Wageningen UR Livestock Research

Partner in livestock innovations

Report 593

Individual methane recordings in dairy cattle

May 2012

LIVESTOCK RESEARCH
WAGENINGEN UR

Colophon

Publisher
Wageningen UR Livestock Research
P.O. Box 65, 8200 AB Lelystad
Telephone +31 320 - 238238
Fax +31 320 - 238050
E-mail info.livestockresearch@wur.nl
Internet http://www.livestockresearch.wur.nl

Editing
Communication Services

Wageningen UR Livestock Research and Central Veterinary Institute of Wageningen UR, both part of Stichting Dienst Landbouwkundig Onderzoek (DLO Foundation), together with the Department of Animal Sciences of Wageningen University comprises the Animal Sciences Group of Wageningen UR (University & Research centre).

Single numbers can be obtained from the website.

Abstract
In this study options are explored for individual methane recordings of dairy cows on large scale under practical circumstances. In a simulation study based on respiration research data, accuracies of methane measurements based on different sampling strategies are calculated. Scenarios showed that measuring methane (or ratio CH_4:CO_2) in the milking parlour or concentrate feeders will provide predictions close to the accuracy reached with scenarios based on sampling in cubicles. However under real farm conditions methane emission patterns may be more variable and overall accuracies decline.

Keywords
Methane, carbon dioxide, emission, dairy production

Reference
ISSN 1570 - 8616

Author(s)
Nico Ogink
Ron Crump
Yvette de Haas
Julio Mosquera
André Bannink
Jan Dijkstra

Title
Individual methane recordings in dairy cattle

Report 593

LIVESTOCK RESEARCH
WAGENINGEN UR

Report 593

Individual methane recordings in dairy cattle

Nico Ogink
Ron Crump
Yvette de Haas
Julio Mosquera
André Bannink
Jan Dijkstra

May 2012

This feasibility study is financed by Agentschap NL

Preface

In Nederland draagt de landbouw voor ongeveer 13% bij aan de uitstoot van broeikasgassen. Het gaat hier vooral om lachgas en methaan. Lachgas komt hoofdzakelijk vrij uit de bodem. Methaan komt met name vrij via pensfermentatie en uit dierlijke mest. Het potentieel om methaanemissie bij melkvee via fokkerijprogramma's terug te dringen wordt geschat op 10-20%. Om dit potentieel op termijn te kunnen benutten dient een basis te worden gelegd door het samenstellen van een dataset met individuele waarden van methaanemissie en genetische informatie van de dieren. Daarvoor is het noodzakelijk een effectieve methode te ontwikkelen voor het op grote schaal, routinematig bemeten van de methaanproductie van individuele dieren onder praktijkomstandigheden, om de individuele methaanconversie over lactatieperiodes te kunnen bepalen.

ROB wil zicht krijgen op de perspectieven van methaanmetingen aan individuele koeien in stallen voor het verkrijgen van bovenbeschreven data. Daartoe heeft AgentschapNL dit onderzoek gefinancierd vanuit het ROB-programma.

Nico Ogink
Projectleider

Summary

Achtergrond en aanleiding
In Nederland draagt de landbouw voor ongeveer 13% bij aan de uitstoot van broeikasgassen. Het gaat hier hoofdzakelijk om lachgas en methaan. Lachgas komt met name vrij uit de bodem; methaan komt deels vrij uit dierlijke mest maar vooral via de pensvertering van melkvee en andere herkauwers. Er zijn aanwijzingen dat verschillen in methaanuitstoot bij herkauwers deels genetisch bepaald zijn (genotypische variatie). Dat zou de mogelijkheid bieden om methaanemissie van melkvee via fokprogramma's terug te dringen. Het potentieel om methaanemissie op deze wijze terug te dringen wordt geschat op 10-20% minder methaan van melkvee. Om deze optie op termijn te kunnen benutten dient een basis te worden gelegd voor een dataset met individuele waarden van methaanemissie en genetische en genotypische informatie van de dieren. Daarvoor is het noodzakelijk een effectieve methode te ontwikkelen voor het op grote schaal, routinematig bemeten van de methaanproductie van individuele dieren onder praktijkomstandigheden. Voor het benutten van genotypische variatie gaat het daarbij om het kunnen bepalen van de gemiddelde methaanproductie per eenheid geproduceerde melk van een individueel dier.

