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LINKING CATTLE GRAZING BEHAVIOR TO METHANE AND CARBON DIOXIDE DYNAMICS

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SUMMARY

Various methods are presently used to measure methane (CH4) emissions of ruminants on pasture. Those measurements are essential to evaluate nutritional strategies to mitigate enteric CH4 emissions as well as addressing the selection of low producing individuals. On pasture and in the barn, variations in CH4 emissions are observed depending on the time of the day. However, no studies have been made to link these diurnal fluctuations to behavioural phases, especially on pasture. The aim of this study was to understand the individual dynamics of CH4 production and their links to the grazing behaviour. For this purpose, a new tool was specifically developed. Five red-pied dry cows were equipped with infrared CH4 and carbon dioxide (CO2) sensors measuring concentrations in the exhaled air at 4 Hz. The animals were equipped with a heart rate belt (HR) and motion sensors to detect their feeding behaviours (grazing vs. rumination) for periods of 8 h/d. Wind speed (WS) was also monitor to verify interference with sampled gas concentrations. Results showed that using the CH4:CO2 ratio reduced the interference with WS that was observed on raw CH4 and CO2 concentration signals. CH4:CO2 ratio average over 5 min periods indicated that CH4 emissions were lower during grazing than rumination ($P<0.01$). The eructation frequency during grazing (0.48 eructation/min, $P<0.01$) was also lower than during rumination (0.65 eructation/min). HR was higher during grazing that rumination. Because HR is usually linked to metabolic CO2 production intensity, hence influencing the denominator of the CH4:CO2 ratio, further investigation should focus on the quantification of changes in fermentative and metabolic CO2 emissions along the day to estimate total CH4 production more accurately and the relationship between CH4 emissions patterns and post-feeding times.

INTRODUCTION

Characterising ruminant enteric methane (CH4) production dynamics is a particular challenge. Firstly methane emitted by ruminants is a greenhouse gas with a global warming potential 32 times higher than that of CO2 over 100 years. Livestock is responsible of 25% of the total CH4 emissions from anthropogenic sources. Secondly methane production represents a loss between 2 to 12% dietary energy for the animal (Johnson and Johnson, 1995; Holmes et al., 2013).

The reference method for measuring methane emissions is the respiration chamber where animals are kept for several hours and changes in air composition following respiration and eructation are monitored (Storm et al., 2012). On pasture, alternative methods rely on tracer ratio techniques with SF6 being most widely used (Johnson and Johnson, 1995) Since 2010, a novel method based on metabolic CO2 production by the animal has been suggested (Madsen et al., 2010). This sniffer technique and its variations such as the GreenFeed (GF) flux method (C-Lock Inc., Rapid City, USA) (Zimmerman et al., 2013) are becoming increasingly used for cattle CH4 measurements on pasture. The CO_2 produced by the animal is used as an internal tracer to quantify CH_4 emissions. The CH_4 and the CO_2 concentrations exhaled are measured when the animal's head is in a feeder. Combining punctual measurements of the CH_4:CO_2 ratio several times a day to an estimation of the total daily CO_2 production allows estimating CH_4 daily production (Madsen et al., 2010; Haque et al., 2015). Peak frequency and mean peak area of CH_4 concentration were also shown to correlate well with total CH_4 emissions (Garnsworthy et al., 2012; Hegarty, 2013). One major drawback of these methods is related to the fact that CH_4 estimations are extrapolated from few short-term measurements spread more or less evenly over the day (Hammond et al., 2015) and when the animal has it head in a feeder. Therefore, monitoring during feeding or milking only provides short insights to determine the daily methane production (Wu et al., 2015), a maximum of CH_4 emissions occurring after eating when the rumen is most congested (Lockyer and Champion, 2001; Garnsworthy et al., 2012). Moreover, the signal acquisition frequency is often too low (1Hz) to allow a deep investigation of the influence of breathing on CO_2 measurements since respiration has a typical frequency of 0.4-0.8Hz (Reece 2004). Therefore, those methods might induce a bias when quantifying methane production over one day and following the diurnal variation pattern in the dynamics of methane emission that are related to the behavioural phases of the animal (Hegarty, 2013; Velazco et al., 2016).

