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# SID 5 Research Project Final Report

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## Project identification

1. Defra Project code: AC0219

2. Project title:
   Methane emissions by individual dairy cows under commercial conditions

3. Contractor organisation(s):
   The University of Nottingham
   School of Biosciences
   Sutton Bonington Campus
   Loughborough
   LE12 5RD

4. Total Defra project costs (agreed fixed price): £ 174,451

5. Project: start date ..... 01 October 2009
   end date ..... 30 June 2010

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## Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.


Dairy systems account for 40% of UK agricultural methane emissions, the majority of which (80%) arise from enteric fermentation in the rumen. Total methane emissions at farm or national level are the product of number of cows and emissions per cow. It is easy to count cows, but it is difficult to measure emissions by individual cows accurately on farms using conventional methods. This presents a problem for evaluating potential mitigation strategies and assessment of their efficacy.
At Nottingham University, we developed a novel technique based on continuous breath analysis during milking. This provides accurate methane concentrations for individual cows and does not affect behaviour. It measures physiological parameters and offers a high level of replication needed for statistical power.

The main objectives of this project were:
**Objective 1** – to collect data on methane emissions by 200 individual cows during milking, which will indicate variation among cows and relationships between methane and production parameters.
**Objective 2** – to calibrate the technique against methane measurements made with respiration chambers and tracer techniques.
**Objective 3** – to compare methane measurements made under Objectives 1 and 2 with predictions from published models.

The main findings of this six-month project are:

Online monitoring of methane in breath during milking provides a low-cost method for estimating daily emissions by individual cows. Importantly, it offers a high level of replication needed for statistical analysis.

The technique calibrates well with daily methane emissions measured subsequently for the same cows in metabolism rooms and fed on the same diet.

Differences between diets designed to alter methane were detected by examining within-cow variation measured by the technique, in agreement with chamber measurements of the same diets.

The on-farm data indicate wide variation in methane emissions both between and within cows, but between-cow variation is considerably greater than within-cow variation. This gives confidence that the technology does measure repeatable parameters that are based on individual cow physiology or behaviour.

Sources of variation include milk yield, live weight, stage of lactation and parity, all of which are related to dry matter intake, which is the main driver of methane emissions. Diurnal variation is another potential source of variation that requires further study, but could be adjusted for by appropriate models.

A limited genetic analysis suggests that there could be a significant genetic component of between-cow variation in methane emissions, offering the potential for future genetic selection.

Comparison with tracer technology indicated that online breath sampling provides estimates of methane emissions that are as accurate, if not more accurate, than the SF6 technique. Importantly, the online technique requires no animal handling, confinement or interference with normal commercial practices.

Comparison of methane emissions measured online with values predicted by published equations, including Tier II used in National Inventories, revealed low accuracy for most prediction equations. This is in agreement with reviews of such equations when tested against chamber measurements and emphasises the need for improved equations coupled with on-farm measurements.

The findings of this study have major implications for National Inventories, mitigation strategies and methane monitoring. For the first time, methane emissions have been measured concurrently in over 200 individual animals fed on the same diet and housed under commercial conditions. This has revealed a high degree of variability between individuals, which needs to be accounted for when compiling an inventory or testing the success of mitigation strategies.

It is suggested that future work is required to deploy the technique on a range of commercial farms to quantify between-farm variation and identify its causes. This would provide evidence for design and monitoring of mitigation strategies to reduce methane emissions by dairy herds. Wide-scale monitoring of individual cows would provide evidence required for genetic analysis to identify low methane emitters that could be selected for use in breeding programmes.

Validation of this technique to monitor methane emissions by individual cows on commercial farms provides a valuable tool for gathering evidence to inform Defra policy decisions and to meet obligations under the UK Climate Change Act.

## Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:
   - the scientific objectives as set out in the contract;
   - the extent to which the objectives set out in the contract have been met;
   - details of methods used and the results obtained, including statistical analysis (if appropriate);
   - a discussion of the results and their reliability;
   - the main implications of the findings;
   - possible future work; and
   - any action resulting from the research (e.g. IP, Knowledge Transfer).

The objectives of the project were:

**Objective 1** – to collect data on methane emissions by 200 individual cows during milking, which will indicate variation among cows and relationships between methane and production parameters.

**Objective 2** – to calibrate the technique against methane measurements made with respiration chambers and tracer techniques.

**Objective 3** – to compare methane measurements made under Objectives 1 and 2 with predictions from published models.
All of these objectives have been met in full.

## Background

Dairy systems account for 40% of UK agricultural methane emissions, the majority of which (80%) arise from enteric fermentation in the rumen. Total methane emissions at farm or national level are the product of number of cows and emissions per cow. It is easy to count cows, but it is difficult to measure emissions by individual cows accurately on farms using conventional methods. This presents a problem for evaluating potential mitigation strategies and assessment of their efficacy. In particular, it is impossible to assign a realistic measure of variation (uncertainty) to the national inventory. There is an urgent need for individual cow data to inform policy decisions and to meet obligations under the UK Climate Change Act.

Conventional methods cannot measure methane emissions accurately in dairy cows kept under commercial conditions. Respiration chambers are accurate, but they distort behaviour and measurements take several days per cow. Polytunnels allow approximation of natural behaviour, but under-predict emissions and are susceptible to temperature fluctuations. Sensors around groups of cows are compromised by variable wind and weather outdoors, and require ventilation control indoors that compromises cow health. Tracer techniques (e.g. sulphur hexafluoride; SF6) give daily values for individual cows, but show great variation and require frequent cow handling and face masks that can interfere with behaviour. At Nottingham University, we developed and tested a novel technique based on continuous breath analysis during milking. This provides accurate methane concentrations for individual cows and does not affect behaviour. It measures physiological parameters and offers a high level of replication needed for statistical power.

Our novel technique uses a methane gas analyzer in a robotic milking system. Individual cows visit the robot between two and six times daily for five to ten minutes per visit and methane concentration in exhaled breath is continuously monitored and recorded at one-second intervals. Preliminary observations of 40 cows showed large increases in methane at approximately one-minute intervals, which corresponded to cows releasing gas from the rumen by eructation. Mean eructation interval per cow varied from 45 to 85 seconds and mean peak methane concentration varied between 800 and 7000 ppm. Importantly, both peak frequency and peak methane concentration were consistent within cows, although they varied markedly among cows.
The aims of the current study were to quantify variation in methane between and within cows and to compare breath measurements with total daily emissions estimated with respiration chamber and tracer techniques.

## Objective 1

## *To collect data on methane emissions by 200 individual cows during milking, which will indicate variation among cows and relationships between methane and production parameters.*

### *Variation in methane emission rate*

Methane concentrations were measured in breath of 219 cows during 66,734 individual robotic milkings at the Nottingham University Dairy Centre between 30 November 2009 and 27 April 2010. An example trace from one milking is shown in Figure 1.1, which shows breath methane concentrations recorded at one-second intervals. Each peak corresponds to one eructation and is characterised by a sudden large increase in methane concentration followed by exponential decay. For each peak, maximum concentration (height above the baseline) and area under the curve (integral) were recorded. For each milking, duration, number of peaks, mean peak height and mean peak integral were recorded. From these data, eructation frequency (peaks per minute) and methane emission rate (mean peak integral times peak frequency) were calculated.