Doelstelling van deze studie
De doelstelling van dit project was om inzicht te krijgen in de wijze waarop metingen van methaanemissie aan individuele koeien in praktijkstallen opgezet kan worden. De meetmethode moet het mogelijk maken per dier de gemiddelde methaanemissie per kg melk over een lactatieperiode met voldoende nauwkeurigheid te kunnen bepalen. Hiervoor is een verkennend haalbaarheidsonderzoek uitgevoerd bestaande uit drie onderdelen:

1. Onderzoek naar de te realiseren nauwkeurigheid van verschillende meetmethoden en bemonsteringstrategieën voor individuele metingen, en optimalisatie van deze strategieën;
2. Nadere specificatie van de uitvoering van het in te zetten meetsysteem, uitgewerkt voor twee scenario's;
3. Invulling van mogelijke vervolgstappen en een schatting van de kosten ervan, zoals:
    - uitvoeren testprogramma's;
    - uitvoeren pilot-onderzoek;
    - uitvoeren grootschalig meetprogramma.

Methode
Voor het onderzoek naar meetmethoden is kennis nodig van verwachte uitstootpatronen van methaan over de dag en over langere perioden. Deze kennis is vereist voor het ontwikkelen van een goede bemonsteringsstrategie. Hiervoor is gebruikt gemaakt van een dataset met continue methaanmetingen aan melkkoeien in respiratiekamers gedurende meerdere dagen. De dataset bevatte ook gegevens over de CO2-uitstoot. Er is een analyse van deze dataset uitgevoerd om inzicht te krijgen in de uitstootpatronen. Aanvullend zijn op basis van deze analyse verschillende meetmethodes gesimuleerd en qua nauwkeurigheid met elkaar vergeleken. Voor het realiseren van de tweede en derde doelstelling is gebruik gemaakt van de aanwezige kennis en ervaring van onderzoekers binnen Livestock Research.

Resultaten doelstelling 1: nauwkeurigheid meetmethoden
De data-analyse van methaanmetingen in de respiratiekamers laat zien dat rondom de dagelijkse voeropname-tijdstippen de methaanuitstoot relatief hoog is. Na de voeropname is er een dalende tendens te zien in de methaanuitstoot. De gemiddelde methaanuitstoot was 1,2 liter per koe per meting van 3 minuten, met een range van 0,4 tot 2,4.

Op basis van de dataset zijn zes scenario's gesimuleerd voor het meten van de gemiddelde individuele dagemissie van methaan. De volgende bemonsteringsmethoden zijn gesimuleerd:

- Melkstal-benadering: twee 15 minuten-metingen gedurende melken (M)
- Voerautomaat-benadering: vijf 6 minuten-metingen op 6.00/10.00/14.00/18.00/22.00 uur (V)
- Ligbox-benadering 1: gedurende een periode van 4 uur elke 9 minuten een meting over 3 minuten (L 1x4)
- Ligbox-benadering 2: gedurende 2 uur en na 8 uur nog eens gedurende 2 uur elke 9 minuten een meting over 3 minuten (L 2x2)

- Ligbox-benadering 3: 10-15-20 willekeurig over de dag verspreide 3 minuten-metingen (L verspreid)

In de simulatie van elke bemonsteringsmethode zijn twee verschillende monitoringsparameters toegepast die dienst zouden kunnen doen in de praktijk: de CH_4 uitstoot, en de CH_4:CO_2 concentratie ratio. Bij meting van de eerste parameter moet in de praktijk zowel het volume van de luchtstroom als de methaan-concentratie worden gemeten. Het aantrekkelijke van de alternatieve tweede parameter is dat enkel de concentratie van methaan en koolzuurgas in de ademlucht van dieren hoeft te worden bepaald. Dit is technisch eenvoudiger dan het bepalen van een volume-uitstoot. De ratio kan een goede voorspeller zijn voor een lage methaanuitstoot van een dier omdat dit gepaard kan gaan met een verschuiving in de uitstoot van gasvormig niet-benut koolstof (C), d.w.z. een verschuiving richting minder C via CH_4 en meer via CO_2.