Tackling these limitations, the objective of this study was to develop a tool to measure CH_4 emission of grazing cattle instantaneously and continuously and assess the limitations of such a device.

MATERIAL AND METHODS

Animals

The experiment was conducted on the AgricultureIsLife research platform of TERRA (University of Liège, ULg). All animal handling procedures were approved by the ethics committee of the ULg [Experiment n°1627]. Five dry red-pied cows weighing 674±36.6kg were set to graze a 2ha ryegrass (Lolium perenne) and white clover (Trifolium repens) pasture in September 2015 in Gembloux, Belgium (50° 33′N 4° 41′E). Each animal was monitored for 2d for 8h/d with 3 types of sensors as described below: (1) gas sensors, (2) movement sensors, and a (3) heart rate sensor. All sensors were synchronized for further data processing. Meteorological data including rainfall, T and wind speed (WS) were obtained for the experimental days.

Gas sensors

The developed device uses two gas infra-red sensors, one for CH_4 and the other for CO_2 (NG Gascard® 0-1 % CH_4 and Gascard® NG 0-10% CO_2, respectively; Edinburgh Sensors, Livingston, UK). Exhaled gas is sucked (24V DC Pump Gascard NG Models) into the sensors (CH_4 sensor being placed upstream from the CO_2 sensor) directly from the nostrils via a 1.85m polyethylene pipe (inner ø 4mm) at a flow rate of 0.4 l/min. A 0.45µm filter ensures protection of both sensors. A major meteorological issue is to collect the expiration air consistently along the day. The position of the tube inlet must remain constant in terms of both distance and orientation. For this purpose, a nostril ring that does not alter the animal's grazing ability has been specifically designed. A microcontroller records data from both sensors at 4 Hz. All components are supplied in power by a 12V battery (XTPower® MP- 24000) yielding 24h of autonomy and placed in two pockets of a rucksack carried on the back of the animal.

Behavior sensor

Simultaneously to gas production dynamics, the behaviour of the animals has been recorded using the method of Andriamandroso et al. (2015) that allows discriminating at a high rate (0.05Hz) grazing and ruminating activities. Briefly, an iPhone (4S Apple Inc., Cupertino, CA, USA) was attached on the halter of the cows. Through a dedicated application (Sensor Data, Wavefrontlabs, available on Apple Store) data from the inertial measurement unit of the iPhone were recorded such as 3D accelerometer, gyroscope and GPS. A Boolean algorithm was finally used to discriminate what the animals have been doing (grazing vs. rumination).

Heart rate sensor

The cows were fitted a heart rate (HR) belt (Equine H7 heart rate, Polar, US). This belt measures the heart rate at 1 Hz and communicates via Bluetooth to a dedicated application (Heart Rate Variability Logger, HRV, available on Apple Store) of an iPhone which records the HR in a CSV format.

Data processing and statistical analyses

CO2, CH4 concentrations, CH4:CO2 ratios, peak frequencies and heart rate data were averaged over 5 min periods. They served as experimental units in the MIXED procedure in SAS (SAS Institute, 2008) testing behaviours (grazing, rumination) as class variable and the individual cows as random variable. Linear regressions between CH4, CO2 concentrations or CH4:CO2 and WS or HR were calculated using the FITLM function in MatLab R2014a (MathWorks, Natick, MA, USA).

RESULTS

As exemplified on Figure 1, CO2 pattern followed that of respiration with maximums and minimums alternating as a consequence of inspiration and exhalation. CH4 pattern was different. When the animal eructed, a sudden rise was measured followed by the convolution of an exponential decay during approximately 25s with the oscillation pattern of the respiration.

