Methane emissions of stored manure from dairy cows fed conventional or brown midrib corn silage

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ABSTRACT

The objective of this study was to examine the effects of feeding conventional corn silage (CCS) or brown midrib corn silage (BMCS) to dairy cows on CH₄ emissions from stored manure. Eight lactating cows were fed (*ad libitum*) a total mixed ration (forage:concentrate ratio 65:35; dry matter basis) containing 59% (dry matter basis) of either CCS or BMCS. Feces and urine were collected from each cow and mixed with residual sludge obtained from a manure storage structure. Manure was incubated for 17 wk at 20°C under anaerobic conditions (O₂-free N₂) in 500-mL glass bottles. Methane emissions and changes in chemical composition of the manure were monitored during the incubation period. The total amount of feces and urine excreted was higher for cows fed BMCS than for cows fed CCS [8.6 vs. 6.5 kg/d of volatile solids (VS)]. Manure from cows fed BMCS emitted more CH₄ than manure from cows fed CCS (173 vs. 146 L/kg of VS) throughout the incubation period. Similarly, VS and neutral detergent fiber losses throughout incubation were higher for manure from cows fed BMCS versus cows fed CCS (37.6 vs. 30.6% and 46.2 vs. 31.2%, respectively). Manure NH₃ concentration (79% of total manure N) was not affected by corn silage cultivar. Results of this study show that using a more digestible corn silage cultivar (BMCS vs. CCS) may increase the contribution of manure to CH₄ emissions, and may offset gain achieved by reducing enteric CH₄ emissions.

Key words: manure, methane emission, brown midrib corn silage

INTRODUCTION

Methane emissions from Canadian agriculture (1.2 Mt of CH₄/yr) originate mostly from enteric fermenta-

tion (83%) and to a lesser extent from manure management (17%). These emissions represent 48% of total greenhouse emissions of the Canadian agriculture (Environment Canada, 2015a). Since these emissions are the major contributor to agricultural CH₄ emissions, several nutritional strategies have been put forward to mitigate enteric CH₄ production from dairy cows (Beauchemin et al., 2009; Benchaar et al., 2014; van Gastelen et al., 2015). However, the effect of these strategies on CH₄ emission of manure needs to be assessed to ensure that reducing enteric CH₄ emissions would not be counterbalanced by increased CH₄ emission from manure management. Studies investigating the effect of enteric CH₄ mitigation strategies on CH₄ emissions from stored manure are scarce.

According to IPCC (2006), CH₄ emission factor (kg of CH₄/cow per year) of manure management depends on quantity of volatile solids (VS) excreted and maximum CH₄-producing capacity of manure VS (B₀). Diets of dairy cow have a direct effect on the amount and degradability of excreted VS (Doreau et al., 2014; Møller et al., 2014; Massé et al., 2016; Hassanat and Benchaar, 2019). Forages are the main component in dairy cow diets. Forage digestibility is affected by several factors, but mainly by the content and ruminal degradability of fiber. For instance, brown midrib corn silage (BMCS) is characterized by lower lignin concentration and higher ruminal degradability of fiber, compared with conventional corn silage (CCS). Feeding more digestible forages can influence the amount and the chemical composition (NDF, ADL, and total N concentrations) of VS of manure.

In a recent study, Hassanat et al. (2017) observed that cows fed BMCS had similar enteric CH₄ emissions (g/d) as cows fed CCS, but DMI and milk yield were higher for cows consuming BMCS than for cows fed CCS. Consequently, CH₄ production expressed on a DMI or milk production basis was reduced. However, an increase in DMI may result in an increase in VS excretion, which is associated with higher CH₄ emission of manure (IPCC 2006). To the best of our knowledge, only 3 studies (Møller et al., 2014; Massé et al., 2016;

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Hassanat and Benchaar, 2019) have investigated the effect of dietary forage source on CH₄ emission of manure. Thus, the objective of this study was to assess the effect of replacing CCS with BMCS in the diet on CH₄ emissions of stored manure from dairy cows.