In elk scenario is de nauwkeurigheid berekend waarmee de gemiddelde dagemissie kon worden bepaald. De nauwkeurigheid is hierbij uitgedrukt als de correlatie tussen het werkelijke daggemiddelde en de volgens de bemonsteringsmethode geschatte daggemiddelde. De coëfficiënt kan tussen 0 en 1 variëren, oplopend van geen voorspellende waarde bij 0 naar een exact juist voorspelde waarde bij 1. In de onderstaande tabel A staan de correlatiecoëfficiënten opgenomen. De bandbreedtes bij 3 methodes hebben betrekking op het van het starten van de betreffende bemonstering op verschillende tijdstippen gedurende de dag.

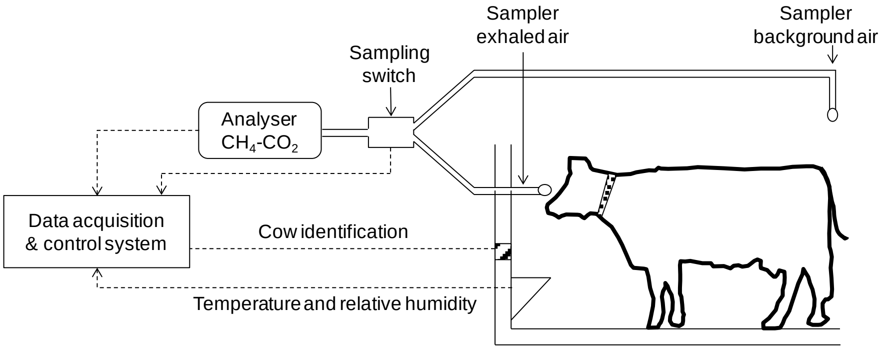
Tabel A *Nauwkeurigheid van verschillende meetmethodes voor het meten van de gemiddelde dagemissie van methaan, uitgedrukt als de correlatie tussen het werkelijke en geschatte daggemiddelde. De gecodeerde bemonsteringmethodes zijn in de tekst toegelicht.*

Bemonsteringsmethode	Meetparameter: CH4-uitstoot	Meetparameter: CH_4:CO_2 ratio
M	0,85 - 0,87	0,31 – 0,32
V	0,89	0,33
L 1x4	0,73 – 0,96	0,35 – 0,39
L 2x2	0,85 – 0,96	0,34 – 0,39
L verspreid n=10	0,88	0,33
L verspreid n=15	0,92	0,36
L verspreid n=20	0,94	0,37

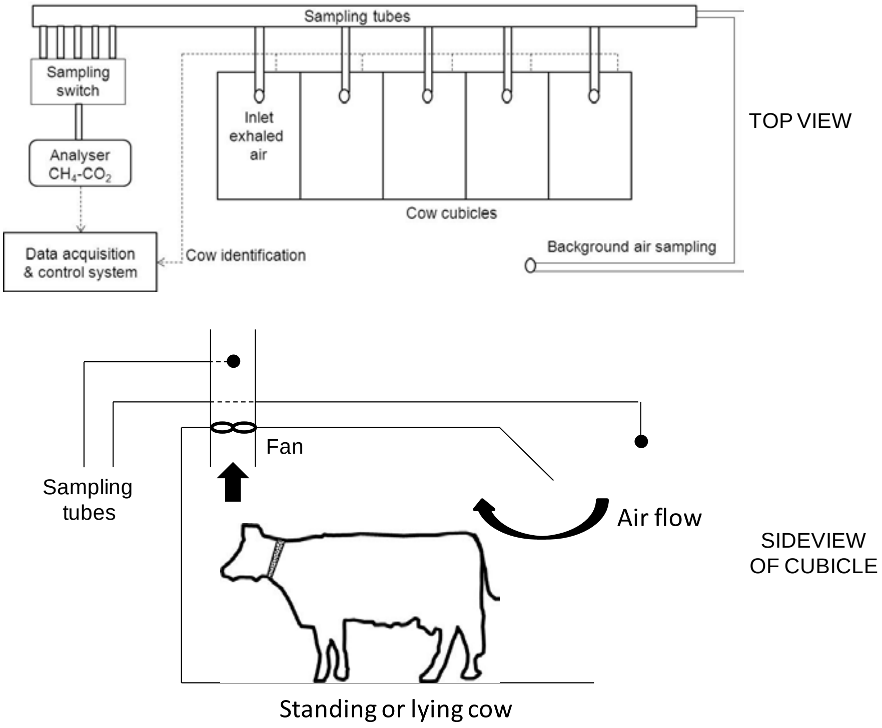
De tabel laat zien dat de meest directe, maar meettechnisch wat complexere parameter CH4-uitstoot bij alle bemonsteringsmethodes een duidelijk hoger nauwkeurigheidsniveau heeft. Binnen de twee parameter-kolommen bezien ontlopen de nauwkeurigheden van de verschillende methodes elkaar niet veel. Een relatief eenvoudige bemonsteringsopzet gericht op meten tijdens het melken zit qua nauwkeurigheid dicht bij het meten verspreid over de dag in ligboxen. Bij het simuleren van diverse methoden is aangenomen dat er geen verstorend effect is van voeropname tijdens de meting (zowel bij methode M als V) op de methaanuitstoot. Deze aanname lijkt niet geheel juist, maar het is onbekend hoe groot het effect zou kunnen zijn. Hier zal rekening mee gehouden moeten worden bij de keuze voor de beste meetopstelling.

