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Influences of feeding behaviour and forage quality on diurnal methane emission dynamics of grazing cows

Y. Blaise[123], A.L.H. Andriamandroso[12], B. Heinesch[13], Y. Beckers[12], E. Castro Muñoz[234], F. Lebeau[13], J. Bindelle[12]
[1]*TERRA Teaching and Research Center, AgricultureIsLife*
[2]*AgroBioChem, Precision Livestock and Nutrition Unit*
[3]*Biosystems Dynamics and Exchanges, Gembloux Agro-Bio Tech, University of Liege, Passage des Déportés 2, 5030 Gembloux, Belgium*
[4]*Facultad de Ciencias Agrícolas, Universidad Central del Ecuador, Universitaria, Quito, 170129, Ecuador*
yblaise@ulg.ac.be, jerome.bindelle@ulg.ac.be

Abstract

This study aimed to evaluate diurnal methane (CH_4) emission dynamics of grazing cattle and highlight their relationships with biotic factors such as the feeding behaviour as well as seasonal changes in pasture characteristics.
Existing methods to assess grazing ruminants' daily CH_4 emissions provide useful insights to investigate mitigation strategies relying on feeding and genetic selection. Nonetheless such methods based on tracer gases (SF_6) or feeding bins equipped with sniffers (e.g. GreenFeed) can hardly cover diurnal CH_4 emission fluctuations which can influence the accuracy of total CH_4 production estimations. Previous studies in barns showed that emission dynamics strongly vary during post feeding time, leading to a possible bias in estimates of daily CH_4 emissions as high as 100%. To investigate whether such fluctuations are also taking place on pasture, a portable device was designed with infrared CH_4 and CO_2 sensors measuring concentrations in the exhaled air at a high sampling rate (4 Hz). Six grazing dry red-pied cows were equipped with the device and motion sensors during runs of 24h to monitor CH_4 and CO_2 emissions and detect their feeding behaviours (grazing, rumination and other behaviours), respectively. This experiment was performed in summer and fall in order to cover seasonal changes in pasture forage quality. Methane emission was estimated from the CH_4:CO_2 concentration ratio and the metabolic CO_2 production of the cows. As for barn studies, variations were observed in total daily CH_4 emission due to the seasons and diurnal variations were also observed due to animal behaviours. Relationships between animal feeding behaviour and CH_4 emissions patterns on pasture were also unravelled.

Keywords: cattle, methane emission, pasture heights, grazing, behaviour.

Introduction

Livestock holds an important share of the anthropogenic greenhouse gases emissions. In cattle, rumen fermentation contributes significantly to this burden through the production of methane (CH_4). Methane is less prevalent in the atmosphere (1851 ppb in 2017) than carbon dioxide (CO_2) (407 ppm in 2017) but has a global warming potential 72 times greater than CO_2 over a 20-year period (IPCC, 2007; NOAA, 2017). Over the past decade, the concentration of CH_4 in the atmosphere grew faster than ever before and some name the expansion of cattle that increased from 1.3 billion heads in 1994 to 1.5 billion heads in 2014 as one of the major causes. There is an urgent need to develop adequate measure to reduce methane emissions or at least mitigate their effects and therefore develop techniques that allow measuring CH_4 emissions at different scales and under different production systems, including the individual level for grazing animals (Saunois et al., 2016). Grazed pastures are indeed important agroecosystems for the multiple ecosystems services they provide. In Belgium, a grazing cattle in a cow-calf operation produces about 50kg of CO_2 eq /year (Dumortier et al., 2016) but the whole pastoral agroecosystem works rather as a carbon sink (Gourlez de la Motte et al., 2016). While CH_4 affects climate change, for animal nutritionists, CH_4 production is also a sign of feed inefficacies. On average, 6% of the energy consumed by cattle is lost as methane (Johnson and Johnson 1995). CH_4 is released from the rumen mainly during eructations (87%) (Saunois et al., 2016).

