Towards a robust protocol for enteric methane measurements using a hand held Laser Methane Detector® in Ruminants

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Abstract

Direct measuring of enteric methane in breath of ruminants is becoming popular. Since the first peer-reviewed publication (Chagunda et al., 2009) showed the potential application of the proprietary Laser Methane Detector® (LMD) in ruminants, it has been shown to have strong relationship with traditional techniques such as respiration calorimetric chambers. For example, Chagunda et al, (2013) reported sensitivity and specificity for cows of 95.4% and 96.5%, and for sheep, sensitivity was 93.8% and specificity was 78.7%. However, there is no joined-up protocol covering all aspects, including, data collection, data extraction, data handling, and estimating methane volume from the measured concentration.

Using data from two studies this paper presents results from tests and analysis to develop a method for data extraction, determine optimal recording duration, differentiating breath from eructation; and conversion of methane concentration to volume. The first study used a group of 71 dairy cows with repeated measurements over a 5 week period. Methane was measured by pointing the LMD at the nostril of the cow from a distance estimated to be 1m in the feed-face after midday milking. Measurements lasted 4 to 5 minutes. For each individual time-series measurement, time of recording and cow's tag number were recorded. In the second study measurements were taken from 18 Holstein Friesian heifers simultaneously by the LMD and the metabolic chamber.

In differentiating eructation from breath, one standard deviation for the individual measurement-window, was used as a threshold. This proved to be a biologically meaningful and statistically effective way of distinguishing methane coming from the rumen through eructation and that from the normal breath. An example is the mean of 395.8 (with a standard deviation of 182.7) ppm. To determine the optimum recording duration, five levels of 60s, 120s, 180s, 240s and 300s were created. Gross average of methane emissions was calculated for each recording window. Significant difference was tested using analysis of variance (ANOVA). In this test the only group that resulted in significantly low measurements ($p<0.001$) was the 60s. Given that eructation episodes in cow breath cycles are estimated to be one to three per minute, measurement windows of less than 3 minutes would risk missing out on some eructation episodes. When methane was measured when animals were standing

the relationship between LMD methane and Chamber methane was highest (r = 0.65) while daily averages had the weakest relationship (r = 0.48). This strong and positive correlation allowed us to build regression equations for estimating methane volume (g/day) from methane concentration (ppm) measured by the LMD.

Key words: enteric methane, measuring protocol, breath cycles.

Introduction

Agriculture faces considerable challenges to limit global warming. Livestock play an important role in greenhouse gases emissions, especially in methane. If livestock farming represents 14.5% of the total GHG of human origin (FAO 2010), cattle is the main emitter with 65% of the sector's total output (FAO 2010). Enteric methane produced during digestion is the largest source as 80 to 85% is exhaled. Methane emissions can be reduced by modifying feed intake, feed ration, and through animal genetics. However, collecting enough measurements on a sufficient number of animals to test different reduction strategies is challenging especially with standard techniques such as respiration calorimetric chambers and tracer gas method (SF6). These techniques, although effective and reliable, are however expensive and time consuming. New approaches and proxies will allow measurement to be taken at farm level, in large numbers and without disturbing the animals.

The LMD is a proprietary hand-held methane detector that measures instantly and specifically methane. It is made by Tokyo Gas Engineering Company. It is generally used in detecting methane leaks in such places like gas transportation networks, old mine pits, and landfill. Methane measurements from the LMD are based on infrared absorption spectroscopy, and measures methane concentration while accounting for the plume thickness in ppm-m. The use of Laser Methane Detector (LMD or LMm), suggested for the first time by Chagunda et al, (2009) seems to be a promising and practical tool. Since the first peer-reviewed publication (Chagunda et al., 2009) showed the potential application of the LMD in ruminants, it has been shown to have strong relationship with traditional techniques such as respiration calorimetric chambers. For example, Chagunda et al, (2013) reported sensitivity and specificity for cows of 95.4% and 96.5%, and for sheep, sensitivity was 93.8% and specificity was 78.7%. However, there is no joined-up protocol covering all aspects, including, data collection, data extraction, data handling, and estimating methane volume from the measured concentration. Using data from two studies this paper presents results from tests and analysis to develop a method for data extraction, differentiating breath from eructation; determine optimal recording duration, and conversion of methane concentration to volume.