Het uiteindelijke meetdoel is om een indicatie te geven van de nauwkeurigheid van de bepaling van de gemiddelde methaanuitstoot per kg melk gedurende de gehele lactatie (overall gemiddelde). Op basis van de beschikbare huidige datasets is dit niet mogelijk. De nauwkeurigheid van dit overall gemiddelde wordt mede bepaald door de verhouding tussen het variatieniveau van methaanuitstoot tussen de daggemiddeldes over de gehele lactatieperiode enerzijds, en de intensiteit van het gehanteerde steekproefschema over de gehele periode anderzijds. Wij beschikken niet over informatie die inzicht in de lange termijnvariatie kan geven. De meetperioden in de geanalyseerde dataset uit de respiratiecellen zijn daarvoor te kort, en meer langdurige individuele reeksen zijn niet beschikbaar. Ook in de literatuur is hierover niets bekend. Bij een sterke variatie in daggemiddelde methaanemissies gedurende de lactatie- en droogstandsperiode speelt de steekproefintensiteit over de lactatieperiode heen een belangrijke rol bij het schatten van de overall gemiddelde methaanuitstoot. Als de steekproef niet intensief genoeg verdeeld over de lactatie plaatsvindt kan dit leiden tot een aanzienlijke fout in het overall gemiddelde, ook al bestaat de reeks uit daggemiddeldes die elk afzonderlijk met grote nauwkeurigheid zijn bepaald. Omgekeerd kan een voldoende intensief uitgevoerd steekproefschema over de gehele lactatieperiode leiden tot een hoge overall nauwkeurigheid, zelfs bij gebruik van minder nauwkeurige methodes voor de afzonderlijke dagemissies, zoals de ratio-methode. Een voldoende aantal herhalingen zal namelijk het effect van de dagemissie-meetfout op het overall-gemiddelde teniet doen. D0or hun toevalskarakter middelen deze zich namelijk grotendeels tegen elkaar uit. Dit betekent dat het mogelijk is dat een benadering gebaseerd op de meetparameter CH_4:CO_2 met een beperkte nauwkeurigheid op dagniveau (zie tabel A) in een meetopzet over de gehele lactatieperiode toch goed zou kunnen voldoen.

Resultaten doelstelling 2: uitwerking meetsystemen
Op basis van de resultaten uit deze studie is het nu dus nog moeilijk om een definitieve beslissing te nemen over de beste meetopstelling. Daarom zijn twee meetopstellingen verder uitgewerkt. Eén methode richt zich op het meten van de concentratie-ratio's in een melkrobot (de melkstal valt af vanwege meetkosten omdat dan iedere melkplaats uitgerust moet worden met een gasmonitor). De andere richt zich op het meten in een ligbox-opstelling waarin zowel de concentratie-ratio's als de methaan-uitstoot kan worden vastgesteld. Zowel de locatiekeuze van de meetopstelling als de keuze van de monitorings-parameter (CH_4:CO_2 ratio, of CH_4 emissie) worden daarmee opengehouden. In de hieronder opgenomen figuren A en B worden beide meetopstelling schematisch weergegeven.



