INTRODUCTION

The concentration of greenhouse gases (GHGs) in the atmosphere has increased abruptly. Methane (CH₄) is a potential GHG, whose emission is attributed to the animal industry. The enteric fermentation of livestock, especially ruminants, releases 0.375 million tons methane year⁻¹ and accounts for 22.7% of the total methane emission in Japan (Takahashi, 2005). The dairy industry in Hokkaido is responsible for 46% of the annual milk production in the country, which exceeds 3.8 million t in this region. The number of dairy cows in Hokkaido has increased to 857,500 and accounts for approximately 52% of all the dairy cows in Japan. Timothy (Phleum pratense) and Italian ryegrass (Lolium multiflorum Lam) are the chief forage grasses available for silage production in Japan. Although Timothy and Italian ryegrass are predominantly used in grassland farming, red clover (Trifolium pratense) can play an increasingly significant role in future silage production because of its N₂-fixing ability. Kume demonstrated that alfalfa (Medicago sativa) is useful for decreasing the methane production from cows in Hokkaido (2001); however, it is unclear whether Italian ryegrass and red clover have a similar effect. To combine sustainable dairy production and environmental conservation, the quantity and quality of forage given to dairy cattle should be such that it helps in mitigating the emission of GHGs, especially methane. Thus, the potential of each forage species for methane production must be evaluated.

In the present study, we compared the methane (CH₄) emission from dry cows fed first-cut Timothy silage (1st TY), second-cut Timothy silage (2nd TY), second-cut Italian ryegrass silage (2nd IR), third-cut Italian ryegrass silage (3rd IR), or second-cut red clover silage (2nd RC) as the sole feed. The methane emission ranged from 258.2 L day⁻¹ to 396.5 L day⁻¹. The methane emission from dry cows fed red clover silage was relatively lower than that from dry cows fed grass silage. However, the methane emission per unit digestible neutral detergent fiber (dNDF) intake (dNDFI) did not differ significantly between the experimental silages. The methane emission was significantly correlated with the NDF intake and digestibility. Methane emission had a significant correlation with the quadratic function of dNDFI. The differences in the daily volume of methane emission from cows fed different forages can be explained by dNDFI. (Key Words: Methane Emission, Cow, Silage, Timothy, Italian Ryegrass, Red Clover)

MATERIAL AND METHODS

Location and animals

All data were collected in the Hokkaido Prefectural Konsen Agricultural Experiment Station (43°N, 144°E, 50 m) and 32 Holstein dry cows (752 (77) kg) were used for the digestion and respiration trials. Experimental animals were cared for according to the guidelines of the Hokkaido Prefectural Konsen Agricultural Experiment Station Committee (2005) for Animal Use and Care.

Experimental forages and digestion and respiration trials

The cows were fed ad libitum 1st TY, 2nd TY, 2nd IR, 3rd IR, or 2nd RC as the sole feed. Silage was given to each animal once a day as the morning meal.

For each of the experimental forage species, the cows
were housed in individual pens for 14 days during the adjustment period, followed by a 7-day collection period. The respiration trial was performed during the last 2 days of each collection period by using an open-circuit respiratory system with a hood over the head of the animal (Takahashi et al., 1999). The methane concentration in the inlet air and exhaust gas from the ventilated hood was automatically analyzed using a dispersive infrared methane analyzer (model VIA-500; Horiba, Kyoto, Japan). Data from the analyzer were entered into the computer (NEC, Tokyo, Japan) at 1-minute intervals and then automatically standardized at 0°C, 101.3 kPa, and zero water vapor pressure.

Chemical analyses

Air-dried forage and feces samples were ground in a Wiley mill (1-mm screen) for analyzing the dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), and neutral detergent fiber (NDF) content according to the methods of AOAC (2006). The Ca, Mg, and K concentrations in the experimental forage were determined by atomic absorption spectrophotometry and P was determined calorimetrically (Kume and Tanabe, 1993; Kume et al., 1998).

Statistical analyses

The general linear model procedure of the JMP® software (2005) was used to analyze the effect of grass silage and legume silage on methane production. The relationships between methane production and nutrient intake or digestibility were examined by regression analysis, using the PROC REG procedure of the JMP® software (2005). Significance was declared at p<0.05.

RESULTS AND DISCUSSION

Table 1 shows the chemical composition of the experimental forages. Among the experimental forages, the Ca content was the highest in 2nd RC, though the NDF content was slightly lower in this forage. A relatively higher content of CP was observed in 2nd IR and 3rd IR, as compared to the other forages. The NDF content was the lowest in 3rd IR. For the dairy industry in Hokkaido, grass silage is commonly used as the main forage during the long winter season. In this study area, the nutrient content of the experimental forages was similar to that found in other areas. Table 2 shows the nutrient digestibility and total digestible nutrients (TDN) of the experimental forages. Among the experimental forages, the DM digestibility of the 1st TY was the highest, the CP digestibility of the 2nd RC was significantly lower than that of the 1st TY, 2nd IR, and 3rd IR, and the NDF digestibility of the 2nd RC was the lowest (p<0.05 for all 3 parameters). With respect to the different harvest seasons for the same forage spieces, the NDF digestibility of the 1st TY was significantly higher than that of the 2nd TY and that of the 2nd IR was significantly lower than that of the 3rd IR (p<0.05). Consequently, the TDN content of the 2nd RC was the lowest among the experimental forages (p<0.05). Table 3 shows the methane emissions from dry cows fed the experimental forages. In terms of the daily volume (L day⁻¹) and dry matter intake (DMI), the methane emission on the 2nd RC was the lowest.