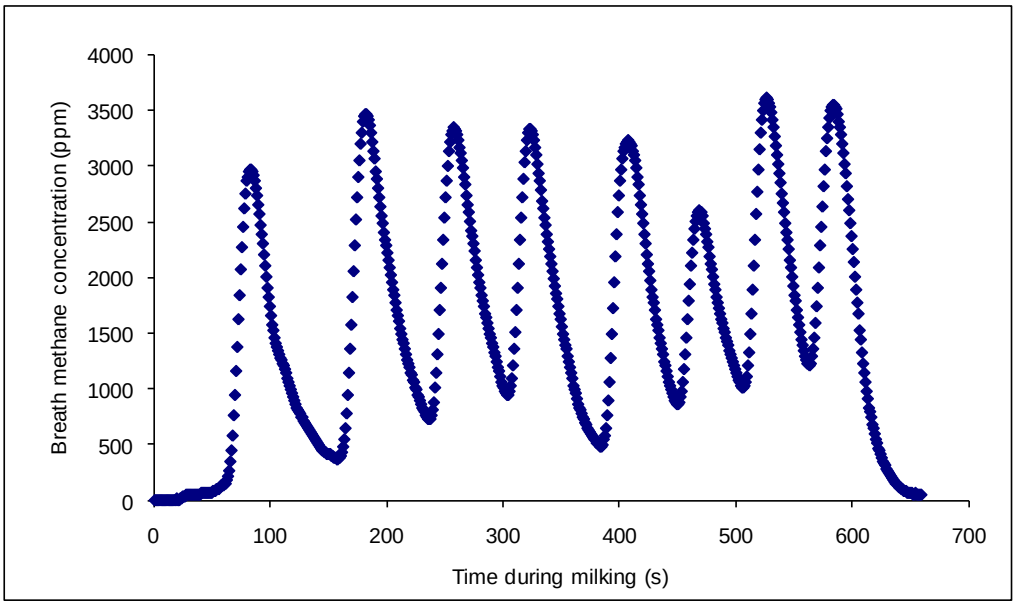


**Figure 1.1** Methane concentration of breath from a dairy cow during milking

The main metric of interest is mean methane emission rate during milking (MERM), which is a measure of quantity of methane produced per unit time. Considerable variation was observed between cows in MERM over the five-month monitoring period (Figure 1.2).

To examine variation in MERM between and within cows, a subset of the data was selected which excluded cows during periods when they were being fed on experimental diets and excluded times during which instruments were offline or being calibrated. The resulting dataset comprised 215 cows with 14533 daily mean values for MERM, milk yield and live weight. All of these cows were fed on the same commercial diet, which consisted of a total mixed ration (TMR; Appendix Table A.1) fed ad libitum, plus concentrates fed in robots during milking at the rate of 1.6 kg/d plus 0.16 kg/kg milk yield above 23 kg/d. Not all cows were present for the whole 5-month period, due to the dynamic nature of the herd (cows calving, being dried off or culled).

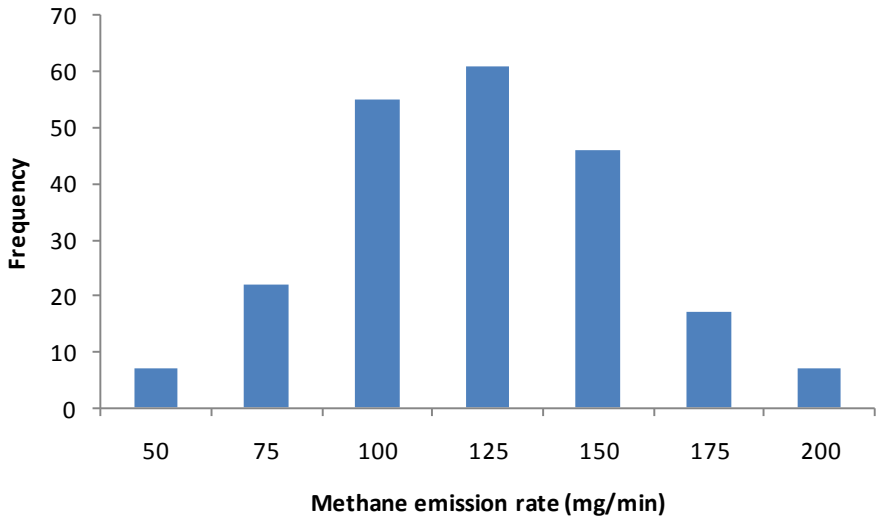


**Figure 1.2** Frequency distribution of individual cows according to average methane emission rate measured in breath during milking over a 5-month period.

Data were analysed using linear models to partition variation between and within cows with accumulated analysis of variance to test the significance of each source of variation. A simple comparison of between-cow versus within-cow variation (Table 1.1a) showed that between-cow variation was 32 times greater than within-cow variation ($P<0.001$), giving confidence that the technology does measure repeatable parameters based on individual cow physiology or behaviour. Much of the between-cow variation can be explained by differences between cows in live weight and milk yield (Table 1.1b) but, even after adjusting for these factors, a significant amount of between-cow variation was still unexplained. Changes in live weight and milk yield during the study period explained significant proportions of the within-cow variation (Table 1.1c). It is likely that live weight and milk

yield act as proxies for dry matter intake, which is the main driver of methane emissions. Stage of lactation, parity and month also affected MERM significantly, but these factors were largely confounded with live weight and milk yield.

**Table 1.1** Analysis of variance tables comparing partitioning of variation in methane emission rate during milking (MERM) between and within cows

| | Source | DF | SS | MS | VR | F prob |
|---|---|---|---|---|---|---|
| a) | Cow | 214 | 261465 | 1222 | 32.5 | <0.001 |
| | Residual | 14318 | 537878 | 37.6 | | |
| | Total | 14532 | 799343 | 55.0 | | |
| b) | Live weight | 1 | 27996 | 27996 | 764 | <0.001 |
| | Milk yield | 1 | 19492 | 19492 | 532 | <0.001 |
| | Cow | 214 | 227181 | 1062 | 28.9 | <0.001 |
| | Residual | 14316 | 524673 | 36.7 | | |
| | Total | 14532 | 799343 | 55.0 | | |
| c) | Cow | 214 | 261465 | 1222 | 33.3 | <0.001 |
| | Live weight | 1 | 8601 | 8601 | 235 | <0.001 |
| | Milk yield | 1 | 4603 | 4603 | 126 | <0.001 |
| | Residual | 14316 | 524673 | 36.7 | | |
| | Total | 14532 | 799343 | 55.0 | | |

## *Genetic effects on methane emission rate*

The size and genetic structure of the herd precludes a full genetic analysis, but some indication of the genetic contribution to variation in MERM can be obtained from examining sire effects. A total of 72 sires were identified for 164 daughters in the selected dataset. Thirty nine sires had only 1 daughter, 11 had 2, 7 had 3, 9 had 4, 2 had 5, 3 had 7, and 1 had 15 daughters. Adding sire to the model shown in Table 1.1b revealed a significant sire effect, which was greater than the effect of daughters within sires (Table 1.2). This suggests that there is a genetic component to variation in MERM which, if confirmed and quantified in a larger study, could be used as a basis for future genetic selection to reduce methane emissions.