Figuur A Schematische weergave (zij aanzicht) van de wijze waarop in een melkrobot de CH_4:CO_2 ratio in de ademhaling kan worden bemonsterd



Figuur B Schematische weergave (boven aanzicht gevolgd door zij aanzicht ligbox) van de wijze waarop in meerdere ligboxen de CH_4:CO_2 ratio en de CH4-uitstoot via de ademhaling kan worden bemonsterd.

Resultaten doelstelling 3: vervolgstappen

Om een definitieve keuze te maken wordt aanbevolen prototypes van beide meetmethoden te ontwikkelen en, als tussenstap, beide meetopstellingen gedurende een jaar in één stal toe te passen, voordat een keuze voor toepassing in de praktijk wordt gemaakt. In deze tussenfase kan de variatie in methaanemissie gedurende de lactatie in beeld worden gebracht, en kan tevens gevalideerd worden welke monitoringsparameter het beste resultaat geeft. In de melkrobot kan alleen de CH_4:CO_2 ratio gemeten worden, terwijl in de ligboxenopstelling ook de individuele CH_4 –uitstoot kan worden bepaald. Het is mogelijk dat een deel van de variatie tussen dieren gemist wordt als enkel naar de ratio CH_4:CO_2 gekeken wordt, aangezien dan mogelijke effecten van een verbeterde voerefficiëntie die leiden tot een verlaagde methaanemissie genegeerd worden (zie hoofdstuk 3.1). Hiermee wordt een deel van de genetische variatie dat van belang is voor het economische kengetal gemist door te kijken naar alleen CH_4:CO_2 in plaats van naar CH_4 emissie. Maar of dit echt zo is, zal moeten blijken uit de validatiestudie waarbij beide monitoringsparameters naast elkaar getoetst worden en waarbij gedurende een jaar data verzameld wordt. De tussenfase-studie biedt tevens de mogelijkheid om met grotere zekerheid een indruk te krijgen van het potentieel aan genetische variatie in de Nederlandse melkveestapel.

De meetopstelling in de melkrobot vraagt qua apparatuur een beperkte ontwikkelingsslag. Door met deze methode op korte termijn te gaan meten, kan ervaring opgedaan worden en komen de moeilijkheden in beeld die niet op voorhand te voorspellen zijn. Deze ervaring kan dan meegenomen worden in de ontwikkeling van de meetmethode voor ligboxen, waarvoor een langere periode noodzakelijk is. De verwachte kosten voor het realiseren en het in bedrijf hebben van beide meetopstellingen zijn uitgewerkt in hoofdstuk 4.2. Na ontwikkeling van de methoden zal een vergelijkende meting in een onderzoeksstal de vereiste informatie opleveren om de beste methode vast te stellen voor toepassing op praktijkbedrijven.

Conclusie

De conclusie van deze haalbaarheidsstudie is dat er variatie tussen dieren is in de methaanproductie en dat die variatie ook opgepikt kan worden als niet de gehele dag de methaanproductie van de koeien gemeten wordt. Om te komen tot de beste meetopstelling om individuele methaanmetingen te gaan doen, is vervolgonderzoek nodig waarbij twee meetopstellingen gelijktijdig getest worden in dezelfde stal, en waarbij twee monitoringsparameters met een voorspellende waarde worden vergeleken. Als de database gevuld wordt met gegevens van beide meetopstellingen, kunnen analyses uitwijzen welke methode geschikt is voor toepassing op praktijkbedrijven.

Table of contents

Preface

Samenvatting

Literature

1 Introduction

In the Netherlands, approximately 13% of the greenhouse gas emission (mainly methane and nitrous oxide) can be related to agriculture. The Dutch agricultural sector did not receive a quantitative aim to reduce the greenhouse gas emission, but it did get a qualitative aim and has to implement cost effective measures in practice. In the convenant “Schone en Zuinige agrosectoren” participants agreed that greenhouse gas emissions are reduced with 4 to 6Mton in 2020 compared to 1990.