MATERIALS AND METHODS

This study was conducted at the Sherbrooke Research and Development Centre (Sherbrooke, Quebec, Canada). Animal procedures were conducted under the approval of the local Institutional Animal Care Committee and were in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

Cows, Experimental Design, and Diets

The study used 8 multiparous lactating Holstein in a completely randomized design. At the start of the trial, average DIM, BW, and milk yield (mean $\pm$ SD) of cows was 138 ± 16 d, 684 ± 76.5 kg, and 35 ± 7.6 kg/d, respectively. During the trial, cows were housed in individual tiestalls, had free access to water, and were fed (ad libitum; 5% orts on an as-fed basis) a TMR (65:35, forage:concentrate ratio; on a DM basis) based on either CCS or BMCS (59% of dietary DM). Chemical composition of the 2 silages and diets (ingredient and chemical) is shown in Table 1. Cows were allowed 2 wk of adaptation to experimental diets as described in Hassanat et al. (2017).

Feces and Urine Collection

Total collection of feces and urine was performed over 2 consecutive days by fitting cows with harnesses and tubes allowing the collection of feces and urine separately. Total urine was collected into reinforced plastic containers, whereas feces was collected from reinforced plastic mats placed behind the cow. Feces and urine were weighed daily, sampled (4% of the amount excreted), mixed based on the daily excreted feces:urine ratio (on a fresh weight basis), and then pooled over the 2 collection days for each cow. Sub-samples were taken and stored at -20°C . They were subsequently freeze-dried and ground to pass a 1-mm screen using a Wiley mill (standard model 4, Thomas Scientific, Swedesboro, NJ) for later determination of DM, VS, NDF, ADF, ADL, and total N concentrations.

Anaerobic Incubation of Manure

The mixture of feces and urine of each cow was mixed manually on a fresh weight basis (1:1) with psychrophilic residual sludge obtained from a manure storage structure at the dairy operation of the Sherbrooke Research and Development Centre. The residual sludge had a pH of 7.38 and was composed (on a DM basis) of 82.1% VS, 43.3% NDF, and 3.4% total N. In this study, the mixture of feces, urine, and residual sludge was identified as manure.

The pH of the manure was measured immediately and a representative sample (50 g) was diluted (1:1)

Table 1. Chemical composition of diets and corn silage conventional and brown midrib cultivars (adapted from Hassanat et al., 2017)

Item (% of DM, unless otherwise noted)	Diet ¹		Silage ¹	
	CCS	BMCS	CCS	BMCS
Ingredient				
Conventional corn silage	58.8	—	—	—
Brown midrib corn silage	—	58.7	—	—
Timothy hay, chopped	6.6	6.6	—	—
Concentrates ²	34.6	34.8	—	—
Chemical composition				
OM	94.1	94.4	96.5	96.6
CP	15.9	15.5	5.54	6.54
NDF	35.1	35.1	34.3	34.4
ADF	19.7	19.8	20.1	20.1
ADL (% of NDF)	6.32	5.53	7.90	6.13
Starch	16.5	17.0	26.9	28.3
Ether extract	2.69	2.76	2.38	2.49
Gross energy (Mcal/kg of DM)	4.36	4.37	4.37	4.38

¹CCS = conventional corn silage; BMCS = brown midrib corn silage.

²Contained (on DM basis) 17.3% soybean meal (48% solvent extracted), 10.6% corn gluten feed, 2.1% corn grain (ground), 1.8% soybean hulls, 1.6% mineral and vitamin supplement, 0.7% calcium carbonate, and 0.5% potassium carbonate.