**Table 1.2** Analysis of variance table including sire effect on between-cow variation in methane emission rate during milking (MERM)

| Source | DF | SS | MS | VR | F prob |
|---|---|---|---|---|---|
| Live weight | 1 | 19900 | 19900 | 530 | <0.001 |
| Milk yield | 1 | 18498 | 18498 | 493 | <0.001 |
| Sire | 71 | 96816 | 1363 | 36.3 | <0.001 |
| Cow | 92 | 79749 | 867 | 23.1 | <0.001 |
| Residual | 10818 | 406291 | 37.6 | | |
| Total | 10983 | 621254 | 56.6 | | |

## *Diurnal variation in methane emission rate*

Some of the between-cow variation in MERM could be due to diurnal variation. It is well established that methane emissions increase after feeding and remain elevated for several hours during feed digestion and rumination (e.g. Grainger et al., 2007). Variation in MERM according to time of day when milking occurred is shown in Figure 1.2. The herd is fed fresh TMR between 07:00 and 09:00 each day, which is when peak feeding activity occurs, even though TMR is available ad libitum throughout the 24 hours. Methane emission rate rose sharply between 08:00 and 10:00, remained relatively steady throughout the day, and declined between 18:00 and 06:00.

In robotic-milking systems, individual cows have their own unique milking pattern that is consistent from day to day. Milking times relative to feeding time will influence MERM, as will the number of milkings per day. More frequent milking will overcome the influence of diurnal variation on daily means, but it could be an issue in cows milked only once or twice daily. Further investigation of within-day variation in the dataset collected during this study will form the basis of future work in this area. Diurnal variation would have more of an influence on absolute values of MERM in cows milked twice-daily through non-robotic parlours, but would have less of an influence on between-cow variation because all cows are milked at about the same time each day.

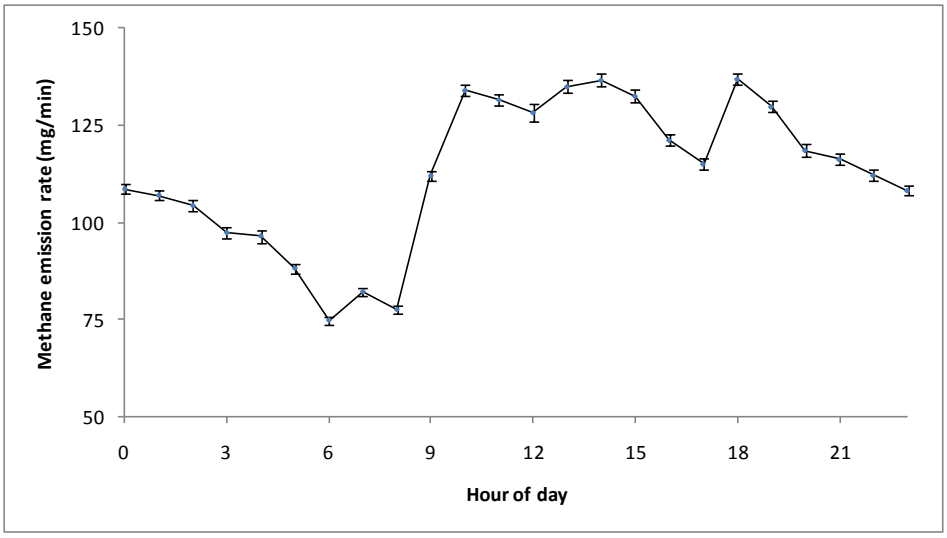


**Figure 1.2** Diurnal variation in average methane emission rate measured in breath during milking of 215 cows over a 5-month period. Data points are means (with standard error bars) of 1000-3000 milkings monitored at each hour of the day.

## *Diet effects on methane emission rate*

An experiment was conducted to test the ability of breath monitoring during milking to detect differences in methane emissions due to diet. The commercial TMR was used as a control (low methane) diet, and an experimental diet was designed to generate higher methane (Appendix Table A.1). The high methane TMR was based on the commercial TMR with an increased proportion of forage from grass silage plus additional sugar beet pulp and peas. All of these ingredients were predicted to increase methane emissions.

Each diet was fed to each of 42 cows for a period of 14 days per diet in a crossover design. Importantly, in view of the between-cow variation observed in the main study, this design used within-cow responses to test diet effects. Diets were fed ad libitum through an automatic feeding system with electronic recording of feed intake. Concentrates were allocated during milking at the rate of 1.6 kg/d plus 0.16 kg/kg milk yield above 23 kg/d. Breath methane concentrations, milk yield and live weight were recorded automatically at each milking.
Data were analysed using linear models to partition variation between and within cows with accumulated analysis of variance to test the significance of each source of variation in MERM. Sources of variation tested included feeding period, date, dry matter intake, milk yield, live weight and diet.

When fed on the high methane diet, cows consumed less dry matter (P<0.001) and yielded less milk (P=0.034) than when fed on the low methane diet (Table 1.2), but live weight was not affected by diet. Methane emission rate during milking was significantly greater when cows were fed on the high methane diet whether expressed as mg/min (P=0.042) or mg/min per kg dry matter intake (P<0.001).Thus, we can conclude that the monitoring system was able to detect diet effects on methane production under farm conditions.

**Table 1.2** Effect of diets designed to alter methane emissions on dry matter intake, milk yield, live weight and methane emission rate measured during milking

| | Low methane diet | High methane diet | sed | P |
|---|---|---|---|---|
| Dry matter intake (DMI; kg/d) | 23.6 | 20.3 | 0.31 | <0.001 |
| Milk yield (kg/d) | 32.7 | 32.1 | 0.28 | 0.034 |
| Live weight (kg) | 622 | 621 | 1.3 | 0.656 |
| Methane emission rate (mg/min) | 110 | 115 | 2.43 | 0.042 |
| Methane emission rate (mg/min/kg DMI) | 4.83 | 5.83 | 0.160 | <0.001 |

## Objective 2

## *To calibrate the technique against methane measurements made with respiration chambers and tracer techniques.*

### *Calibration against respiration chambers*

Twelve lactating cows (milk yield 20-40 kg/d) were monitored at the Nottingham University Dairy Centre for at least 10 days before transfer to two metabolism rooms at the research unit. At the Dairy Centre, methane concentration was determined at one-second intervals in breath of individual cows during milking in robots (approximately 30 milkings per cow). Data were analysed for peak frequency (eructation rate), maximum methane concentration in each eructation (peak height), and total methane release per eructation (peak integral). Methane emission rate during milking (MERM) was calculated as the product of peak frequency and peak integral.
Cows were housed individually in one of two metabolism rooms for 3 to 7 days per cow. Methane emissions by cows in rooms were calculated as the difference between inlet and outlet methane concentrations multiplied by volumetric air flow at the outlet and corrected to standard temperature and pressure. Cows were milked, fed and cleaned twice-daily in the rooms. An airlock system was installed to minimise loss of methane from the rooms during entry and exit by personnel, and abnormal data around these times were discarded.
Both at the Dairy Centre and in metabolism rooms, all cows were fed ad libitum on the commercial maize/grass silage TMR (Appendix Table A.1) plus concentrates at the rate of 1.5 kg/d plus 0.16 kg/kg milk yield above 23 kg/d.