The reduction of enteric methane of cattle, being the major source of methane emission in animal production, is therefore important. Recent studies have shown that natural variation among animals exists in enteric methane emission (Grainger et al., 2007). This variation can be used to breed cows with low methane conversion, achieving an expected progress in terms of methane reduction ranging between 10 and 20% (Waghorn et al., 2006). To be able to use this potential in the long term, a database is needed with both genetic information of the individual animals (pedigree, markers) and their individual methane conversion, expressed as the methane emission per produced unit of milk during the lactation cycle. To determine individual methane conversions, individual methane emission recordings across the lactation cycle are required. Currently it is not possible to measure individual methane emission on large scale at affordable costs. Therefore, there is an urgent need for an effective method to determine individual methane emissions routinely on large scale under practical circumstances.

In 2010, Wageningen UR Livestock Research has performed a first internal study to list the possibilities to measure methane on individual cows under practical circumstances with an optimal balance between the costs and the obtained information (Ogink et al., 2010). The conclusion of that study was that there are possibilities to set up such a system, but that the development of sampling and measuring designs could benefit from utilizing information on methane emission characteristics from research in respiration chambers. On request of AgentschapNL (ROB programme), the work plan for further exploration of the options and opportunities for measuring individual methane records in dairy cattle is developed in this current study.

1.1 Objective of project

The aim of this study is to explore the possibilities for individual methane recordings on large scale under practical circumstances. This is achieved in three parts:

(1) Estimate the accuracies that can be achieved with several possible measuring strategies, based on the variation characteristics that are derived from an analysis of available cow data from research in respiration chambers
(2) Specification of the set up of the measuring equipment, for 2 different scenario’s
(3) Indication of further research, including an indication of the costs

Chapter 2 will present the structure of the data of respiration chambers that is used in the analyses and the outcomes of the analyses of methane recordings, and the results of six different sampling strategies are presented as well. Chapter 3 describes the possible set-ups of the measuring equipment based on the results of the data analyses of Chapter 2. Finally in Chapter 4 an outlook in the costs of the equipment and required future research is given, and in Chapter 5 the conclusions of this project are drawn.

2 Prediction of daily methane production

The prediction of total daily methane production is based on observations taken throughout the day on methane (CH_4) or the ratio of methane to carbon dioxide ($CH_4:CO_2$), and the impact of different recording strategies on the accuracy of prediction is investigated.

Daily methane (or $CH_4:CO_2$) production curves are described using experimental data from respiration chambers. Using the results of this data analysis, index calculations are carried out to determine the effect of using different combinations of the observations to predict the total daily production.

2.1 Data

Data were available from ten trials, a trial involving a pair of cows within one of two respiration chambers. Each trial lasted for five days, with data reported over a 72 hour period spanning 4 calendar days. The recording equipment alternated between the two respiration chambers and a reset period such that each observation within a trial represented a three minute yield with six minute intervals between them. There were a small number of both random and systematic (associated with feeding/milking events) missing observations in the data.

Animals remained in tie-stalls for 12d to become accustomed to the diet and restriction in movement. After this period, animals were housed in 1 of 2 identical respiration chambers to determine gaseous exchange, energy balance, and diet digestibility. Because 2 chambers were available, measurements were obtained in 10 periods, staggered in time. Within each period, 2 cows receiving the same treatment were housed in one chamber, and 2 cows receiving a different treatment were housed in the other chamber. Within each chamber, the 2 cows originated from a different block. The experimental unit for data measured in the respiration chambers (e.g., methane production, diet digestibility parameters) therefore consisted of a pair of cows. Feed intake was restricted per block to 95% of the *ad libitum* feed intake of the animal consuming the lowest amount of feed during d5 to d8 (i.e. still in the tie-stall) within a block (Zijderveld et al., 2011). This is not completely comparable with Dutch circumstances, where cows are usually fed *ad lib*. The diet consisted of 40% grass silage, 26% corn silage, and 34% concentrates on a DM basis, which is comparable to Dutch circumstances. Four different additives were fed, that were assumed to lower the methane emission of the cows. However, the results did not show significant differences with the control group, and therefore the data can be assumed to be representative for normal Dutch circumstances (Zijderveld et al., 20110. The additives were handmixed into the diet at the time of feeding. Because their inclusion rate was low, this did not affect the average chemical composition of the total mixed ratio.