Materials and methods

LMD data extraction and data agreement with metabolic calorimetric chamber

Metabolic calorimetric chambers are the gold standard for measuring enteric methane emissions in ruminants. For both the validation of the LMD and developing a robust data extraction protocol, there ought to be a strong agreement between data from the LMD and the metabolic chamber. In the current study, eighteen heifers were used at AFBI (Agri-Food and Biosciences Institute) in Hillsborough, Northern Ireland. Methane was measured simultaneously by the Chamber and the LMD from the same animals. The chamber took a measure every 4 minutes meaning that the dataset from the chamber had one measurement in ppm at every 4 min interval. The emissions per day were calculated in g/day. Data from the Chambers and those from the LMD were aligned in a single file using the unique timestamp. The closest measurements in time from the Chamber were associated to the ones from the LMD. In contrast to the chamber, the LMD takes measurement every 0.5s so there is no direct match as

intervals are different. There were 93,184 LMD individual measurement and 85,270 measurements from the chamber, representing a total of 128 recording windows. Heifer activity and behaviour at the time of LMD measurement were recorded. These activities were lying, lying and ruminating, standing and eating, standing, standing and restless, standing and ruminating, drinking and eating. The normality of the distribution was tested using mean (μ), standard deviation (σ), and quantiles. On the high end of the distribution, 99%, 95% and 90% quantile while 1% and 5% quantiles, on the low end, were used to test normality. On the low end, standard deviation was also used to separate methane measurements from normal breath from those culminating from eructation. In this regard, the standard deviation for each measurement window for each cow was calculated. Only individual measurements above one standard deviation were used to calculate the average for each measurement window (Ricci *et al.*, 2012). In order to determine the agreement and best fitted equation between the LMD enteric measurement and the Chamber daily emissions, Pearson correlation and regression analysis were used.

Measuring duration

In order to examine the optimal recording duration, data from a group of 71 dairy cows measured over a five-week period were used. In each week, methane measurements were carried out on 3 consecutive days. Fifteen cows had methane measured every week from the feed-fence after midday milking. The distance between the LMD and the cow was maintained at an estimated 1 m. Measurements were taken for up to 5 minutes per measurement window from each cow. The cow id as well as time of recording was recorded to allow joining the LMD data to the individual-cow specific data. From the original data set, 5 new dataset are created in order to calculate a gross average of methane emissions relative to 60s, 120s, 180s, 240s and 300s. The first one contains all the measurements taken between 0 and 60s,; the second one on the same pattern but for measurements up to 120s, and so on until 300s. The following mixed model was used to test the difference in the methane measurements for the different duration windows:

$$Y_{ijkl} = \mu + \alpha_i + \tau_j + \beta_{jk} + \varepsilon_{ijkl}$$

where,

Y_{ijkl} = average value of the α_i measurement time, the τ_j cow and the l^{th} sample.
μ = the grand mean.
α_i = the effect of the ith measurement time.
τ_j = the effect of the j^{th} cow. τ_j effects are independent and follow a Normal distribution with a variance σ^2CowID and represent the variation due to individuals.
β_{jk} = nested factor with "CowID" i. β_{jk} effects follow a Normal Distribution with a variance σ^2.
ε_{ijkl} = random residual N(0, σ^2).

Results and discussion

LMD data extraction and LMD data agreement with chamber

The descriptive statistics for the enteric methane concentration measured by the LMD and the metabolic chamber are presented in Table 1. Although the averages for the methane measured using the different methods were not massively different, the raw LMD data indicated very high variation. When the LMD measurements were processed as described in the methodology section, both the distribution and the variance normalized.

In general, the measurements of enteric methane concentration from the LMD were higher than those measured using the metabolic chamber. Enteric methane emissions measured by the chamber displayed a normal distribution (Figure 1).

Initial inspection of the raw data indicated a highly skewed distribution. Although the value at 99% quartile was 562.3 ppm, the maximum value was 49287 ppm. As a results two tests were carried out, one by keeping 99% of the dataset and another one by keeping only 95%. For the 99% dataset, measurements above 562 ppm were removed while for the 95% dataset, the threshold of 238 ppm was used. This procedure normalized the distribution without need to transform the data (Figure 2).