Relationships between methane measurements made during milking at the Dairy Centre and subsequently in metabolism chambers were examined by simple linear regression. Total daily emissions in the chambers were not related significantly to peak frequency, but highly significant linear relationships were found between chamber emissions and peak height, peak area and MERM (Table 2.1). Each of these prediction equations accounted for more than 60% of the variation in total daily emissions. The best predictor of daily emissions was MERM (Figure 2.1).

**Table 2.1** Predictions of total daily methane emissions (g/d) measured in metabolism chambers from characteristics of methane peaks measured previously in the same cows during milking under commercial conditions over a 10-day period prior to chamber measurements

| | Constant | s.e. | Slope | s.e. | %var | P |
|---|---|---|---|---|---|---|
| Peak Height (mg/l) | 292.7 | 17.6 | 0.240 | 0.047 | 69.2 | <0.001 |
| Peak Area (mg) | 275.1 | 23.8 | 0.776 | 0.175 | 63.0 | 0.001 |
| Peak area x frequency (mg/min) | 278.6 | 16.9 | 0.853 | 0.140 | 76.7 | <0.001 |

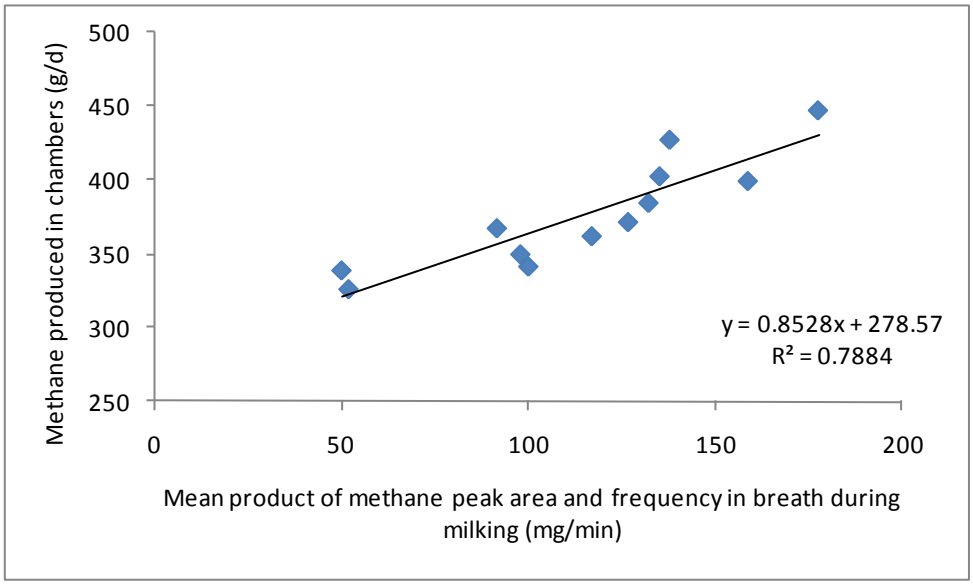


**Figure 2.1** Relationship between total daily methane emissions measured in metabolism chambers and methane emission rate (peak area x frequency) measured previously in breath during milking over a 10-day period.

## *Diet effects*

After methane measurements in metabolism rooms were completed for cows fed on the commercial (low methane) TMR, each cow was transferred onto a diet designed to increase methane production by inclusion of peas and greater proportions of grass silage and sugar beet pulp (Appendix Table A.1). Cows were fed on the high methane diet for 10 to 14 days before being returned to metabolism rooms for a second period of methane measurements. One cow was eliminated between periods due to illness.

Daily total methane emissions increased from 377 ±10.7 g/d for the low methane diet to 395 ±12.7 g/d for the high methane diet. This increase was not significant (P = 0.097) and was lower than expected because dry matter intake was reduced by the high methane diet. Average dry matter intake was 15.3 ±0.46 kg/d for the low methane diet and 13.7 ±0.58 kg/d for the high methane diet. To overcome the confounding effects of dry matter intake and diet, methane emissions measured in chambers were expressed as g/kg DMI for comparison with breath data collected during milking at the Dairy Centre. Mean methane emissions measured in chambers were 24.9 ±0.89 g/kg DMI for the low methane diet and 29.0 ±0.95 g/kg DMI for the high methane diet; the difference was highly significant (P <0.001). Interestingly, this increase in methane emissions in chambers (16%) is similar to the increase in MERM (20%) observed in the diet trial of Objective 1.

Relationships between methane measurements made during milking at the Dairy Centre and measurements in metabolism chambers for both diets are shown in Table 2.2. Again, significant relationships were found for peak height, peak area and MERM. For each predictor, the slope of the equation was similar for low and high methane diets; only the constant changed significantly. This is illustrated for MERM in Figure 2.2.

**Table 2.2** Relationships between methane emissions (g/kg DMI) measured in metabolism chambers and characteristics of methane peaks measured previously in the same cows during milking over 10 days prior to chamber measurements for cows fed on diets designed to induce low or high methane emissions

| | Diet | Constant | s.e. | Slope | s.e. | %var | P |
|---|---|---|---|---|---|---|---|
| Peak Height (mg/l) | Low | 17.9 | 1.51 | 0.020 | 0.004 | 67.4 | <0.001 |
| | High | 22.8 | 1.86 | 0.018 | 0.005 | 53.5 | 0.006 |
| Peak Area (mg) | Low | 16.3 | 1.96 | 0.065 | 0.014 | 64.1 | 0.001 |
| | High | 19.3 | 1.35 | 0.073 | 0.009 | 84.6 | <0.001 |
| Peak area x frequency (mg/min) | Low | 16.8 | 1.46 | 0.071 | 0.012 | 75.1 | <0.001 |
| | High | 21.4 | 1.60 | 0.067 | 0.013 | 71.1 | <0.001 |

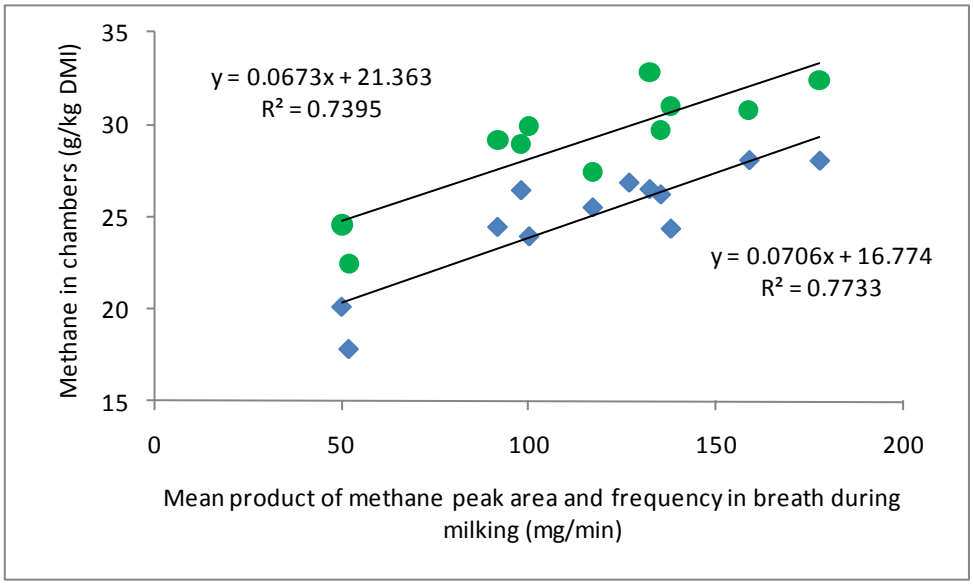


**Figure 2.2** Relationship between daily methane emissions (g/kg DMI) measured in metabolism chambers and methane emission rate (peak area x frequency) measured previously in breath during milking over a 10-day period. Cows were fed on diets designed to produce low (diamonds) and high (circles) methane emissions.