with water, subsampled (15 mL), and frozen at -20°C immediately for later determination of VFA concentrations. The manure was also sampled for subsequent determination of DM, VS, and NDF contents. The manure was transferred under anaerobic conditions (O_2 -free N_2) into 500-mL glass bottles (6 replicates/cow; 250 g in each bottle), sealed with butyl rubber stoppers and aluminum seals (Wheaton, Millville, NJ), and incubated at 20°C for 17 wk in a controlled environment chamber. Of the 6 replicates, 3 replicates were used for gas production measurements, whereas 3 other replicates were used for determining fermentation characteristics of manure (pH and VFA concentrations). Blank bottles ($n = 3$) containing residual sludge (250 g) were treated similarly for measurement of gas production. Thus, in total, 51 bottles were used in this study. Manure was incubated for 17 wk at 20°C to simulate the period of the year (late spring, summer, and early fall) in Canada during which the monthly average temperature of the manure storage structure is 20°C (Massé et al., 2016). Beyond this period (early spring or late fall), temperature would be considerably lower than 20°C and CH_4 production from stored manure would decline substantially.

Gas Production Measurement

Gas pressure in the bottle headspace was measured daily using a digital pressure gauge ($0\text{--}15\text{ psi} \pm 0.05\%$; Ashcroft 2089, Ashcroft Inc., Stratford, CT) and the volume of gas produced was calculated according to the following equation:

$$P_1 V_1 = P_2 V_2,$$

where P_1 = atmospheric pressure, V_1 = volume of gas produced at atmospheric pressure, P_2 = pressure of gas measured at specific incubation day, and V_2 = volume of headspace of the bottle.

Headspace gas was sampled using a syringe connected to the pressure gauge by a sampling valve. The collected gas sample was then forced into 6-mL screw-capped vacuum vials (Labco Ltd., Lampeter, UK) covered with a rubber septum (National Scientific, Rockwood, TN) for measurement of CH_4 concentration. Gas pressure in the bottle head space was then reduced to room pressure by leaving the sampling syringe opened for 2 min. Methane production from blanks was not subtracted from gas produced from the experimental bottles.

After 17 wk of anaerobic incubation, bottles ($n = 3$) used for gas production measurements were weighed, their contents pooled, and sub-samples were frozen at -20°C for later determination of DM, VS, and NDF

concentrations. Losses of VS and NDF after 17 wk of anaerobic incubation were calculated as the difference between VS and NDF contents at the beginning and at the end of the incubation period.

Fermentation Characteristics of Manure

Manure from each bottle was sampled under anaerobic weekly up to wk 10 and then on wk 12, 14, and 17 of incubation. Bottle contents were homogenized by swirling, sampled (3.5 g/bottle) using a peristaltic pump (Accu-Jet, BRAND, Wertheim, Germany) and plastic tubing under continuous purging with O_2 -free N_2 , and then pooled across replicates. The pH was measured immediately and a subsample (5 mL) was stored at -20°C for later determination of VFA concentrations. On wk 17 of incubation, a subsample (5 mL) of manure was acidified to pH 2 with 50% sulfuric acid and frozen at -20°C for later determination of NH_3 concentrations.

Chemical Analyses

Dry matter content was determined by drying samples in a vacuum oven at 100°C overnight (AOAC, 2005; method 934.01). Ash content was determined by incineration at 550°C overnight in a muffle furnace (AOAC International, 2005; method 942.05) and the VS content was calculated as the difference between 100 and the percentage of ash. Total N was determined using the macro-Kjeldahl procedure (AOAC International, 2005; method 954.01). The concentration of NDF was determined as described by Van Soest et al. (1991). The ADF and ADL contents were determined according to AOAC International (2005; method 973.18). The NDF, ADF, and ADL procedures were adapted for use in an Ankom200 Fiber Analyzer (Ankom Technology Corp., Fairport, NY). Analysis of VFA was performed using a gas chromatograph equipped with a flame ionization detector and auto-injector (Perkin-Elmer, Norwalk, CT) fitted with DB-FFAP column ($30\text{ m} \times 0.53\text{ mm} \times 1\text{ }\mu\text{m}$; J&W Scientific, Folsom, CA) with He as the carrier gas. The gas flow in the column was fixed at 5 mL/min, oven temperature was fixed at 135°C , and total run time was 8 min. Injector and detector temperatures were 200 and 250°C , respectively. Gas flows at the detector were 45 and 450 mL/min for H_2 and air, respectively. Ammonia was determined as in Weatherburn (1967). Analysis of CH_4 and CO_2 was performed using GC (490 Micro GC, Agilent Technologies, Amstelveen, North Holland, the Netherlands) equipped with a 10 m PPQ column (Agilent Technologies) and a thermal conductivity detector. Column temperature was fixed at 70°C , while injector and detector temperatures were