Another of the important factors to take into account during enteric methane measurement is the animal activity. Animals always exhibit different behavioral activities. The concentration of methane measured during these activities is also shown to differ

Table 1. Descriptive Statistics for enteric methane measured by the LMD and the metabolic chamber

	Raw LMD data (ppm)	Processed LMD data (ppm)	Chamber methane (ppm)	Chamber methane (g/day)
Minimum	0	24	12	85
Maximum	49287	237	177	170
Mean	125.3	114	84.7	127.8
Variance	294656.9	2020.0	519.3	240.2
SD	542.8	45	22.8	15.5

Number of observations 44208 for LMD and 44208 for the indirect open-circuit respiration calorimetric chamber.

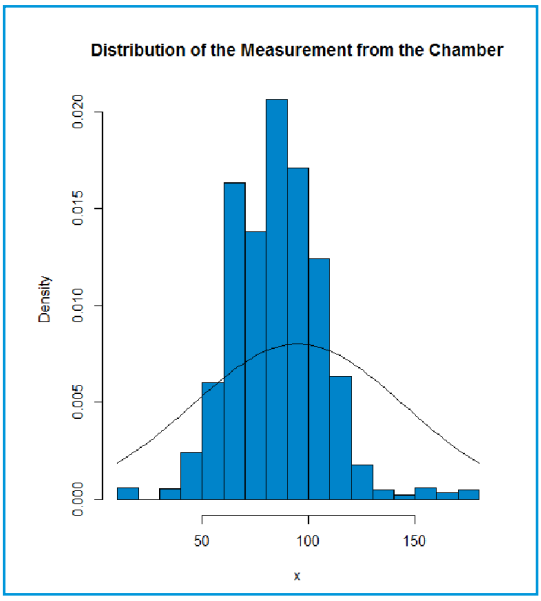

Figure 1. Gaussian distribution curve for enteric methane measured using an indirect open-circuit respiration calorimetric chamber

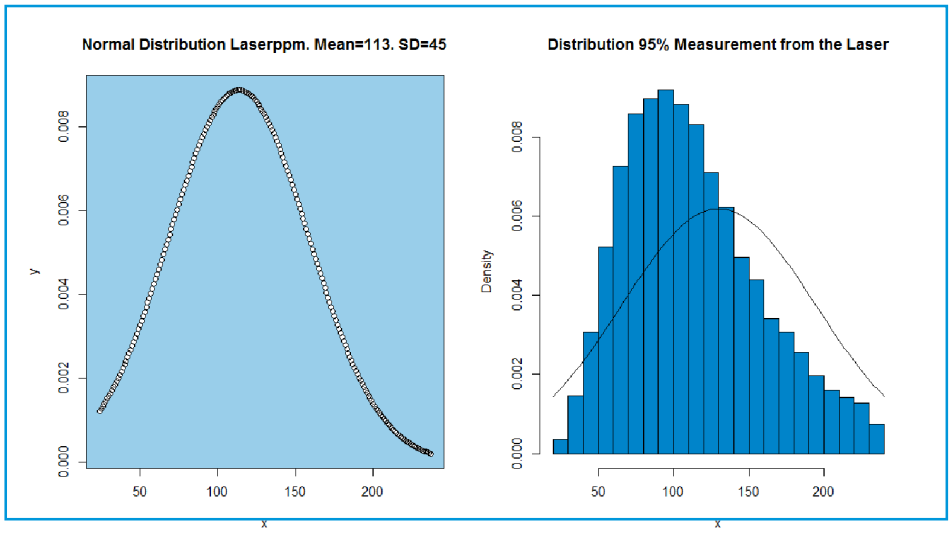

Figure 2. Gaussian distribution curve for enteric methane measured using a Laser Methane Detector.

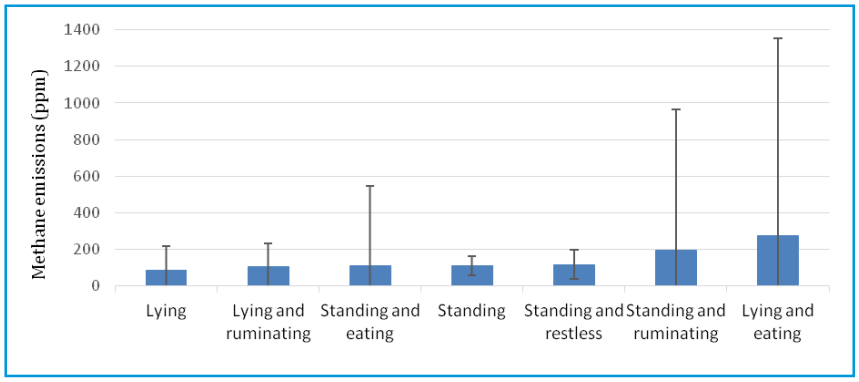

Figure 3. Enteric Methane Emissions (ppm) measured with the LMD depending on the activity.

