Report of the Short Term Scientific Mission of Sarah Mühlbach

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COST Action FA1302

Host institution: Department of Molecular Biology and Genetics - Center for Quantitative Genetics and Genomics, Tjele, Denmark
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1. Purpose of the STSM

Methane emissions from dairy cows are measured with a series of different techniques like the respiration chamber, SF6, GreenFeed or other sniffers. So far, not much is known about how those data can be compared or combined and especially how data from a Laser Methane Detector are to be treated. The aim of this STSM was to compare measurements from a Laser Methane Detector (LMD) and from a Fourier transform infrared (FTIR) detector in Automated Milking Systems (AMS) in order to analyze their agreement. Methane was simultaneously measured while the cows were milked (LMD & FTIR) or only by LMD while they were ruminating either lying or standing. Thus, methane data under the same condition as in the AMS and under different conditions (location, activity, time) were compared. Furthermore, the influence of distance on LMD measurements was examined with calibration gas containing methane to better characterize this technique.

2. Work carried out

Experiment I: influence of distance of LMD measurement
A calibration gas mixture containing 1% methane was released from a gas bottle with a constant flow rate (2 l/min). At different distances methane was measured with the LMD to find out whether distance was influencing the recorded methane values. The smallest distance was 0.25 m and increased in 0.25 m steps until 2.50 m. For every distance three repeats were performed. Additionally the background concentration of methane was measured at every distance before and after methane was released. The experiment was carried out under windless, laboratory conditions and in the barn where the LMD is normally used. The laboratory was flushed with fresh air before each distance step was examined. The purpose of this was to make sure that no accumulation of the released methane would influence the measurements. Figure 1 shows the experimental set up.

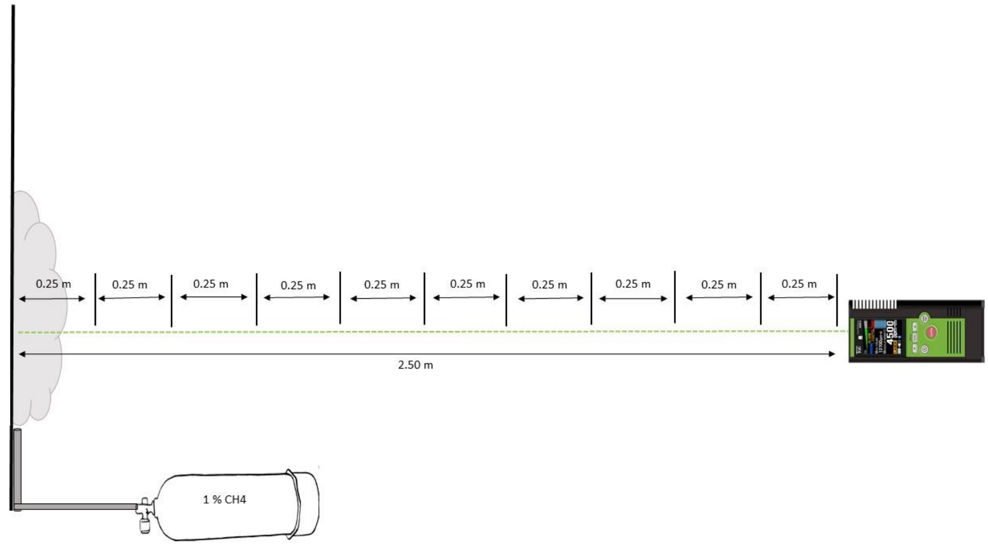

Figure 1: experimental set-up of the distance gas measurement

Experiment II: measurement of methane concentration in an AMS with FTIR and LMD

The sampling tube from FTIR for measuring methane was located in the feed bin of the AMS. For comparing FTIR and LMD under equal conditions the target for the LMD was also the feed bin. For this the LMD was installed at the front of the AMS entrance and was pointed to the feed bin. Every time a cow entered the AMS a measurement was started and ended when the cow finished the milking process. See figure 2 for an impression.

In the data from FTIR all values were removed from those times when the cow's head was out of the feed bin so that no breath methane supposedly reached the sampling tube.

Figure 2: methane measurement in an AMS with LMD

Experiment III: measurement of methane with the LMD while the cows were ruminating and comparison with FTIR

For routine LMD measurements cows are measured while performing a specific activity. We preferred to measure the cows when they were lying ruminating, but also standing ruminating cows were measured. Profiles of the breath of the cows where measured for five minutes with three repeats within four days. The distance between LMD and cow was 2 m.

In addition to the methane measurement the air temperature and humidity was also recorded continuously with a portable data logger. The measurements were performed between 9:00 h and 16:00 h each day.

From the FTIR data all values were removed from those times when the cow's head was out of the feed bin. Then an average methane concentration in ppm for each cow and day was calculated and compared with the LMD phenotypes as described for experiment II.

