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Forage and Flax Seed Impact on Enteric Methane Emission in Dairy Cows

[1,4]V. Sejian, [2]J. Lakritz, [3]T. Ezeji and [4]R. Lal

[1]Division of Physiology and Biochemistry, Central Sheep and Wool Research Insitute, Avikanagar, Rajasthan-304501, India

[2]Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH 43210, Columbs, USA

[3]Department of Animal Sciences, The Ohio State University and OARDC, Wooster, OH-44691, USA

[4]Carbon Management and Sequestration Centre, The School of Environment and Natural Resources, The Ohio State University, OH-43210, USA

Corresponding Author: Veerasamy Sejian, Adaptation Physiology Laboratory, Division of Physiology and Biochemistry, Central Sheep and Wool Research Institute, Avikanagar, Via-Jaipur, Rajasthan, India Tel: +91-9828624721 Fax: +91-01437-220163

ABSTRACT

Methane (CH_4) is a potent greenhouse gas (GHG) and its release into the atmosphere is directly linked with animal agriculture, particularly ruminant production. The primary objective of the study is to identify whether high quality forage and flax seed feeding can reduce enteric CH_4 emission in non-lactating dairy cattle. Two 8 year old Jersey cows weighing 545 kg were used in the present study. The animals were fed with a total mixed ration (corn silage, alfalfa hay and grain mixture) with the proportion consisting of 70% roughage and 30% grains. The flax seed were provided of 7% added fat on DM basis. In both experiments, gas samples were collected through the rumen fistula. Gaseous samples were obtained at 6:00, 8:30, 10:30, 12:30, 13:30, 15:30 and 17:30 h. The results of the experiments showed that both high proportion forage feeding and flax seed supplement reduced the enteric CH_4 emission. The highest CH_4 emission % after forage feeding was recorded at 15:30 h and the lowest at 6:30 h. Flax seed feeding started reducing CH_4 from 10:30 h onwards and this reduction continued until end of sampling period. The data presented show that both high quality forage and flax seed supplement reduced enteric CH_4 production. However, detailed studies are required to identify the exact quantity of flax seed to be fed to avoid its negative influence on production efficiency of the cow.

Key words: Methane, carbon dioxide, forage feeding, flax seed, enteric fermentation

INTRODUCTION

The global release of methane (CH_4) from agricultural sources accounts for two-thirds of the anthropogenic CH_4 sources (Moss *et al.*, 2000). These sources include rice (*Oryza sativa*) cultivation, enteric fermentation, biomass burning and animal wastes. Being a potent greenhouse gas (GHG), CH_4 release into the atmosphere is directly linked with animal agriculture, particularly ruminant production (Beauchemin and McGinn, 2005). Livestock, produced throughout the world, are an important agricultural product in virtually every country. Ruminant animals (particularly cattle, buffalo, sheep, goat and camels) produce significant amounts of CH_4 under the anaerobic conditions of the digestive processes. This microbial fermentation process, referred to as 'enteric fermentation', produces CH_4 as a byproduct which is released mainly through eructation and normal respiration and small quantities as flatus (Lassey, 2007; Chhabra *et al.*, 2009).

Several factors play a major role in enteric fermentation and in controlling the overall CH_4 emission from livestock. There is an urgent need to understand factors affecting enteric CH_4 production, variables that reduce the uncertainty in GHG emission inventories and strategies which reduce emission (Beauchemin and McGinn, 2005). The composition of diet fed to the livestock is an important factor which influences CH_4 emission (McCrabb *et al.*, 1997; Yurtseven and Irfan, 2009), especially from enteric fermentation in lactating dairy cows. The proportion of forage in livestock diet and the source of grain influence enteric CH_4 production by ruminants (Beauchemin and McGinn, 2005; Yurtseven *et al.*, 2009). There are several feed supplements which alter significantly the level of CH_4 emission (Leng, 1991; Moss *et al.*, 1995). But additional feed supplement should be used with caution as they can reduce animal productivity when the wrong dosage is used (Beauchemin and McGinn, 2006; Foley *et al.*, 2009).