The monitoring of CH_4 fluxes is usually carried out in metabolic chambers, i.e. in a controlled environment. It is regarded as the standard method (Storm et al., 2012). For grazing cattle, the chamber is not adequate. On pasture, three techniques can be used to estimate CH_4 production: (1) the eddy covariance method allowing the measurement of the CH_4 production of an entire herd and over time steps of 30 min (Dumortier et al., 2016); (2) the tracer method involving sulphur hexafluoride allowing the measurement of one individual's methane production over periods of, typically, 1 to 5 days (Hammond et al., 2016); and (3) short infra-red CH_4 and CO_2 measurements of the air exhaled that are achieved on individual animals and used to estimate their daily CH_4 production. In the latter, measurements are performed in a feeding bin and last for a few minutes. They can be repeated for a same individual between two to four times per day (Madsen et al., 2010 Garnsworthy et al., 2012). Such short term measurements can induce a bias when quantifying CH_4 production if there is important diurnal variation pattern in the dynamics of CH_4 emission that are possibly related to the behavioural phases of the cows (Velazco et al., 2016). In barns, cows fed on a restricted diet displayed strong fluctuations of their CH_4 emission rates according to the post-feeding time (Blaise et al., 2017). On pasture, the feeding behaviour is different since animals realise longer and more frequent meals and forage intake rate during the meals is lower (Andriamandroso et al., 2017). Hence, in order to contribute to management practices which could limit the CH_4 emissions of grazing cattle, an experiment was designed to measure how CH_4 emission rates of grazing cows vary along the day and whether such variations depend on the animal's behaviour and the changes in pasture characteristics across the seasons.

Material and methods

The experiment was run on the *AgricultureIsLife* experimental farm of TERRA Teaching and Research Centre of Gembloux Agro-Bio Tech in Gembloux, Belgium (50°33'59.06"N 4°42'07.97"E). All the experimental procedures and handling of the animals were approved by the Animal Care Committee of the University of Liege [protocol n°14-1627].

Experimental set up

Six dry red-pied Holstein cows between 4 and 7 years old and weighing 697.3 ± 82.9 kg were used during two data acquisition sessions: one in the summer (July 2016) and the second during the fall (September 2016). The herd was set to graze a permanent ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*)-based pasture and water was freely available to the animals. The pasture was divided in adaptation and measurement paddocks whose size and grass height allowed to reach forage allowances letting cows graze ad libitum. During both measurement campaigns i.e. in summer and fall, animals were grazing the same pasture and forage allowance (approx. 17 kg/100 kg BW/d) was similar, as measured using a rising plate pasture meter. After one week of adaptation to the sensors and to the pasture in an adaptation paddock, the cows wearing the equipment described below were placed in a measurement paddock for a measurement period that lasted 24 hours. The experimental scheme was performed in summer and repeated in fall.

Sward and ingestion characteristics

Before each measurement periods on a paddock, grass height (n=20) was measured and grass was sampled by randomly cutting eight quadrats of 30 × 30 cm^2. This grass was taken for chemical composition and nutritive value determination. Faeces were collected individually by rectal grabbing for faecal near-infrared reflectance spectrometry (F-NIRS) analysis. Faecal and grass samples were oven dried at 60°C. After moisture determination, samples were ground at 1 mm in a hammer mill (Cyclotec, FOSS Electric, Hillerød, Denmark). Each sample of ground forage and faeces were read by a NIRS-system 5000 monochromator spectrometer (XDS Rapid Content Analyser XM-1100 Serie, FOSS Electric, Hillerød, Denmark) (Decruyenaere et al., 2015). The absorption spectrum of each sample was recorded as log 1/R for wavelengths ranging from 1100 to 2498 nm, every 2 nm (WINISI 1.5, FOSS Tecator Infrasoft International LCC, Hillerød, Denmark). Prediction equations used to convert spectral data were provided by the Reference Laboratory Network REQUASUD (Wallonia, Belgium). Prediction by F-NIRS for CP, OM, NDF, ADF, ADL and DMI were considered as good since the standardized Mahalanobis distance (H) which evaluates the correspondence between the faeces spectra and the F-NIRS database was always lower than 3, ensuring an accurate prediction.

Sensors

Three types of sensors were worn by the animals and synchronized for further data processing: (1) gas sensors, (2) movement sensors and heart rate (HR) belt (3) (Figure 1).

Gas Sensor. A pump (24V DC Pump Gascard NG Models) sucked at a flow rate of 0.5 l/min the exhaled gas in a flexible PVC hose (1.85 m, inner ø 4mm) in front the nostril. The gas measurement sensors were placed on the animal's back (Figure 1), the CH4 infra-red sensors coming upstream from the CO_2 infra-red sensor (NG Gascard® 0-1 % CH4 and Gascard® NG 0-10% CO_2, respectively; Edinburgh Sensors, Livingston, UK). A 1-µm filter ensured the protection of both sensors. The concentrations of CH_4 and CO_2 were recorded at 4 Hz on a SD-card connected to a microcontroller.