fixed at 110 and 70°C, respectively. The column was operated at a carrier gas (He) pressure of 138 kPa, with elution time of 1 min. Calibration was done using standard gas mixtures containing different proportions of CH₄.

Statistical Analyses

Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) and the statistical model included diet as a fixed effect, and cow was the experimental unit. Data of CH₄ production were summarized by week. Data of CH₄ production and manure pH were analyzed as repeated measures using the same model with the addition of week and week × diet interactions as fixed effects. The appropriate covariance structure used for repeated-measures analyses was chosen to achieve lowest Akaike, corrected Akaike, and Bayesian information criterion values. Cow within treatment was the subject of the repeated measures. The appropriate covariance structure was specific for each variable measured in repeated measures. Significant effects of treatments were declared at $P \leq 0.05$ and tendencies were declared at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Chemical Composition of the Mixture of Feces and Urine

The effect of feeding cows CCS or BMC on the amount excreted and chemical composition of the mixture of feces and urine is presented in Table 2. Cows fed BMCS excreted 31% more ($P < 0.01$) VS than cows fed CCS (8.6 vs. 6.5 kg). This is mainly due to an increase (1.7 kg/d) in OM intake and no change in OM digestibility (average of 71.3%) when cows were fed BMCS versus CCS as reported in Hassanat et al. (2017). The lower lignin concentration in BMCS compared with CCS is the main reason explaining the increase in OM intake when cows were fed BMCS versus CCS. In agreement, a meta-analysis by Ferraretto and Shaver (2015) showed that feeding cows BMCS versus CCS increases DMI without changing OM digestibility. An increase in DMI without change in OM digestibility would result in an increase in the amount of VS excreted. Other studies reported a positive relationship between OM intake and VS excretion (Hassanat et al., 2013; Massé et al., 2016; Appuhamy et al., 2018).

Concentrations of VS, NDF, ADF, and total N in feces and urine mixture were not affected by the dietary treatment and averaged 86, 52, 27, and 5.4% on a DM basis, respectively (Table 2). Lignin concentration in

the fiber fraction was lower ($P = 0.04$) in the mixture of feces and urine from cows BMCS (9.0% of NDF) compared with the mixture from cows fed CCS (10.4% of NDF). This difference is due to the lower lignin concentration in BMCS compared with CCS (5.5 vs 6.3% of NDF) because fiber digestibility was not affected by replacing CCS by BMCS in the diet (Hassanat et al., 2017).

Fermentation Characteristics of Incubated Manure

Interaction between dietary treatment and incubation week was significant ($P < 0.01$) only for manure pH. Indeed, manure of cows fed BMCS had lower pH on wk 2 of incubation (6.94 vs. 7.16; $P = 0.01$) and higher pH on wk 14 of incubation (7.98 vs. 7.79; $P = 0.02$) compared with manure of cows fed CCS (Figure 1). Overall, manure pH averaged 7.70 (Table 3). This pH sustains growth and activity of acidogen, acetogen, and methanogen populations in stored manure (Angelidaki et al., 2003; Pind et al., 2003). Concentration of total VFA tended to be higher ($P = 0.07$) in manure of cows fed BMCS compared with manure of cows fed CCS (55.4 vs. 41.8 mM). In contrast, acetate concentration in manure was not affected by corn silage cultivar in the diet and averaged 33.5 mM (Table 3). Concentrations of propionate and other minor VFA were higher ($P \leq 0.03$) in manure of cows fed BMCS compared with cows fed CCS.