Dietary fat aleters the ruminal microbial ecosystem and, in particular, the competition for metabolic hydrogen between the CH_4 and propionate production pathways (Fonty and Morvan, 1996; Giger-Reverdina *et al.*, 2003). In addition, fatty feeds seem to be the most promising dietary alternative to synthetic CH_4 inhibitors (Jouany, 1994; McAllister *et al.*, 1996; Spears, 1996). Supplementation of diets with lipids that are not protected from ruminal digestion is one strategy recognized to lower enteric CH_4 emissions (Boadi *et al.*, 2004; Monteny *et al.*, 2006). However there are conflicting reports on the effect of fat supplementation on CH_4 production (Johnson *et al.*, 2002; Woodward *et al.*, 2006; Eugene *et al.*, 2008; Martin *et al.*, 2008).

Understanding the relationship of diet to enteric CH_4 production is essential to reducing uncertainty in GHG emission inventories and to identifying viable reduction strategies. Hence, the present study was undertaken to establish the effect of forage and flax seed feeding on enteric CH_4 emission. The primary objective of the study is to identify whether high quality forage and flax seed feeding can reduce enteric CH_4 emission in dairy cattle.

MATERIALS AND METHODS

Site of study: The experiment was conducted in May-June 2010 at the Ohio State University veterinary hospital, which is located at Columbus (40°02"00"N, 83°02"30"W), Ohio. The mean annual precipitation is 1016 mm and means annual air temperature is 11°C. The average annual precipitation, relative humidity, wind velocity and solar radiation are 77.54 cm, 58.25%, 9.5 MPH and 306, respectively.

Animals: Two 8 year old Jersey cows weighing around 545 kg were used in the present study. The animals were housed in well-ventilated free stall barn. The barn was maintained under proper hygienic conditions. The animals were fed twice daily at 6:00 and 16:00 and had *ad libitum* access good quality drinking water.

Feeding pattern: The animals were completely stall fed twice daily (6:00 and 15:00) without allowing for grazing. In the first experiment, animals were fed with a Total Mixed Ration (TMR) consisting of 70% roughage and 30% grains. The composition of forage used is corn (*Zea mays* L.) silage and alfalfa (*Medicago sativa* L.) hay. In the second experiment, the same feeding schedule was followed in addition to flax (*Linum usitatissimum* L.) seed, provided @ of 7% added fat on DM basis. The whole flax seed were mixed with TMR and fed to the cows. This respective feeding schedule was followed for the entire duration of both the experiment.

Experimental design: Both experiments were carried out for a period of 10 days each. In the first experiment, the animals were fed with high quality forage. In the second experiment, apart from the feeding schedule followed in the first experiment, animals were fed additionally flax seed @ 7% of total feed intake. In both experiments, gas samples were collected through the rumen fistula. Gaseous samples were collected at 6:00, 8:30, 10:30, 12:30, 13:30, 15:30 and 17:30 h. The sample at 6:00 h is prior to feeding while rest all samples are after feeding.

Gas collection: The rumen of the animals was fistulated from the left paralumbar fossa. A 15 mL syringe attached with a stopper needle was used for the gas collection. The needle was inserted into the fistula and a representative gas sample of 10 mL was withdrawn from the fistula and stored in the evacuated 15 mL glass vial. After collection, the gas vials were kept stored under dark pending analysis.

Gas analysis: Gaseous samples from both the experiments were analyzed using gas chromatography (GC System, model No. 7890A; Agilent Technologies). A volume of 30 μL was injected into the GC system and the reading time for each sample was set at 3.5 min. The volume of air, CH_4 and CO_2 were recorded for each sample. Gaseous concentrations were determined from peak areas and identified from their different retention times relative to the known standards.