Motion Sensors. **C**ows were fitted with a halter on which an iPhone (4S Apple Inc., Cupertino, CA, USA) was attached at the level of the neck of the animal (Figure 1). The built-in inertial measurement unit (IMU) was used to record head and jaw position and movements and converted into a behaviour matrix via an open-source algorithm to differentiate grass intake, ruminating and other behaviours (Andriamandroso et al., 2017).

Heart rate sensor. A transmitter heart rate (HR) belt was placed around the cows' chest (Equine H7 heart rate, Polar, US). Contact areas were moistened with water and electrocardiography gel. The transmitter belt communicated via Bluetooth with a dedicated application (Heart Rate Variability Logger, HRV, available on Apple Store) of an iPhone placed on the animal which recorded the HR in a CSV format at 1 Hz.

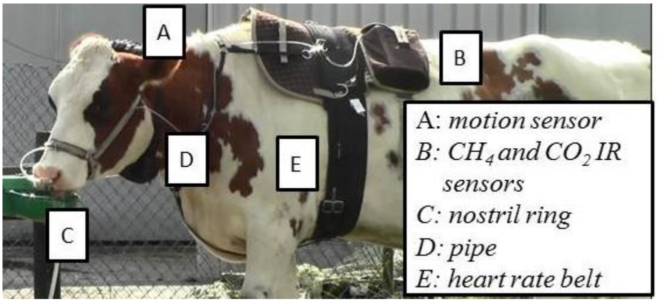

Figure 1. Equipment installed on a grazing cow. The motion sensor of an iPhone 4S is placed inside a waterproof box (A) attached on a halter on the top of the neck. The opening of the pipe (D) is attached to a nostril ring (C) to pump the gas exhaled (A). At the exhaust pipe there are two IR gas sensors to measure CH_4 and CO_2 concentrations.

<u>Signal analysis</u>

Data from the IMU were used to classify the cows' behaviour by time windows of 60 seconds in MatLab R2014a (MathWorks, Natick, MA, USA). MatLab R2014a was also used for the processing of the HR and the analysis of the gas concentration. All these data were synchronized during the processing analyses. HR was averaged over 60 seconds. The calculation of CH_4 DER (daily emission rate, L/day) as described by Madsen et al. (2010) was calculated for each 60-s time windows. For this purpose, every minute, the minimum CH_4 and CO_2 values were considered as background concentrations and subtracted from all the other raw values. Then, CO_2 and CH_4 concentrations were averaged over 60 s. Subsequently, all values below 400 ppm of CO_2 were discarded to avoid samples with very low concentration of breath (Haque et al., 2014). Such rejection (6%) of data was mainly ascribed to clogging of the pipe with grass or water. The technique to estimate the CH_4 DER consisted in using metabolic CO_2 as an internal tracer and multiplying it by the ratio between CH_4 and CO_2 (Equation 1 and 2) (Madsen et al., 2010). The total daily metabolic CO_2 produced by the animal is calculated from the daily heat production (Equation 4). For a dry and non-pregnant cow, the heat production is estimated according to the BW (Equation 3) (Haque et al., 2014).

$$CH_4{:}CO_2 = \frac{\left([CH_4]_{\text{exhaled air}} - [CH_4]_{\text{background}}\right)}{\left([CO_2]_{\text{exhaled air}} - [CO_2]_{\text{background}}\right)}, \qquad Equation\ 1$$

$$CH_4PMR\ (L/day) = \left(CO_{2_{metabolic}}\right) \times CH_4{:}CO_2, \qquad Equation\ 2$$

$$\text{HP} = 5.6 \times BW^{0.75}, \qquad Equation\ 3$$

$$CO_{2_{metabolic}}(L/day) = HPU \times 180 \times 24, \qquad Equation\ 4$$

where:

$[CH_4]$ and $[CO_2]$ $_{in\ exhaled\ air}$	are the concentrations of gas in the air, ppm;
$[CH_4]$ and $[CO_2]$ $_{background}$	are the minimum concentrations in each time window, ppm;
HP	is the heat production from the animals, watt (W);
BW	weight of the animals, kilograms (kg);
HPU	is the heat producing unit (HP/1000);
180	L of CO_2/HPU/h.

Statistical analyses

The CO2, CH4 concentrations, CH_4:CO_2 ratios, CH_4 DER, HR were compared using PROC MIXED in SAS (SAS Institue, Inc., Cary, NC, USA) in a general linear model where the fixed effect of behaviours (grazing, rumination, other), season (fall and summer) and their interaction were tested and the individual cow was used as a random variable. Each time window served as experimental unit. The chemical composition of the faeces (CP, OM, NDF, ADF, ADL) and the DMI of the cows (N=6), as well as forage allowance and the nutritive values of the grass (N=20) (Table 1) in summer and fall were also compared using an one-way ANOVA model in SAS.