At the end of the incubation period, NH₃ concentration of manure was not affected by replacing CCS with BMCS in the diet and averaged 79% of total N (Table 4). Up to 85% of manure N can be in the form of NH₃ after an extended period of storage (Petersen, 2018).

Methane Production of Manure

Methane production (L/kg of VS) of manure throughout the 17 wk of anaerobic incubation is presented

Table 2. Volatile solids and composition of the mixture of feces and urine from lactating cows fed diets based on conventional corn silage (CCS) or brown midrib corn silage (BMCS)

Item	Diet		SEM	P-value
	CCS	BMCS		
Volatile solids				
kg/d	6.54	8.55	0.324	<0.01
% of DM	86.4	86.3	0.17	0.71
Composition				
NDF (% DM)	51.8	51.8	0.62	0.99
ADF (% DM)	27.1	26.1	0.49	0.22
ADL (% NDF)	10.38	8.95	0.384	0.04
Total N (% DM)	5.52	5.25	0.176	0.32

Figure 1. Changes in pH of incubated manure from lactating cows fed diets based on conventional corn silage (▲) or brown midrib corn silage (□). Error bars indicate SEM.

in Figure 2. A significant interaction ($P < 0.01$) was observed between corn silage cultivar and incubation week. Methane production started from the onset of incubation. From the onset of incubation up to wk 4, CH_4 production of manure was not affected by corn silage cultivar. However, from wk 5 to 17, manure from cows fed BMCS produced more CH_4 ($P \leq 0.04$) than manure from cows fed CCS. Peak of CH_4 production of manure was observed at wk 5 of incubation: 19.4 and 25.1 L/kg of VS for cows fed CCS and BMCS, respectively (data not shown).

After 17 wk of incubation, manure from cows fed BMCS produced 19% more CH_4 ($P = 0.01$) than manure from cows fed CCS (173 vs. 146 L/kg of VS; Table 4). This is principally due to lower lignin concentration in manure from cows fed BMCS compared with manure from cows fed CCS, which allowed more extensive fiber degradation during anaerobic incubation of manure.

Table 3. Fermentation characteristics of manure from lactating cows fed diets based on conventional corn silage (CCS) or brown midrib corn silage (BMCS)

Item	Diet		SEM	<i>P</i> -value
	CCS	BMCS		
pH	7.67	7.72	0.07	0.13
Total VFA (mM)	41.8	55.4	4.37	0.07
Acetate (mM)	29.6	37.3	3.63	0.18
Propionate (mM)	9.7	14.2	1.08	0.01
Minor VFA ¹ (mM)	2.59	3.89	0.307	0.03

¹Minor VFA include iso-butyrate, butyrate, iso-valerate, valerate, and caproate.

Under similar experimental conditions, Hassanat and Benchaar (2019) observed higher maximal CH_4 production potential (L/kg of VS) associated with lower lignin concentration in manure of cows fed corn silage-based diets compared with red clover silage-based diets. According to Triolo et al. (2011) and Kifle and Chen (2016), a strong negative relationship ($R^2 = 0.91$ and 0.85 , respectively) exists between lignin concentration in manure and CH_4 emissions. Results of this study clearly show the effect of the diet on manure VS chemical composition, and its consequent effect on CH_4 production of manure.

The IPCC (2006) suggested a default value (240 ± 36 L/kg of VS) of maximal CH_4 -producing capacity of manure to calculate CH_4 emissions of manure of dairy cows in North America. However, this approach does not take into consideration changes in the chemical composition of manure VS as affected by the type of diet fed to dairy cows. Values of maximal CH_4 production potential of manure reported in this study (146 and 173 L/kg of VS) are within the range of values (85–197 L/kg of VS) observed in previous studies (Massé et al., 2016; Hassanat and Benchaar, 2019) under similar experimental conditions (17 wk of anaerobic incubation; 20°C).