For both diets, dry matter intake in metabolism rooms was approximately 30% lower than similar cows fed on the same diets under semi-commercial conditions at the Dairy Centre. This was accompanied by a 30% lower in milk yield. Reduced intake and milk yield are usually reported for cows housed in metabolism chambers and result from confinement and, in this instance, reduced milking frequency. It is likely that actual methane emissions under commercial conditions are 20-30% higher than those measured in chambers, but we have not adjusted the calibration in Figure 2.1 for two reasons. Firstly, we want our data to be comparable with other chamber studies;

secondly, individual intakes are not available for these cows whilst at the Dairy Centre. Calibrations in Figure 2.2 do allow for differences in dry matter intake and predict that emissions would be 200 g/d greater on-farm than predicted in Figure 2.1.

### *Diurnal variation*

Whilst cows were in metabolism rooms, methane concentrations in breath were measured continuously using the same system as that employed during milking in robots, except that breath samples were obtained via a SF6 sampling harness (see below) instead of via a fixed sampling point. Breath sampling allows comparison of diurnal variation concurrently in chamber and breath measurements.

As expected, diurnal variation in methane emissions followed a clear and consistent pattern (Figure 2.3a). Methane production was lowest before morning feeding, rose sharply to a plateau that was maintained through the day, rose sharply to a maximum following the evening feed, and then declined steadily throughout the night. Methane production measured by breath sampling followed a similar diurnal pattern. Figure 2.3a shows that the 2-hour rolling average for chamber methane changed in parallel with the product of breath methane peak area and frequency, in response to recorded eructations (Figure 2.3b).

Despite the high degree of diurnal variation, it was interesting to confirm that measurements made during milking are representative of total daily emissions.

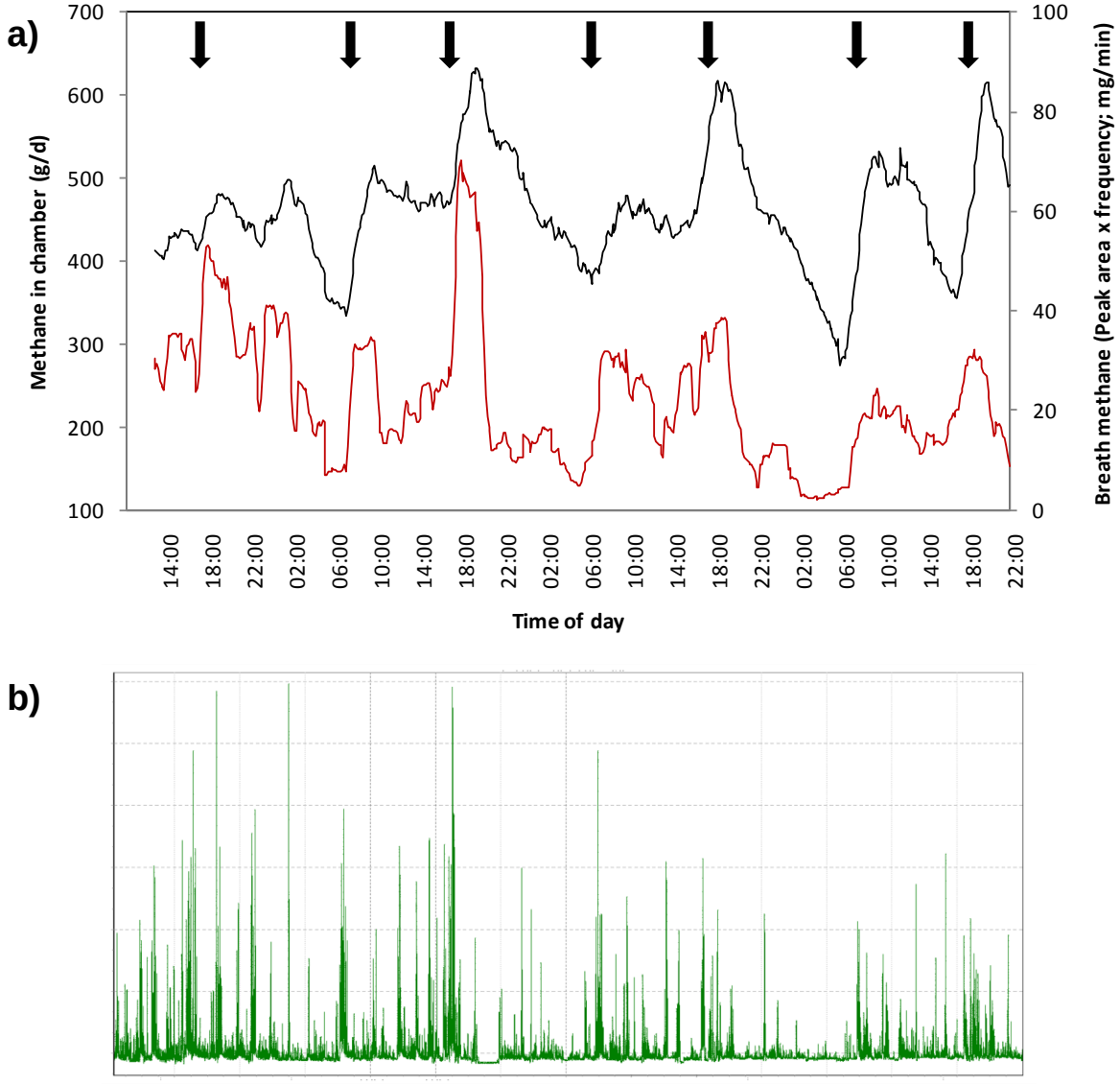


**Figure 2.3** Example of diurnal variation in average chamber methane (a, black line) and average breath methane (a, red line). Data are moving 2-hour averages for an individual cow housed in a metabolism chamber for 3.5 days; arrows indicate feeding times. Panel b shows a trace of breath methane concentration over the same time period.