Table 1. Chemical composition of experimental forages

<table>
<thead>
<tr>
<th></th>
<th>1st TY</th>
<th>2nd TY</th>
<th>2nd IR</th>
<th>3rd IR</th>
<th>2nd RC</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (%)</td>
<td>34.1</td>
<td>42.4</td>
<td>30.2</td>
<td>18.6</td>
<td>39.7</td>
<td></td>
</tr>
<tr>
<td>CP (% DM)</td>
<td>13.1</td>
<td>13.9</td>
<td>18.1</td>
<td>20.5</td>
<td>16.7</td>
<td></td>
</tr>
<tr>
<td>NDF (% DM)</td>
<td>53.8</td>
<td>55.1</td>
<td>52.4</td>
<td>48.6</td>
<td>49.1</td>
<td></td>
</tr>
<tr>
<td>Ether extracts (% DM)</td>
<td>3.6</td>
<td>3.9</td>
<td>4.5</td>
<td>7.2</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Crude ash (% DM)</td>
<td>8.0</td>
<td>9.4</td>
<td>15.3</td>
<td>17.3</td>
<td>12.2</td>
<td></td>
</tr>
<tr>
<td>Ca (% DM)</td>
<td>0.52</td>
<td>0.63</td>
<td>0.44</td>
<td>0.43</td>
<td>1.01</td>
<td>0.1</td>
</tr>
<tr>
<td>P (% DM)</td>
<td>0.28</td>
<td>0.32</td>
<td>0.42</td>
<td>0.43</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>K (% DM)</td>
<td>2.41</td>
<td>2.28</td>
<td>3.71</td>
<td>3.82</td>
<td>3.15</td>
<td></td>
</tr>
<tr>
<td>Mg (% DM)</td>
<td>0.18</td>
<td>0.24</td>
<td>0.21</td>
<td>0.20</td>
<td>0.30</td>
<td></td>
</tr>
</tbody>
</table>

Means with different superscripts are significantly different (p<0.05).

Table 2. Digestibility of experimental forages

<table>
<thead>
<tr>
<th></th>
<th>1st TY</th>
<th>2nd TY</th>
<th>2nd IR</th>
<th>3rd IR</th>
<th>2nd RC</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (%)</td>
<td>71.6a</td>
<td>63.6b</td>
<td>61.2c</td>
<td>60.2b</td>
<td>58.0b</td>
<td>1.03</td>
</tr>
<tr>
<td>CP (%)</td>
<td>64.4a</td>
<td>61.8ab</td>
<td>68.3a</td>
<td>67.3b</td>
<td>55.4b</td>
<td>1.08</td>
</tr>
<tr>
<td>NDF (%)</td>
<td>70.1ab</td>
<td>62.8c</td>
<td>64.6ac</td>
<td>71.8a</td>
<td>51.1d</td>
<td>1.53</td>
</tr>
<tr>
<td>Ether extracts (%)</td>
<td>77.0a</td>
<td>66.8b</td>
<td>72.6ab</td>
<td>78.2a</td>
<td>71.5ab</td>
<td>1.07</td>
</tr>
<tr>
<td>TDN (%)</td>
<td>67.4a</td>
<td>60.1b</td>
<td>59.8b</td>
<td>65.8ab</td>
<td>48.4a</td>
<td>1.37</td>
</tr>
</tbody>
</table>

Means with different superscripts are significantly different (p<0.05).
On the other hand, the methane emission on the basis of NDF intake was the highest in the 3rd IR. However, the methane emission on the basis of digestible NDF intake (dNDFI) did not significantly differ between the experimental forages. The methane emissions from animals fed most legume forages have been reported to be lower than those from animals fed grass silage (McCaughey et al., 1999; Waghorn et al., 2002). However, Van Dorland et al. (2007) reported that methane emissions from animals fed some legume forages were higher than those from animals fed grass forages. This lower emission from animals fed legume forages is often explained by the presence of condensed tannins, lower fiber content, higher DMI, and faster rate of passage from the rumen (Beauchemin et al., 2008). The 2nd RC in this study did not show the abovementioned characteristic pattern of methane emission for legume forages.

Significantly high correlation coefficients were observed between methane emissions and NDF intake \( (r = 0.73, p<0.01) \) and between methane emissions and dNDFI \( (r = 0.84, p<0.01) \). Therefore, we performed regression analyses between NDF intake \( (x_1) \) and methane emission \( (y_1) \) (Figure 1). The regression equation was as follows:

\[
y_1 = 5.14x_1^2 - 39.3x_1 + 360.00 \quad (R^2 = 0.56, p<0.01)
\]

Figure 2 shows the regression curve between dNDFI and methane emission. Methane emission had a significant correlation with the quadratic function of dNDFI \( (x_1) \). The regression equation was as follows:

\[
y_1 = 10.52x_1^2 - 44.89x_1 + 325.24 \quad (R^2 = 0.76, p<0.01)
\]

Methanogens in the rumen are hydrogenotrophic (Takahashi, 2005). When hydrogen accumulates in large quantities in the rumen, the proliferation and activities of rumen microorganisms are inhibited because of the high hydrogen pressure. The generation of methane by methanogens is important for rumen microorganisms because hydrogen is harmful to the latter (interspecies hydrogen transfer). Fiber-digesting bacteria that use structural carbohydrates such as cellulose and hemicelluloses produce this hydrogen. dNDFI is a measure of the intake of digestible structural carbohydrates. Therefore, it is evident from this study that methane emission occurs because of the digestion and fermentation of feed fiber in the rumen of dry cows that are fed only forage. The differences in the daily emission of methane from cows fed different forages can be explained by dNDFI.

**CONCLUSION**

Our results clearly show that methane emission from
ruminant animals occurs because of the digestion and fermentation of feed fiber in the rumen. The daily methane emission per cow, in particular, is closely related to the dNDFI and differences in methane emission from cows fed different forages can be explained by the dNDFI.

REFERENCES