3. Main results

Experiment I:

The course of the methane values are shown in figure 3. The background methane concentration in the laboratory was smaller than in the barn. On the other hand, the methane concentration of the gas released from the bottle was bigger in the laboratory. Table 1 shows the mean methane concentrations (+SD) for both locations.

Table 1: methane concentrations recorded with an LMD before and after releasing calibration gas containing 1% methane at two different locations

	laboratory (ppm-m)	barn (ppm-m)
background	10 ± 6	27 ± 19
gas released	94 ± 15	45 ± 17

In a linear mixed model distance and location where used as fixed effects. Locations had a significant influence on methane concentrations ($p<0.05$).

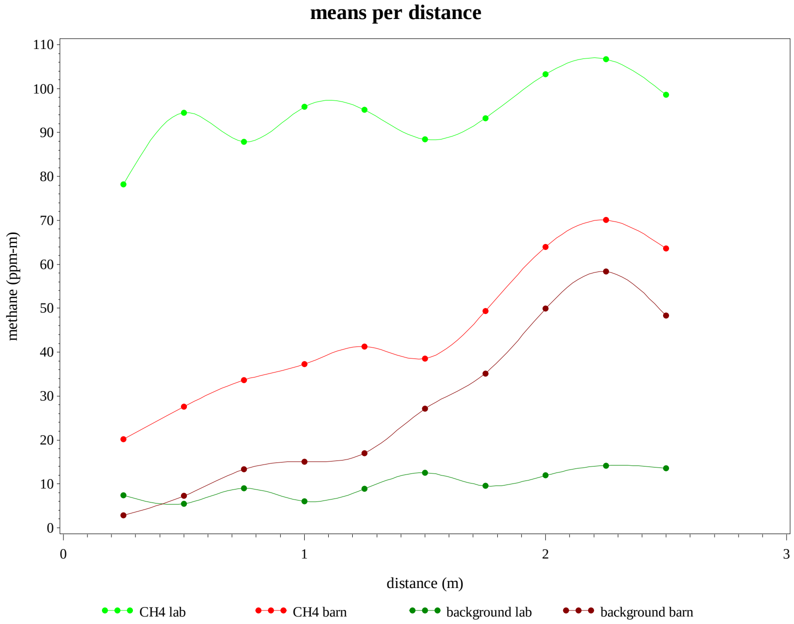

Figure 3: methane concentrations recorded with an LMD before and after releasing calibration gas containing 1 % methane at two different locations (lab=laboratory, barn) and at different distances from the source

Methane concentrations were also significantly different between distances, but only between distances <1.5 m to 2 m and more. Relevant differences between LSMeans and p-values are shown in table 2.

Table 2: differences between LSMeans and p-values of different distances

distance A	**distance B**		
	2.00	**2.25**	**2.50**
0.25	-34 (0.0002)	-39 (<.0001)	-32 (0.0006)
0.75	-23 (0.0500)	-28 (0.0052)	n.s.

n.s. = not significant

Experiment II:

In the AMS methane was measured with the LMD and FTIR over two days. Table 3 gives an overview of all measured data.

Table 3: total measured data from the direct comparison between LMD and FTIR

day	**LMD**		**FTIR**	
	data points	**profiles**	**data points**	**profiles**
1	15536	23	9116	19
2	15554	18	7335	17
total	31090	41	16451	36

For the direct comparison in the AMS 36 profiles with a corresponding FTIR profile were used. The other 5 LMD profiles were recorded to test the experimental setup. For the descriptive statistics of the raw values see table 4 below.

Table 4: descriptive statistics of the mean values from LMD & FTIR measurement

technique	**n (profiles)**	**mean ± SD (ppm-m)**	**min (ppm-m)**	**max (ppm-m)**
LMD	36	129 ± 113	0.0	1149
FTIR	36	312 ± 520	0.0	7200

The Pearson correlation coefficient of FTIR and LMD profile means was 0.397 and significant ($p<0.05$).

Figure 4 illustrates a profile of a cow measured in an AMS with the LMD and FTIR simultaneously. It can be seen that the FTIR values were lagging behind the LMD values. In average the time difference between LMD and FTIR was about 30 seconds due to the transportation to the analyzer unit of the FTIR.