Table 1 Each trial was subject to one of four dietary treatments, which were nested within chamber:

Trial	Chamber:	1					2				
	Pair:	1	2	3	4	5	1	2	3	4	5
Treatment:		A	C	A	C	A	B	D	B	D	B

Given the low number of trials, treatment and chamber effects were ignored (Table 1). Estimates of variance components associated with cows will include some portion of the between treatment and between chamber variation.

Methane data were provided as estimates of daily methane production for the pair of cows. These data were converted back to three minute CH_4 yields in litres per cow by division by 960, there being 480 three minute periods in a day and 2 cows in each respiration cell.

Carbon dioxide data were estimates of the daily yield of CO_2 per kg of metabolic weight. These observations were converted to three minute yields of CO_2 in litres per cow by multiplying by the provided metabolic weight and dividing by 960.The ratio of CH_4 to CO_2 production within a three minute period was calculated as CH_4 yield divided by CO_2 yield. The day was divided into three minute segments, this being the duration of each record. These segments were known as samples and each observation was assigned to one of the 480 samples within a day based on its associated timestamp.

Figure 1 shows the three-minute yields of methane production per cow plotted against time of day (sample within day) for each of the ten trials. There are two clear events within the day that result in elevated methane production, and there is a brief sequence of missed observations at each of these times on each day in each trial. Figure 2 is a similar plot to Figure 1 except for CH_4:CO_2, the two feeding/milking events result in an elevated CH_4:CO_2 ratio.

Correlations between daily mean values of CH_4 production and CH_4:CO_2, and coefficients of variation are listed in Table 2a. The data for three-minute yield of methane and carbon dioxide plus the ratio between CH_4 and CO_2 yields are summarised in Table 2b within trial and day of trial.

Table 2a Correlation within days across daily means of cow pairs: CH_4 production versus CH_4:CO_2 , and coefficients of variation (CV, %) within days of cow pair means: CH_4 production and CH_4:CO_2.

	Correlation CH_4 - CH_4:CO_2	CV (CH_4 production)	CV (CH_4:CO_2)
Day 1	0.43	9	5
Day 2	0.45	8	5
Day 3	0.42	8	4
Day 4	0.63	8	4
Overall	0.64		

Table 2b Summary of methane and carbon dioxide production (litres per cow per three minute period) observations and the ratio of methane to carbon dioxide production observations by trial and day of recording.