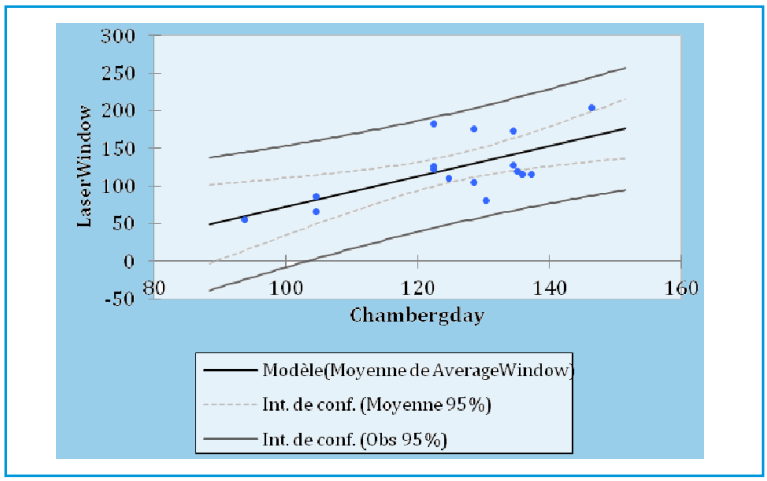

Figure 4. Regression between Laser methane Detector and indirect open-circuit respiration calorimetric chamber.

(Figure 3). When the heifers were standing and eating, standing and ruminating, and lying and eating, the standard error associated with these measurements were very high.

Agreement with the indirect open-circuit respiration calorimetric chamber

For all data, the highest Pearson correlation coefficient was between the Average Laser measurements per day (ppm) and chamber measurements expressed as g/day (r = 0.47). Although this value may not seem very high, the correlation is good. This is because it is a fact the Chamber measures a total methane emission whereas the LMD focuses on enteric methane as it targets the nostrils. The relative strong and positive correlation allowed us to build regression between the LMD and the Chamber data. Inverse regression was used as a calibration method: the value from the LMD is used to estimate the value in g/day that would have been obtained from the Chamber, the gold standard. The LMD measurements were therefore regressed on the Chamber measurements. For prediction, equations were inverted. Two models were built to predict methane emissions in g/day: the first one used an average calculated on one individual-measurement and the second one used the average of the four measurements realized on the day for each heifer. As heifers were involved in different activities while measurements were taken, activities were reported and compared. The activity that showed the best coefficient of determination was the Standing, R^2 = 0.42 and r = 0.65 (Figure 4).

The equation of the model is: LMD (ppm) = -127.21 + 2.00*Chamber (g/day). Therefore, the predictive g/day from a LMD (ppm) from a standing activity is:

$$\mathbf{Chambergday} = \frac{\mathbf{LaserWindow} - \mathbf{127.21}}{\mathbf{2}}\mathbf{.00}$$

Measuring duration

Results indicated that the measurement duration had a significant effect (P<0.01) on the concentration of methane measured by LMD. The results from the ANOVA demonstrate acceptance of the homoscedasticity hypothesis. Pair-wise analysis showed that the only group that resulted in significantly low measurements (P<0.001) was the 60s. This meant that all measurement duration equal to or more than 120s would give results that are not significantly different from each other. However, as eructation happens every minute in cows (Garnsworthy *et al.*, 2012) we suggest that LMD measurement window should be at least 3 minutes long.

Conclusions

The current study was one in a series aimed at generating enough evidence to contribute to developing a robust protocol for enteric methane measurements using a hand held Laser Methane Detector® in Ruminants. Methane measurements from the LMD have shown strong agree with those from the gold standard, the indirect open-circuit respiration calorimetric chamber. Results have also demonstrated the importance of testing and normalizing the distribution of the data before further analysis. Measurement windows longer than 3 minutes are recommended in order to get robust data which capture the required number of eructation.

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