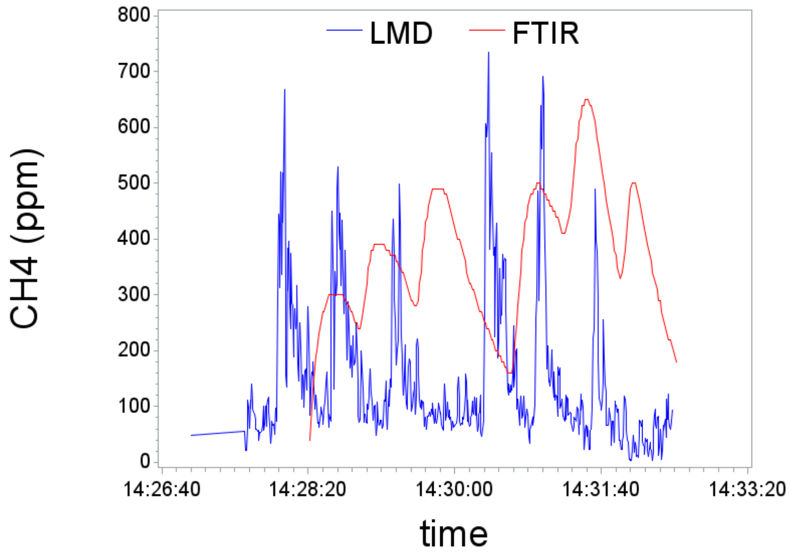

Figure 4: methane profile of a cow with a high agreement between LMD and FTIR

Experiment III:

For the indirect comparison between LMD and FTIR cows were measured on four days. The overview of all measured data is shown in table 5.

Table 5: total measured data from the indirect comparison between LMD and FTIR

day	LMD		FTIR
	data points	**profiles**	**profiles**
1	24 141	33	29
2	32 692	47	43
3	33 079	48	43
4	20 116	27	21
total	110 028	155	136

For the indirect comparison with the FTIR 44 ruminating cows with three repeats were analyzed. The difference between profiles means for lying and standing was tested in a linear mixed model. Both activities had no significantly different methane concentrations ($p=0.428$), so that profiles from both activities were combined.

For an overview of the LMD and FTIR data see table 6.

Table 6: descriptive statistic of methane profiles measured by LMD and FTIR under different conditions

technique	n (profiles)	mean ± SD (ppm-m)	min (ppm-m)	max (ppm-m)
LMD	132	67 ± 57	6	175
FTIR	132	491 ± 274	76	1603

The Pearson correlation coefficient of FTIR and LMD profile means was not significant ($p>0.05$).

4. Conclusion

General conclusion

Three experiments were successfully conducted during this STSM and a unique dataset from two methane measuring techniques, taken under different conditions, was created. The results will likely help other METHAGENE scientists who want to measure methane with the LMD or with the FTIR to analyze their data or to design new experiments.

Experiment I:

The methane concentration measured from a constant methane source differed significantly between laboratory and barn. In the barn lower methane concentrations where measured when gas was released out of the bottle. This could be a result of the different environmental conditions. The laboratory is a closed, small and windless room where methane accumulates easily. The barn is a huge building under outdoor climate conditions so that methane dilute faster. A further example for the influence of the weather can be seen in figure 3. It started to rain heavily between the measurements at distances 2.25 m and 2.5 m. This could have increased the movement of the ambient air so that the released methane was faster diluted than before. As a result the measured methane concentration decreased, in contrast to the earlier trend. Similarly, the other variation in the trend could be explained with varying climate conditions.
In contrast to this, background methane values were higher in the barn. A reason for this were presumably cows producing methane while in the laboratory there was no other methane source.

The higher methane background concentration in the barn was probably the reason for the significant influence of the distance in the barn. The LMD measures the cumulative concentration of along the laser path. With increasing distance, more methane molecules are counted between LMD and target. Vice versa there was only a very low methane background concentration in the laboratory so that the measured methane concentration did not rise significantly even with increasing distance.
This results indicate that the location – and, associated with location, the background methane – plays an important role for methane measurements with an LMD.
Nevertheless these results should be handled with caution because of the low number of observations (3 repeats per distance), so that no final conclusion can be drawn.

Experiment II and III:

Please note that these are only preliminary results. So far only the mean concentration of each profile has been analyzed. A more thorough analysis will follow. Especially the non-significant relationship between LMD profiles taken under routine conditions with FTIR profiles should be investigated in more detail. More methane traits could be derived from the profiles and compared. Also, the data set was very small and should be expanded to derive a more valid conclusion.
A significant relationship was found between FTIR and LMD when measured simultaneously. This is a promising result. However, it is difficult to exactly compare the two techniques. The simultaneous measurement was impaired by the construction of the AMS which prevented a good aiming at the cow's nostrils.

5. Future collaboration with the host institution

Further analysis of the data and the collaborations with the host institute will continue. This work may be the foundation for more joint research projects in the future. A continued work on methane data within METHAGENE is foreseen.

6. Foreseen publications

The investigated results from this comparison of LMD and FTIR shall be published in a scientific peer-reviewed journal. In a second study with another FTIR more data will be collected. So there will be a broader basis for the comparison of the two techniques.

7. Confirmation of the host institution of the successful execution of the STSM

See the attached letter from the host institution.