Results and Discussion

Pasture nutritive values

The cows grazed the same pasture in summer and fall, but, as expected the characteristics of the forage changed (Table1). While forage allowance remained similar (approx. 17 kg/100 kg BW/d), the nutritional value decreased between the summer and fall as highlighted by an increase in fibre contents and a decrease in crude protein and energy (Table1).

Table 1: Pasture forage allowance and nutritive value in summer and fall.

		Seasons	
	unit	Summer	Fall
DM[1]	g/kg	233.8±23.2[b]	338.0±36.2[a]
FA[2]	kg DM/100 kg BW/d	17.2±7.3[a]	17.3±6.7[a]
Ash	g/kg DM	81.6±6.0[a]	98.7±9.3[a]
Ca	g/kg DM	5.37±1.42[a]	7.19±2.79[a]
P	g/kg DM	3.33±0.17[a]	3.62±0.39[a]
NDF[3]	g/kg DM	388±15.9[b]	570.5±25.3[a]
ADF[4]	g/kg DM	233±9.9[a]	366.8±20.4[a]

ADL[5]	g/kg DM	19.9±3.6[b]	31.7±1.7[a]
DOM[6]	g/kg DM	794.4±7.98[a]	666.8±14.85[b]
DCP[7]	g/kg DM	66.21±11.2[a]	52.44±11.24[b]
VEM[8]	g/kg DM	1054±13.73[a]	847.8±23.46[b]
DVE[9]	g/kg DM	89.6±1.85[a]	64.83±4.3[b]
OEB[10]	g/kg DM	-43.3±9.42 [a]	-35±8.01[a]

[1]DM = dry matter; [2]FA = forage allowance; [3]NDF = neutral detergent fibre; [4]ADF = acid detergent fibre; [5]ADL = acid detergent lignin; [6]DOM = digested organic matter; [7]DCP = digested Crude protein. [8]VEM = Dutch standard for NEL (1 VEM = 6.9 kJ of NEL); [9]DVE = truly digested protein in the small intestine; [10]OEB = degraded protein balance calculated as the difference between the amounts of microbial proteins synthesized in the rumen as a function of the nitrogen inputs and the energy inputs. According to the Dutch Feed Evaluation Scheme (Tamminga et al., 1994).
[a b] means within a line with different superscript letters differ.

Results displayed in Table 2 indicate a shift across the seasons in the faeces characteristics, which contained more fibre and less protein during the fall, matching with the changes observed in forage quality (Table 1). During the fall, the animals ate less, probably as a consequence of the increase in NDF content and the decrease in CP, making the grass less digestible and reducing rumen passage time. Another possible explanation might be due to the increase in selectivity or as an additional consequence of the higher fibre content of the forage, an increase in the difficulty for animals to perform defoliation bites required to fulfil easily their daily forage intake.

Table 2: Bodyweight of the cows and chemical composition of the cows faeces and dry matter intake of grazing cows according to the seasons as estimated by F-NIRS.

period	Bodyweight	CP[1]	OM[2]	NDF	ADF	ADL	DMI[3]
	kg	g/kg DM	g/kg DM	g/kg DM	g/kg DM	g/kg DM	g DM/kg BW
Summer	697.3±82.9[a]	198±12[a]	773±20[a]	402±21[b]	227±17[b]	102±11[a]	25.7±2.2[a]
Fall	696.8±70.8[a]	154±6[b]	814±5[a]	533±12[a]	299±3[a]	100±3[a]	18.3±1.3[b]

[1] total protein content; [2] organic matter; [3] dry matter intake.
[a b] means within a row with different superscript letters differ.

Behaviour and diet/season effect on average methane emission

Table 3 illustrates the impact of season (summer and fall) and behaviours (grazing, ruminating and all other behaviours called “other”) on different items: the HR, the CH_4:CO_2 ratio measured continuously in the animal’s breath, the CH4 DER estimated from the ratio and the metabolic CO_2 and the CH_4 DER corrected by the DMI of individual cow estimated from the F-NIRS. The values were comparable to Madsen et al. (2010) who observed ratio between 0.06 and 0.1 using typical Danish feeding levels. Whereas, Martin et al. (2016) calculated on dairy cows in milk higher values for methane production per unit of feed intake with 32.7 l CH_4 DER / kg of DMI. The cows used in this study were dry.