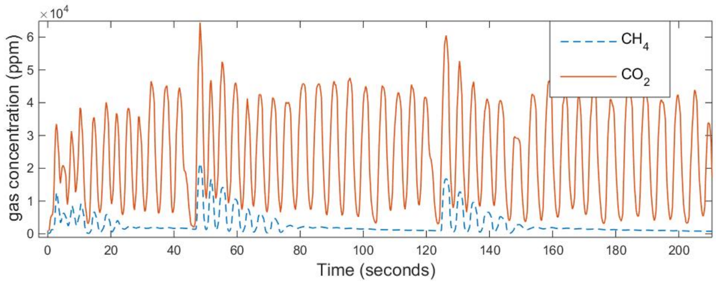

Figure 1. Example of patterns of CH4 (dotted line) and CO2 (solid line) concentration changes in the exhaled air during 3 consecutive eructations of a grazing cow

Gas concentrations and ratio were influenced by the behaviour (P<0.01). When animals were ruminating, both gases were measured with higher concentrations than during grazing (Table 1). The CH4:CO2 ratio followed the same trend with higher values when the animals were ruminating than grazing, however, the extent of the difference seemed to be lower (approx. 20%) than when comparing individual gases concentrations. Heart rate (HR) was affected by the behaviour of the animals, with 59 bpm when ruminating as opposed to 68 bpm when grazing (P<0.01). The observation of the variance parameter indicates that high differences were observed between the cows. Such cow-induced variability was confirmed via visual observation of the data. Finally, in terms of belching frequency, methane peaks were more frequent with 0.652 eructation/min when cows were ruminating than grazing (0.480 eructation/min) (P<0.01) (data not shown).

Table 1. CH4 and CO2 concentrations, CH4:CO2 ratio and heart rate (HR) during grazing and rumination

Main effects	N	CH4 (ppm)	CO2 (ppm)	CH4:CO2 (-)	HR (bpm)
Behaviour					
Grazing	146	724	7.51e3	0.100	68.4
Rumination	54	1317	1.17e4	0.117	60.0
Standard error of the mean		32.8	298	2.20e-3	1.17
Source of variation		P-values			
Behaviour		<0.001	0.005	<0.001	<0.001
Variance parameter estimates					
Cow		8.71e4	1.44e7	1.96e-4	121
Residual		9.73e4	6.92e6	7.81e-4	173

Measured gas concentrations were negatively correlated to wind speeds (WS) (R^2 ranging from 0.102 to 0.571, P<0.01), but its influence was stronger during rumination than grazing (Figure 2). Nonetheless, WS influence on the CH4:CO2 ratio was not significant during rumination (P=0.422) and rather limited for grazing (R^2=0.083, slope=0.0072, P<0.01).

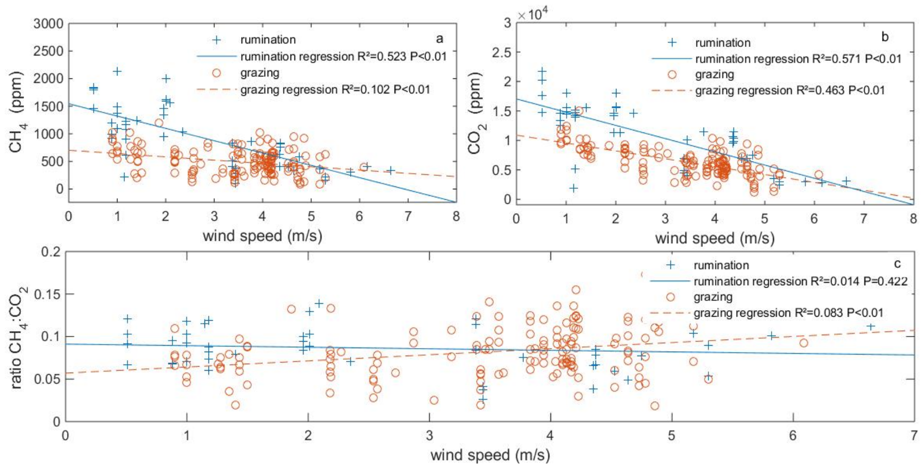

Figure 2 Relationship between concentrations of CH4 (A), CO2 (B) and their ratio (C) measured on 5 cows and the wind speed (m/s), (n=200). The lines show the different regressions for rumination (solid line) and grazing (dotted line).

