. The remaining sample was strained through four layers of cheesecloth and transferred to sterile 50 mL containers. The pH of the ruminal fluid samples was immediately measured using a portable pH-meter (IQ150, America), which was calibrated with pH 4.0 and pH 7.0 buffer solutions. The ruminal fluid was prepared for VFA and ammonia-N analyses by centrifuging at 12,000 × *g* for 5 min, and the supernatant was transferredto 2 mL tubes and then stored at −20 °C.

The VFA concentration was analyzed using the method described by Hoskin et al. (1995). The frozen ruminal fluid samples were thawed at room temperature, and 25% metaphosphoric acid solution was added at