Table 3: Measurement and estimation of the HR (beat per minute), the CH_4:CO_2 ratio, the CH_4 DER estimated and the CH_4 DER per Kg of DMI.

Main effects		N	HR	CH_4 : CO_2	CH_4 DER	CH DER/ DMI
Seasons	Behaviour		Bpm	-	l/day	l/kg/DMI
Summer	Grazing	1110	93.7±15.3[b]	0.055±0.033[c]	179±104[d]	10.0±6.0[c]
	Rumination	635	73.6±8.7[d]	0.056±0.040[c]	187±132[d]	10.3±7.0[c]
	Other	5694	80.7±17.9[c]	0.055±0.037[c]	180±120[d]	10.1±6.7[c]
Fall	Grazing	1304	97.0±22.7[a]	0.095±0.075[a]	276±212[a]	23.1±18.6[a]
	Rumination	782	73.9±12.4[d]	0.072±0.044[b]	211±129[c]	17.7±10.9[b]
	Other	3164	95.5±26.7[ab]	0.077±0.061[b]	233±178[b]	18.8±15.1[b]
Standard error of the mean			0.26	4.5 E-4	1.34	0.11
Source of variation						
	Season × Behaviour		<0.001	<0.001	<0.001	<0.001
Variance parameter estimates						
	Cow		67.4	1.96E-4	1073	9.5
	Residual		305	22.4E-4	21027	116

a b c d Means within a row with different superscript letters differ.

This work shows a combined effect of season and behaviours on CH_4 emissions, but the part of the variance due to individual cows is low. Indeed, the SD for the CH_4 emissions is important, reflecting a variability of the emission during the day whatever the individual. In summer, there is no difference according to the feeding behaviours, whereas there are differences in the CH_4 production per day and per kg of DMI during fall. In fall, the animals produced more CH_4 during grazing. As the heartbeat rate varies, systematically, within a season according to the behaviour, the HR being higher during grazing than during ruminating, one cannot rule out an additional interaction with CH_4 estimates as metabolic CO_2

production might increase with higher HR, reducing the CH_4 estimates by decreasing the CH_4:CO_2 ratio.
During the summer, regardless of the behaviour, CH_4 emissions were smaller. The grass is richer in energy and proteins and the cows ate more but the feed probably stayed less longer in the rumen. Longer residency times in the rumen are associated with higher CH4 emissions. It is indeed well documented that a diet that is richer in NDF decreases DMI and increases CH_4 production (Hammond et al., 2016).
On pasture studying the impact of specific behaviour is not easy, because the animals achieve many small behavioural sequences. This is why, it is difficult to observe the impact of a specific behaviour on CH_4 emission or to analyse precisely the impact of the post-feeding time on CH_4 kinetics. In stable-fed animals, with a restricted diet given twice a day, during and after the meal a rapid increase of the emission is observed (Blaise et al., 2015). In this study, during fall, CH_4 emission is higher during grazing. As cows spent less time grazing during fall, the impact of post-feeding on CH_4 emission is detectable because the impact of a meal on ruminal fermentation is more pronounced. Lockyer and Champion (2001) also found that CH_4 emission rates tended to follow the feeding activity whereas emission rate fell during ruminating. They explained that CH_4 is emitted when the rumen is congested, so when feed enters the rumen, CH_4 production continues during rumination but in smaller quantities and decreases gradually as the fermenting rumen content gets progressively drained. Hegarty (2013), also reported variations in CH_4 emission rates with an increase matching with grazing bouts.
With this tailor-made device, CH_4 emission of grazing cow at each moment could be monitored. Howerer, the technique is an mere estimation of the CH_4 emission because the method is based on the assumption that the emission of the internal tracer (CO_2) is stable. In this experiment, cows on pasture express grazing cattle behaviours and have physical activities. Hence, a higher HR during grazing than during other behaviours is noticed. As stated before, it means that metabolic CO_2, and hence CH_4 DER, may be undervalued during grazing and overestimated during more quite phases.

Conclusions

This paper shows the possibility of improving the estimation of enteric CH_4 emission monitoring on pasture. Combining this innovative technique to a device monitoring animal behaviour at a high-frequency showed that emissions displayed diurnal evolution that is linked to behaviours and, for the present study, particularly in fall. The CH_4 emission is higher during grazing. The main explication is the impact of immediate post-feeding CH_4 production which occurred when grass reach the rumen. A seasonal evolution was also present, with emissions increasing from summer to fall. This increase was due to a lower forage quality that compensated for the decrease in dry matter intake.

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