Statistical analysis: The data generated from this study was statistically analyzed by paired t-test using SAS software. The level of significance was set at $p \leq 0.05$.

RESULTS

Forage feeding on enteric CH_4 emission: Table 1 depicts the effect of forage feeding on CH_4 emission at regular interval in a day. A clear trend was established in both animals for CH_4 emission over different time of gas collection in a day. There is a gradual increase in CH_4 emission after forage feeding from morning towards noon. At 12:00 h there was a reduction in emission in

Table 1: Effect of forage feeding on enteric CH_4 and CO_2 emission of dairy cows

	Time of sample collection						
Enteric emission	6:00	8:30	10:30	12:30	13:30	15:30	17:30
CH_4 (%)							
Animal 1	10.29 ± 2.34^a	13.77 ± 4.11^a	17.80 ± 2.54^b	8.61 ± 3.52^c	14.75 ± 1.99^a	18.25 ± 2.58^d	15.61 ± 1.27^e
Animal 2	14.95 ± 2.09^a	15.87 ± 1.22^a	13.15 ± 2.57^a	12.11 ± 3.46^a	15.80 ± 1.22^a	16.27 ± 1.50^a	15.01 ± 1.96^a
Overall	12.62 ± 1.67^a	14.82 ± 2.05^{ab}	15.48 ± 1.71^{ab}	10.36 ± 2.40^{ab}	15.28 ± 1.12^{ab}	17.26 ± 1.55^b	15.31 ± 1.10^{ab}
CO_2 (%)							
Animal 1	19.2 ± 4.40^a	32.16 ± 3.51^b	38.69 ± 2.95^c	22.98 ± 1.07^a	24.91 ± 5.59^a	38.64 ± 4.25^d	35.35 ± 1.16^e
Animal 2	20.02 ± 1.69^a	32.52 ± 2.81^b	30.31 ± 2.75^c	24.15 ± 1.42^a	27.97 ± 0.83^d	35.70 ± 3.14^e	28.73 ± 1.60^f
Overall	19.70 ± 2.70^a	32.34 ± 2.12^b	34.50 ± 2.36^c	23.56 ± 0.86^d	26.44 ± 2.71^a	37.17 ± 2.54^e	32.04 ± 1.45^f

The values are averages of five day samples collected at a particular time. The values bearing different superscript within a row as compared to 6:00 h value differs significantly at $p \leq 0.05$

Table 2: Effect of flax seed feeding on enteric CH_4 and CO_2 emission of dairy cows

	Time of sample collection						
Enteric emission	6:00	8:30	10:30	12:30	13:30	15:30	17:30
CH_4 (%)							
Animal 1	12.71±5.00[a]	20.12±4.52[b]	21.75±2.43[c]	20.77±3.24[d]	20.28±3.18[e]	16.18±3.72[a]	15.01±3.86[a]
Animal 2	10.75±5.70[a]	23.86±2.61[b]	23.17±1.85[c]	16.87±4.41[a]	20.09±1.72[d]	20.41±1.35[e]	16.50±1.71[a]
Overall	11.73±3.82[a]	21.99±2.54[b]	22.46±1.46[c]	18.82±2.66[a]	20.19±1.72[d]	18.29±1.99[a]	15.76±2.01[a]
CO_2 (%)							
Animal 1	54.90±9.10[a]	45.48±9.65[a]	50.55±5.42[a]	42.34±6.92[b]	49.33±5.43[a]	39.23±9.27[c]	35.33±8.81[d]
Animal 2	37.61±9.95[a]	53.32±6.57[b]	51.35±5.30[c]	41.55±9.67[a]	45.84±6.36[a]	44.27±7.50[a]	40.74±5.37[a]
Overall	46.26±8.86[a]	49.41±5.66[a]	50.95±3.57[a]	41.95±5.60[a]	47.59±3.98[a]	41.75±7.22[a]	38.04±6.51[a]

The values are averages of five day samples collected at a particular time. The values bearing different superscript within a row as compared to 6:00 h value differs significantly at $p \le 0.05$

both animals and then again these values increased from 13:30 h. However this effect was significant ($p \le 0.05$) only in animal 1. The highest CH_4 emission recorded in both the animals is at 15:30 while the lowest at 6:30 h. A similar trend as that of individual basis was established for the overall average of these animals.