### *Comparison with tracer measurements*

The sulphur hexafluoride (SF6) technique (Johnson et al., 2007) was adapted for use whilst cows were in metabolism chambers. The technique estimates methane emission rate as the product of SF6 release rate and the ratio of methane to SF6 in breath samples. Three days prior to transportation to the chambers, a permeation tube was placed in the rumen of each cow via a bolus gun. Permeation tubes were supplied pre-calibrated by the Department of Animal Sciences, Washington State University, with an average SF6 release rate of 1.246 µg/min. As with the standard technique, each cow was fitted with a halter to hold an 8mm I.D. copper pipe in close proximity to the cow's nostrils in order to collect breath samples. Instead of collecting breath samples in an evacuated yoke around the cow's neck, however, breath was transferred to the laboratory side of the chambers via a long plastic tube. This tube was connected to an infra-red methane analyser, as used for online breath monitoring during milking. To mimic the yoke collection system of the standard SF6 technique, breath was collected from the exhaust outlet of the methane analyser into an evacuated 4-litre bottle attached to a stainless steel capillary tube (0.005" I.D.; 30cm long) which regulated gas flow over a 12-hour sampling period. Preliminary tests had showed no difference in methane concentration of gas collected via the analyser exhaust or directly into bottles.

Following the standard SF6 technique, bottles were re-pressurised with nitrogen to 0.3 bar at the end of each sampling period. Sub-samples of gas (15ml) were collected using a syringe and injected into 10ml evacuated plastic tubes. Breath samples collected into tubes were then analysed for SF6 and methane using gas chromatography. Daily methane production rate was estimated by multiplying the methane to SF6 ratio in sampled gas by the SF6 release rate for the bolus inserted into the rumen of each cow. Estimates were compared with mean methane production measured in the chambers over the same time period as the sampling period for each bottle. This eliminated any influence of diurnal variation.

Two cows had to be eliminated from the study because SF6 could not be detected in their breath samples. We assume that their boluses were lost by regurgitation. Some bottles had to be eliminated because either they lost vacuum or the sampling tube became blocked by feed or drinking water. The final dataset comprised 2 to 4 bottles collected from each of 10 cows during each diet period.

Mean methane emissions predicted by the SF6 technique were 356 ±9.7 g/d for the low methane diet and 369 ±12.5 g/d for the high methane diet, both of which are approximately 20 g/d lower than chamber measurements of methane emissions. The relationship between methods was analysed by a linear mixed model with chamber methane as the dependent variable, SF6 methane and diet as fixed effects, and cow as a random effect. There was no difference between diets in the relationship, but the combined prediction of methane by SF6 (Figure 2.4) was significant (P=0.014). The correlation between the two techniques (0.58) is lower than that observed at AFBI (0.76; McCourt et al., 2008), possibly due to between-cow DMI variation in the current study; McGinn et al (2006) found correlations of 0.53 with unrestricted feeding and 0.76 with restricted feeding.

These results confirm that online breath sampling during milking provides estimates of methane emissions that are as accurate, if not more accurate, than the SF6 technique.

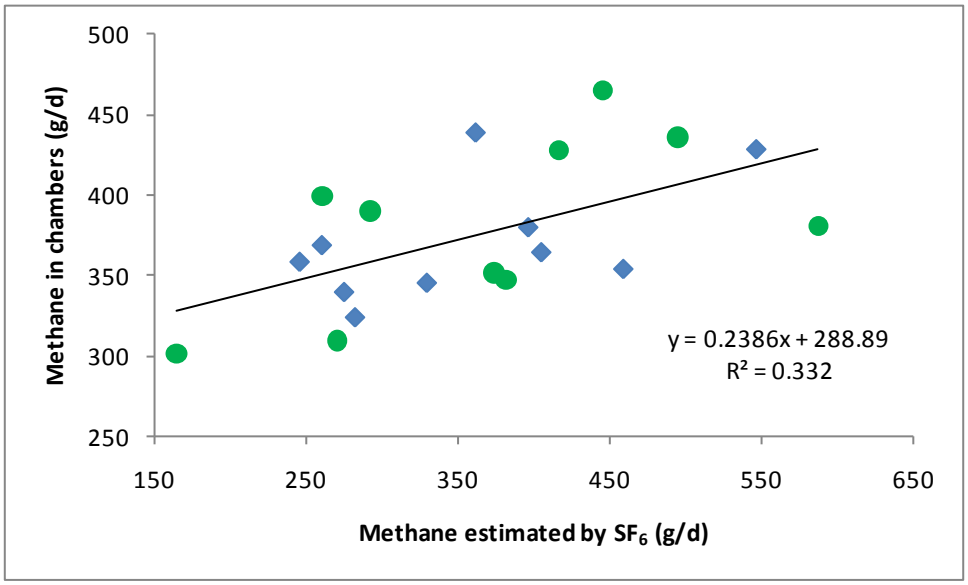


**Figure 2.4** Relationship between total daily methane emissions measured in metabolism chambers and estimated by using the SF6 technique in 10 cows. Cows were fed on diets designed to produce low (diamonds) and high (circles) methane emissions.

## Objective 3

## *To compare methane measurements made under Objectives 1 and 2 with predictions from published models.*

The aim of this study was to see how well published equations that predict methane from parameters such as milk yield, feed intake and diet composition would agree with methane measured using breath monitoring under commercial conditions.

A review of the literature revealed remarkably few equations that might be used to predict methane emissions by dairy cows from factors that could be recorded on farms. This topic was reviewed by Ellis et al. (2010) who evaluated 9 prediction equations against methane emissions measured by indirect calorimetry, mass balance or hood calorimetry. They found that predictions were generally poor, with prediction errors ranging from 20 to 52 % of observed means.

For the current study, seven prediction equations (Table 3.1) were evaluated. The first four equations were included in the review of Ellis et al. (2010); the other three equations were generated by Reading University in Defra projects CC0239 (Yates et al., 2000) and WA0320 (Mills et al., 2003).

**Table 3.1** Equations evaluated for their ability to predict methane emissions measured during milking

| Source | Equation |
|---|---|
| Corré (2002) | CH4 (g/d) = [50 + 0.01 x Milk yield (kg/d) x 365]/365 x 1000 |
| IPCC (1997) Tier II | CH4 (g/d) = [0.06 x Gross energy intake (MJ/d)]/0.05565 |
| Kirchgeßner et al. (1995) | CH4 (g/d) = 10 + 4.9 x Milk yield (kg/d) + 1.5 x Body weight (kg0.75) |
| Schils et al. (2006) | CH4 (g/d) = 20 x Concentrate intake (kg/d) + 22 x Corn silage intake (kg DM/d) + 27 x Grass intake (kg DM/d) |
| Yates et al. (2000) | CH4 (MJ/d) = 1.36 + 1.21 x Dry matter intake (kg/d) – 0.825 x Concentrate intake (kg/d) + 12.8 x NDF (g/kg DM) |
| Mills et al. (2003) - 1 | CH4 (MJ/d) = 5.93 + 0.92 x Dry matter intake (kg/d) |
| Mills et al. (2003) - 2 | CH4 (MJ/d) = 1.06 +10.27 x Dietary forage proportion + 0.87 x Dry matter intake (kg/d) |

Prediction equations were tested using data from the 42 cows used in the diet study of Objective 1 and the calibrations against chamber measurements from Objective 2. Equation parameters were calculated for each cow from its mean milk yield, live weight, and/or feed intake, plus appropriate values for diet composition. Observed daily methane emissions were calculated from individual MERM values in two ways; firstly by using the direct calibration equation (Table 2.1) to convert from MERM directly to emissions in g/d; secondly, by using the separate calibration equation for each diet (Table 2.2) to convert from MERM to emissions as g/kg DMI, which were then multiplied by DMI to give emissions in g/d. Because DMI appears on both sides of the second observed/predicted comparison, a third comparison used predictions from published equations divided by DMI.