Table 4. Methane emissions, volatile solid (VS) and NDF losses, and NH_3 concentration of incubated manure from lactating cows fed diets based on conventional corn silage (CCS) or brown midrib corn silage (BMCS)

Item	Diet		SEM	<i>P</i> -value
	CCS	BMCS		
CH_4				
Maximal production (L/kg of VS)	146	173	5.4	0.01
Emissions (g/d) ¹	128	198	9.20	<0.01
VS loss (%)	30.6	37.6	1.26	0.01
NDF loss (%)	31.2	46.2	2.79	0.01
NH_3				
mM	240	227	5.5	0.17
% of N	78.9	79.3	1.55	0.87

¹Calculated according to equation 10.23 (IPCC, 2006) using VS excretion (Table 2), CH_4 maximal production (L/kg of VS) of manure from cows, and the CH_4 conversion factor as suggested by Environment Canada (2015b).

Figure 2. Methane emissions of incubated manure from lactating cows fed diets based on conventional corn silage (▲) or brown midrib corn silage (□). Error bars indicate SEM.

Daily manure CH₄ emissions (Table 4) were calculated with equation 10.23 of the IPCC (2006) as follows:

$$\text{manure CH}_4 \text{ emission} = \text{VS} \times B_0 \times 0.67 \times \text{MCF},$$

where manure CH₄ emission is the daily manure CH₄ emission per cow (g/d), VS is the quantity (kg/cow per d) of VS reported in Table 2, B_0 is the maximal CH₄ production potential (L/kg of VS) reported in Table 4, 0.67 is the conversion factor of L of CH₄ to g of CH₄, and MCF is the methane conversion factor (0.2) for liquid manure suggested by Environment Canada (2015b).

Manure from cows fed BMCS produced 55% more ($P < 0.01$) CH₄ than manure from cows fed CCS (198 vs. 128 g/d; Table 4). The higher daily manure CH₄ emissions from cows fed BMCS diet compared with emissions from cows fed CCS are due to the higher VS excretion (kg/d) and the higher maximal CH₄ production (L/kg of VS) for cows fed BMCS versus cows fed CCS. In our previous study (Hassanat et al., 2017), enteric CH₄ emission of cows fed BMCS and or CCS were 470 and 483 g/d, respectively. Thus, total CH₄ emissions (sum of enteric and manure emissions) were 668 and 611 g/d for cows fed BMCS and CCS, respectively. Calculated manure CH₄ emissions of cows fed BMCS and CCS represent 30 and 21% of the total emissions, respectively, which is within the range of emissions reported in other studies (Doreau et al., 2014; Hassanat and Benchaar, 2019). Compared with CCS, feeding cows BMCS did not significantly affect enteric CH₄ emissions (−13 g/d), but increased manure CH₄ emissions by 70 g/d, resulting in a net increase in total CH₄ emissions by 57 g/d (+9%).

Degradation of VS and NDF (i.e., VS and NDF losses) during incubation (Table 4) was more extensive ($P < 0.01$) in manure from cows fed BMCS compared with manure from cows fed CCS diet (38 and 46 versus 31 and 31%, respectively). This is consistent with differences in CH₄ emissions observed between manure from cows fed BMCS compared with cows fed CCS. In agreement, Aguerre et al. (2012) and Hassanat and Benchaar (2019) reported up to 33% VS losses of stored manure. Niu et al. (2017) also reported 40% carbon loss from manure stored for 29 wk. Maximum methane production potential expressed on kilogram of degraded VS basis averaged 467 L/kg of VS and were not affected by corn silage cultivar. This value is within the range of values (450–850 L/kg of VS degraded) estimated by Lory et al. (2010).

CONCLUSIONS

Results of this study show that manure contribution to CH₄ emissions may increase when more digestible forages are used in dairy cow diets. Compared with CCS, feeding BMCS to dairy cows increased VS excretion (kg/d) and maximal methane production (L/kg of VS) when manure was incubated under anaerobic conditions for 17 wk. Therefore, it is necessary to assess CH₄ emissions from stored manure when dietary CH₄ mitigation options are suggested to ensure reductions in enteric CH₄ do not increase CH₄ emissions from stored manure. In this case, strategies to reduce or eliminate CH₄ emissions from stored manure should be considered.

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