DISCUSSION

The developed device successfully allowed measuring both CO2 and CH4 emission patterns at a high rate (4Hz) with accuracy. According to Madsen et al. (2010), the average concentration of CO2 in air leaving the lungs ranges from 30,000 to 50,000 ppm. These values are in line with the ones obtained in this study and approx. 10 times higher than the concentrations measured using other sniffer-based techniques (Madsen et al., 2010; Zimmerman et al., 2013). They show that dilution during sampling using this device is low.

Some recorded values were above 50,000 ppm. They corresponded to the conjugation of expiration with the peak of eructation (Figure 1) combining ruminal CO_2 from fermentation to metabolic exhaled CO_2 (Haque et al., 2015). Moreover, to avoid the problems faced by Wu et al. (2015), the use of a nose ring is of utmost importance since according to Haque et al. (2015) and our own observations, instability in CH_4 and CO_2 measurements may originate from the relative position of the pipe inlet to the nostril.

Results from this experiment tend to show that the influence of WS might be neglected when working with CH_4:CO_2 ratios, but not when working directly on CO_2 or CH_4 concentrations. The influence of WS differed according to the behaviour, possibly because of the position of the head. As the animal grazes, it holds its head down, with the nose close to the sward canopy, protected from the wind. During rumination, the animal holds its head in upright position (Andriamandroso et al., 2015), exposing it to the wind, making measurements during rumination more sensitive to WS. Therefore, methods with very low data acquisition frequency integrating CH_4 or CO_2 measurements over several hours or days (Savian et al., 2014) when animals display a range of different unitary behaviours, might oversample gases during grazing as compared to rumination, depending on WS. Consequently, it seems advisable to use the CH_4:CO_2 ratio method since results showed its independence to WS as opposed to CH_4 and CO_2 concentrations.

With 0.105 as average value, CH_4:CO_2 ratios were higher than reported elsewhere (e.g. 0.083 in Aubry and Yan, 2015). However, because HR is usually correlated to metabolic CO_2 production in mammals, differences in HR according to the behaviours suggest that using daily averages for metabolic CO_2 emissions as usually practised in sniffer methods might not be appropriate for short-term measurements. Observed SD's were also higher than in Aubry and Yan (2015) (0.032 vs. 0.011) as a consequence of the variations according to (1) the behaviours along the day, and, (2) more importantly from a selection point of view, to the individual (Table 1). Currently, the diurnal general pattern for CH_4 emissions of grazing ruminants is known. Nonetheless, for research protocols using short-term breath analyses, the duration of the measurement required to satisfactorily detect such variations is not clearly defined yet (Velazco et al., 2016). Our findings support previous observations showing that methane emission dynamics are not steady (Lockyer and Champion, 2001) and diurnal changes of 20% in the CH_4:CO_2 ratio are observed, on average. In this study for instance, CH_4 emissions were higher during rumination than during grazing. This was consistently observed for CH_4 concentrations (1317 vs. 724 ppm), CH_4:CO_2 (0.117 vs. 0.100) and eructation frequencies (0.652 vs. 0.480 eructation/min).

CONCLUSION

It can be concluded that the developed device provided new insights in the dynamics of CH_4 and CO_2 emissions at the individual level consolidating the relevancy of the CH_4:CO_2 approach in sniffer-based techniques to avoid excessive interference from wind speed. However, because HR measurements differed with the behaviour phases, HR data should be considered to correct estimates of metabolic CO_2 emissions. Finally, owing to the limited number of observations, results from this study should be extended to longer grazing periods and over different seasons in order to better understand the impact of postprandial time and time elapsed since the beginning of a behavioural phase on the emission dynamics of grazing cows. Increased knowledge regarding these dynamics should allow the testing of feeding strategies targeted at specific moments of the day to mitigate CH_4 emissions.

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