Forage feeding on enteric CO_2 emission: Table 1 depicts the effect of forage feeding on CO_2 emission at regular interval in a day. A similar trend as that of CH_4 was established in the animals for CO_2 emission after forage feeding in the present study. There is a gradual increase in CH_4 emission after forage feeding from morning towards noon. At 12:00 h there was a reduction in emission in both animals and then again these values increased from 13:30 h. This trend is statistically significant ($p \le 0.05$) in both animals as well as their overall average. The highest CO_2 was established at 15:30 h while the lowest at 6:00 h.

Flax seed feeding on enteric CH_4 emission: Table 2 depicts the effect of flax seed feeding on CH_4 emission at regular interval in a day. After flax seed feeding, initially, there was an increase in CH_4 until 10:00 h and after that it decreased gradually. This trend was similar in both the animals. The lowest emission rate was recorded at 17:30 h. The overall average also showed similar pattern as that of individual basis. This effect of flax seed feeding on CH_4 emission is statistically ($p \le 0.05$) significant at 8:30, 10:30 and 13:30 h, respectively.

Flax seed feeding on enteric CO_2 emission: Table 2 depicts the effect of flax seed feeding on CO_2 emission at regular interval in a day. Initially after feeding from 6:30 to 12:30 there is a gradual increase in CO_2 emission in the experimental animals. Flax seed feeding had reducing trend on CO_2 emission. However this effect is not statistically significant. This reduction is evident after 12:30 onwards. This trend was similar in both the animals as well as their overall averages.

DISCUSSION

In ruminants the effect of feed composition is much higher. CH_4 emission decreases when feeding level increases or when digestibility of the ration is improved. It is a general finding that improving the quality of forage and feed resources improves nutritional value and results in more productive animals. Improved nutrition reduces CH_4 emissions per unit product by optimizing animal performance factors and converting more food energy to beneficial activities, which include weight gain, milk production, work production and reproductive performance. Increased digestibility of feed also reduces CH_4 emissions because more food energy is used by the animal and less is used to produce CH_4 (O'Mara, 2004).

The forage feeding in the present experiment reduced the enteric CH_4 emission. There are several reports which suggests improved forage quality reducing enteric CH_4 emission (Johnson and Johnson, 1995; Boadi and Wittenberg, 2002; Kulling *et al.*, 2002; Hindrichsen *et al.*, 2004). It has also been established that high forage: grain ratio in the ration lowers enteric CH_4 emissions (Boadi *et al.*, 2002). The observed lower CH_4 production from high forage:grain diet can be attributed to the effect of the high content of fat in the diet, which could potentially reduce fiber degradation and amount of feed that is ferrmentable (Mathison *et al.*, 1998). This was in agreement to our finding of reduced CH_4 emission by feeding high forage: grain diet. Further Boadi *et al.* (2004) demonstrated that replacement of 50% of the cereal grain in a typical feedlot ration with forage and oilseed will produce lower enteric CH_4 emissions per unit gain compared to traditional high concentrate diets. Beneficial forage fermentation characteristics resulting in a CH_4 reduction can be attributed to higher biomass availability and better pasture quality (Ominski and Wittenberg, 2006). It is recognized that CH_4 production in ruminants generally increases with forage maturity and that CH_4 yield from the ruminal fermentation of legume and legume plus grass forages is also generally lower than the yield from grass forages (McAllister *et al.*, 1996; Moss *et al.*, 2000). Explanation for the reduced CH_4 emissions can be attributed to the lower proportion of structural carbohydrates in legumes and faster rate of passage, which shift the fermentation pattern towards higher propionate production (Johnson and Johnson, 1995).