Accuracy of prediction equations was evaluated by calculating the root mean square prediction error (RMSPE), which is a standard indicator of accuracy, and its constituent errors due to bias (ECT), slope (ER) and random disturbance (ED). Pearson correlation coefficients (R) were calculated to show strength of relationships between predicted and observed values. These statistics are in Tables 3.2-3.4, together with RMSPE values published by Ellis et al. (2010) or the original authors when comparing prediction equations against chamber measurements.

When methane emissions per day were calculated by direct calibration (Table 3.2), predictions were generally poor and none of the correlation coefficients was significant (critical R for $P < 0.05 = 0.362$). Inaccurate prediction cannot be attributed to the technique for measuring methane because prediction accuracy was comparable with published error values. The equations with the smallest errors were Kirchgeßner et al. (1995), which is based on milk yield and live weight, and Schils et al. (2006), which is based on intakes of concentrates and forage types. The former is in agreement with our analysis of MERM under Objective 1, where we found that milk yield and live weight accounted for significant proportions of MERM. The latter is appropriate for the feeding system used in the study herd (and a large proportion of UK herds), where higher-yielding cows are fed increasing proportions of concentrates. The equation of Yates et al. (2000) also performed relatively well because it includes concentrate proportion. The equations of Corré (2002), IPCC (1997) and Mills et al. (2003) all produced high RMSPE values because they are based on linear predictions from milk yield or dry matter intake with no adjustment for diet composition. It should be noted that the low RMSPE values published by Mills et al. (2003) are for prediction of their own dataset and the high RMSPE values are for prediction of independent data from US studies.

**Table 3.2** Ability of published equations to predict daily methane emissions measured during milking (direct calibration of methane)

| Equation | R | ECT | ER | ED | RMSPE | RMSPE (published) |
|---|---|---|---|---|---|---|
| Corré (2002) | 0.148 | 49.3 | 1.59 | 49.7 | 31.7 | 34.2 |
| IPCC (1997) Tier II | 0.185 | 50.0 | 1.88 | 48.8 | 27.3 | 20.9 |
| Kirchgeßner et al. (1995) | 0.230 | 20.0 | 13.91 | 67.0 | 13.0 | 20.9 |
| Schils et al. (2006) | 0.253 | 5.3 | 4.59 | 91.3 | 15.8 | 39.5 |
| Yates et al. (2000) | 0.267 | 40.0 | 1.98 | 58.7 | 20.8 | 9.7 |
| Mills et al. (2003) - 1 | 0.185 | 70.4 | 2.30 | 27.7 | 29.3 | 7.0 - 43.7 |
| Mills et al. (2003) - 2 | 0.217 | 93.8 | 0.61 | 5.6 | 56.0 | 6.4 – 58.8 |

R = Pearson correlation coefficient; ECT = Error due to bias; ER = error due to slope; ED =random error; RMSPE = Root mean square prediction error; RMSPE (published) = RMSPE published by Ellis et al. (2010) or authors.

When methane emissions per day were calculated by calibration as g/kg DMI, correlations were all significant and RMSPE values were generally lower, indicating more accurate predictions (Table 3.3). This is because the majority of prediction equations relate to DMI in some way. This highlights the need for caution when using emissions factors based linear relationships with DMI, such as IPCC (1997) Tier II methodology, which states that methane emissions are 6.5% of gross energy intake.

**Table 3.3** Ability of published equations to predict daily methane emissions measured during milking (calibration of methane per kg DMI)

| Equation | R | ECT | ER | ED | RMSPE |
|---|---|---|---|---|---|
| Corré (2002) | 0.521 | 7.7 | 7.97 | 85.4 | 17.9 |
| IPCC (1997) Tier II | 0.725 | 4.2 | 6.47 | 90.5 | 12.1 |
| Kirchgeßner et al. (1995) | 0.600 | 71.5 | 17.87 | 11.0 | 22.6 |
| Schils et al. (2006) | 0.904 | 87.7 | 2.71 | 9.7 | 18.4 |
| Yates et al. (2000) | 0.959 | 30.1 | 9.16 | 61.6 | 5.1 |
| Mills et al. (2003) - 1 | 0.725 | 29.9 | 17.99 | 52.9 | 12.7 |
| Mills et al. (2003) - 2 | 0.828 | 93.6 | 2.67 | 3.8 | 33.6 |

R = Pearson correlation coefficient; ECT = Error due to bias; ER = error due to slope; ED =random error; RMSPE = Root mean square prediction error.

When predictions were adjusted for DMI and compared against emissions estimated using calibration of methane emissions as g/kg DMI (Table 3.4), most values for R and RMSPE were similar to the previous analysis. A notable exception was the IPCC (1997) Tier II where there was no correlation and all of the error moved to the slope (ER). This is because IPCC (1997) Tier II uses one fixed emission factor for all cows.

**Table 3.4** Ability of published equations to predict daily methane emissions measured during milking (calibration of methane per kg DMI and predictions per kg DMI)

| Equation | R | ECT | ER | ED | RMSPE |
|---|---|---|---|---|---|
| Corré (2002) | 0.384 | 8.8 | 6.02 | 86.3 | 18.3 |
| IPCC (1997) Tier II | -0.042 | 2.0 | 99.19 | 0.0 | 11.6 |
| Kirchgeßner et al. (1995) | 0.493 | 71.7 | 7.31 | 21.3 | 21.7 |
| Schils et al. (2006) | 0.803 | 86.6 | 4.53 | 9.0 | 18.7 |
| Yates et al. (2000) | 0.920 | 27.1 | 7.11 | 66.7 | 5.3 |
| Mills et al. (2003) - 1 | 0.393 | 29.2 | 61.29 | 10.4 | 12.6 |
| Mills et al. (2003) - 2 | 0.564 | 89.8 | 1.67 | 8.6 | 35.0 |

R = Pearson correlation coefficient; ECT = Error due to bias; ER = error due to slope; ED =random error; RMSPE = Root mean square prediction error.

A feature of any prediction equation is that it performs best when used on the dataset from which it is derived. Equations are often derived from chamber measurements with cows fed on a range of diets. In the current study, only two diets were used, so the potential range of emissions was smaller than might be encountered in practice. However, this is offset by the fact that cows were kept under commercial conditions rather than the controlled conditions of respiration chambers.

The overall conclusion from Objective 3 is that published prediction equations were generally poor at predicting methane emissions on a daily basis, but performed better when adjusted for dry matter intake. Although poor prediction accuracy is disappointing, it is entirely commensurate with similar reviews and studies involving prediction of methane emissions in independent datasets.

## Conclusions

The main findings of this six-month project are:

Online monitoring of methane in breath during milking provides a low-cost method for estimating daily emissions by individual cows. Importantly, it offers a high level of replication needed for statistical analysis.