The study also reveals that forages in grinded form when mixed and fed with other supplement reduced the CH_4 emission. Grinding of forages to improve the utilization by ruminants has been shown to decrease CH_4 losses per unit of feed intake by 20-40% when fed at high intakes (Johnson *et al.*, 1996). The explanation for the decline in CH_4 production is due to the lower fibre digestibility, decreased ruminally available organic matter and faster rate of passage associated with ground or pelleted forages (Le Liboux and Peyraud, 1999).

The feeding of TMR with high quality forage when supplemented with flax seed reduced CH_4 emission considerably. This finding was in line with Dong *et al.* (1997) and Machmuller and Kreuzer (1999) who reported that the addition of dietary fats to increase the energy density of high-forage diets has been shown to depress CH_4 production. The CH_4 suppressing effects of supplemental fats may depend upon several factors, including the amount added and the resulting total concentration of fat in the diet, the FA profile of the fat source, the form in which the fat is administered and the composition of the diet (Beauchemin *et al.*, 2009). The reason for reduced CH_4 production after flax seed feeding is due to high content of the essential dietary Poly Unsaturated Fatty Acids (PUFA) linolenic and linoleic acid in flax seed. It is well known that the presence of long-chain PUFAs inhibits CH_4 production in the rumen through two ways: provision of an alternative metabolic H acceptor to reduction of CO_2 and direct toxic effects on ruminant microorganisms (Johnson and Johnson, 1995; Dong *et al.*, 1997; Giger-Reverdina *et al.*, 2003). Supplementation of ruminant diets with PUFA may provide an alternative hydrogen sink within the rumen and as a result may reduce enteric CH_4 production.

Supplemental fats can reduce CH_4 emissions, but in many cases the CH_4 suppressing effects are caused by a decrease in DMI (Eugene *et al.*, 2008), a decrease in ration digestibility (Martin *et al.*, 2008), or both (Hess *et al.*, 2008). Beauchemin *et al.* (2009) reported that the reduction in CH_4

production due to flax seed feeding is due to reduction in ration digestibility. This reduced feed digestibility might lower milk production of high-producing dairy cows. Care should be taken to include the fatty supplementation, as apart from being costly it also contains some side effects such as increasing the amount of refusals and shift the volatile fatty acid composition to a propionate basis (Mathison *et al.*, 1998; Sauer *et al.*, 1998). Furthermore, feeding fats high in PUFA can alter the FA composition of milk (Bu *et al.*, 2007). Hence, further research should consider providing the minimum possible levels of flax seed to achieve reduction in enteric CH_4 emission. In addition, while conducting such fat supplemental studies it is very pertinent to consider the form of adding the flax seed to the diet and their interaction with the nature of the basal diet.

In addition, much of the research on supplementing feed with fats and oils has been short term and the positive effects observed in studies have not yet been conclusively established over the long term (EPA, 2008). The reason for the non-persistent positive effects could be because of fermentation adaptation, which occurs when the methanogenic bacteria in the rumen adapt to changing feed. The supplements have short-term positive effects that decrease over time as bacteria in the rumen adapt. Alternating feeding strategies might alleviate this fermentation adaptation. When used in typical dairy diets, oilseed incorporation should be based on their nutritional value and costs and not as a strategy to reduce CH_4 emissions.

CONCLUSION

The study shows that both forage and flax seed feeding did reduced the enteric CH_4 emission in cows. Adding flax seed to the diet can be an effective means of reducing CH_4 emissions. However, over supplement of flax seed may reduce CH_4 but at the expense of diet digestibility in addition to possible negative effects on milk production of high-producing dairy cows. This critical point should not be overlooked while targeting reduction of enteric CH_4 emission. Further studies are required to arrive at the exact proportion of flax seed feeding to target CH_4 reduction without affecting the productive efficiency of the cows.

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