The technique calibrates well with daily methane emissions measured subsequently for the same cows in metabolism rooms and fed on the same diet.

Differences between diets designed to alter methane were detected by examining within-cow variation measured by the technique, in agreement with chamber measurements of the same diets.

The on-farm data indicate wide variation in methane emissions both between and within cows, but between-cow variation is considerably greater than within-cow variation. This gives confidence that the technology does measure repeatable parameters that are based on individual cow physiology or behaviour.

Sources of variation include milk yield, live weight, stage of lactation and parity, all of which are related to dry matter intake, which is the main driver of methane emissions. Diurnal variation is another potential source of variation that requires further study, but could be adjusted for by appropriate models.

A limited genetic analysis suggests that there could be a significant genetic component of between-cow variation in methane emissions, offering the potential for future genetic selection.

Comparison with tracer technology indicated that online breath sampling provides estimates of methane emissions that are as accurate, if not more accurate, than the SF6 technique. Importantly, the online technique requires no animal handling, confinement or interference with normal commercial practices.

Comparison of methane emissions estimated by the technique with values predicted from published equations, including the Tier II method used in National Inventories, revealed a low level of accuracy for most prediction equations. This is in agreement with similar reviews of equations currently available.

## Implications

The findings of this study have major implications for National Inventories, mitigation strategies and methane monitoring. For the first time, methane emissions have been measured concurrently in over 200 individual animals fed on the same diet and housed under commercial conditions. This has revealed a high degree of variability between individuals, which needs to be accounted for when compiling an inventory or testing the success of mitigation strategies.

Variability among individuals has been reported from chamber studies and attempts are often made to control this by restricted feeding. The findings of the current study suggest that such accurate measurements made under controlled conditions might not be appropriate in the commercial situation. The tracer technique offers a higher level of replication than chamber measurements, but requires animal handling and harnesses that can disrupt normal cow behaviour, which could offset advantages in replication. The online technique developed in this study acknowledges a high degree of variation within and between animals, but replication is no problem. We estimate that a representative profile of a whole herd could be obtained in 7 to 10 days.

Acknowledging and identifying variability can have benefits. Variability offers the potential for genetic selection of animals that have lower methane emissions per day or per unit of product; genetic improvements are cumulative and permanent. Further study of the causes of variability between and within cows could suggest strategies by which overall methane emissions can be reduced.

The implications of this study for Defra, levy bodies and dairy farmers are that online methane monitoring might be used on representative dairy farms throughout the UK to provide evidence for benchmarking and to measure the success of mitigation strategies. Further validation for different dairying systems will be required before this can be implemented. After validation, however, the resulting information would assist the Government in meeting its legal obligations for the National Inventory and under the Climate Change Act.

## Future work

This study has met its objectives in full and represents a significant advance in methodology for quantifying methane emissions by dairy cows. Although many research questions have been answered, new gaps in knowledge have been revealed that simply could not be considered using previous methodology. Future work is needed in development, application and interpretation of the technique.

Using the data gathered in the current study, we need to explore further the influence of diurnal variation on methane emissions measured during milking. This will require a significant time commitment for data mining that will characterise individual cows according to milking pattern. We have additional data on individual meal patterns and rumination times that would make a valuable contribution to understanding the basic biology and behavioural influences. The results will be beneficial for designing and interpreting future studies.

We need to install the instrumentation on a range of additional farms so that variation due to farming system can be quantified. We have already proved the technology works in two makes of robotic milker (Fullwood and Lely) at the Nottingham University Dairy Centre. Robots have the advantage of monitoring many cows at each milking with a single analyser. Testing in conventional herringbone parlours would open up a wider range of farming systems, but sampling frequency will be lower and cow identification might present a challenge.

Further calibration work is required for different types of diet. The current study showed good agreement between on-farm measurements and chamber measurements when cows were fed on the same diet in both locations. When diet was changed, however, calibration of daily emissions was not possible due to intake differences. Further work is needed also on possible dietary influences on diurnal variation; slowly digested feed ingredients might produce different patterns compared to rapidly digested feeds.

A long-term goal would be to breed cows for lower methane emissions. Proposed work in the Ruminant GIN consortium aims to study the influence of feed efficiency on methane emissions. This will provide only a proxy for methane emissions, whereas the online technique can provide primary data to verify relationships. Due to its low cost and high level of replication, the online technique could provide phenotypic data required for large-scale genetic studies coupled with genomic analysis to identify superior animals for selection programmes.

## IP and knowledge transfer

A fundamental requirement of this project was development of a computer programme to handle the vast amount of raw data generated by the data loggers. Much of the success of this study stems from the ability of that programme to convert methane concentrations measured at one-second intervals during milking into peak characteristics. We would be willing to discuss the use of this programme in research collaborations, but commercial deployment would require a licensing agreement.

Knowledge transfer has been direct to visiting parties of farmers, students and others who have visited the Dairy Centre over the past six months. In addition, presentations have been made at conferences and workshops as listed in section 9. A major paper is in preparation for publication in a peer-reviewed journal and we anticipate further papers will follow from this study.

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## Appendix – Diet formulation and composition

**Table A.1** Formulation and composition of the commercial TMR (low methane) fed to cows during variation studies and the experimental TMR (high methane) used in the diet comparison studies

| | Diet | |
|---|---|---|
| Ingredient (g/kg DM) | Low methane | High methane |
| Grass silage | 132 | 316 |
| Maize silage | 319 | 191 |
| Whole crop silage | 126 | 78 |
| Rape straw | 50 | 30 |
| Sugarbeet pulp | 96 | 113 |
| Rapeseed meal | 132 | 78 |
| Soyabean meal | 84 | 50 |
| Peas | | 107 |
| Megalac | 23 | 14 |
| Minerals | 37 | 23 |
| | | |
| Dry matter (g/kg) | 461 | 389 |
| Metabolisable energy (MJ/kg DM) | 11.2 | 11.1 |
| Crude protein (g/kg DM) | 168 | 171 |
| Neutral detergent fibre (g/kg DM) | 359 | 403 |
| Starch (g/kg DM) | 136 | 128 |
| Oil (g/kg DM) | 48 | 39 |
| Forage (% of DM) | 63 | 62 |

## References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

The following presentations have included material generated by this project or the preliminary study:

Garnsworthy, P.C. (2009) Nutritional strategies to reduce the environmental impact of dairy systems. DSM 2nd Ruminant Symposium, 28-29 April 2009, Copenhagen.

Garnsworthy, P.C. (2009) Methane, maternity and milk: challenges in dairy cow nutrition. Inaugural Lecture, 17 December 2009, University of Nottingham.

Garnsworthy P.C. (2010) Challenges in measuring methane emissions in agriculture. Sensors and Instrumentation KTN Workshop on Instrumentation for Greenhouse Gas Inventory. 4 May 2010, London.

Garnsworthy P.C. (2010) Sustainable intensive farming systems. 11th biennial Mediterranean Symposium of EAAP and CIHAEM, Animal Farming and Environment Interactions in Mediterranean Regions. 27-29 October 2010, Zadar (Croatia).