Trait	Trial[1]	Day 1					Day 2					Day 3					Day 4				
	(C.P)	N	Mean	Sd	Min	Max	N	Mean	sd	Min	Max	N	Mean	Sd	Min	Max	N	Mean	sd	Min	Max
CH_4 (l)	1.1	95	1.13	0.18	0.75	1.5	150	1.08	0.19	0.71	1.49	151	1.10	0.21	0.64	1.60	56	0.98	0.15	0.74	1.39
	1.2	96	1.06	0.20	0.69	1.68	153	1.03	0.24	0.60	1.65	153	1.05	0.23	0.57	1.60	55	1.00	0.25	0.69	1.64
	1.3	95	1.45	0.24	0.85	2.08	152	1.33	0.26	0.76	1.93	152	1.34	0.26	0.69	1.92	54	1.10	0.24	0.53	1.70
	1.4	97	1.27	0.21	0.78	1.68	153	1.23	0.26	0.76	2.04	153	1.21	0.27	0.58	1.92	55	1.07	0.27	0.42	1.63
	1.5	96	1.34	0.30	0.84	2.07	152	1.29	0.31	0.64	2.07	152	1.29	0.29	0.73	2.12	54	1.15	0.34	0.74	1.95
	2.1	95	1.32	0.27	0.69	1.99	153	1.24	0.31	0.55	1.89	153	1.24	0.32	0.53	2.30	56	1.10	0.37	0.64	2.13
	2.2	97	1.29	0.27	0.86	2.32	154	1.21	0.31	0.61	2.05	154	1.12	0.29	0.61	1.86	56	0.95	0.31	0.55	1.70
	2.3	96	1.42	0.26	0.96	2.03	153	1.33	0.26	0.83	2.06	153	1.36	0.25	0.76	2.12	55	1.23	0.23	0.81	1.77
	2.4	96	1.38	0.30	0.80	2.41	151	1.33	0.33	0.68	2.36	151	1.33	0.31	0.76	2.38	53	1.15	0.28	0.78	2.00
	2.5	96	1.34	0.32	0.41	2.05	149	1.19	0.35	0.57	2.17	149	1.22	0.37	0.30	2.11	52	1.00	0.35	0.53	2.04
CO_2 (l)	1.1	95	11.7	1.42	8.7	15.1	150	11.6	1.49	8.8	15.3	151	11.8	1.51	8.1	16.6	56	10.9	1.07	8.6	13.9
	1.2	96	11.7	1.33	9.6	16.3	153	11.5	1.48	8.7	15.6	153	11.6	1.52	8.5	15.7	55	11.2	1.57	9.2	15.1
	1.3	95	14.9	1.70	10.6	18.7	152	14.4	1.72	10.8	18.4	152	14.4	1.63	10.2	18.7	54	12.6	1.34	9.9	16.6
	1.4	97	14.2	1.51	10.4	17.7	153	13.7	1.72	9.9	18.3	153	13.4	1.84	9.1	18.0	55	12.5	1.95	9.5	17.3
	1.5	96	13.1	1.81	9.6	18.6	152	12.7	1.99	9.5	18.4	152	12.6	1.86	9.2	18.3	54	11.6	1.90	9.4	17.2
	2.1	95	12.9	1.86	8.3	16.9	153	12.4	2.09	8.1	18.4	153	12.5	2.13	7.8	18.1	56	11.6	2.37	7.8	17.2
	2.2	97	12.4	1.95	9.4	19.2	154	12.0	2.13	8.7	18.0	154	11.4	1.95	7.6	17.3	56	10.8	2.29	8.2	17.7
	2.3	96	14.8	1.77	11.0	19.0	153	13.9	1.84	9.9	18.8	153	14.1	1.79	10.2	18.5	55	13.2	1.64	10.4	17.6
	2.4	96	13.9	1.96	9.8	18.8	151	13.7	2.24	9.2	19.4	151	13.6	1.92	9.6	20.4	53	12.3	2.00	9.4	17.6
	2.5	96	13.9	2.06	8.9	18.5	149	13.0	2.33	8.2	18.9	149	13.1	2.47	8.1	19.5	52	11.6	2.34	8.7	17.9
CH_4:CO_2	1.1	95	0.096	0.0082	0.076	0.117	150	0.093	0.0088	0.070	0.115	151	0.093	0.0097	0.068	0.114	56	0.090	0.0083	0.076	0.112
	1.2	96	0.090	0.0094	0.070	0.116	153	0.088	0.0120	0.064	0.121	153	0.089	0.0107	0.060	0.114	55	0.088	0.0113	0.073	0.121
	1.3	95	0.097	0.0088	0.071	0.124	152	0.092	0.0097	0.065	0.113	152	0.092	0.0106	0.056	0.118	54	0.087	0.0135	0.053	0.120
	1.4	97	0.090	0.0100	0.069	0.126	153	0.089	0.0115	0.067	0.117	153	0.090	0.0116	0.065	0.122	55	0.085	0.0124	0.042	0.108
	1.5	96	0.102	0.0126	0.076	0.142	152	0.100	0.0122	0.065	0.129	152	0.101	0.0119	0.071	0.131	54	0.098	0.0164	0.071	0.137
	2.1	95	0.102	0.0102	0.079	0.133	153	0.099	0.0124	0.059	0.126	153	0.098	0.0127	0.068	0.129	56	0.093	0.0142	0.068	0.131
	2.2	97	0.104	0.0114	0.064	0.136	154	0.100	0.0131	0.060	0.139	154	0.097	0.0133	0.065	0.134	56	0.087	0.0122	0.062	0.109
	2.3	96	0.096	0.0101	0.077	0.130	153	0.095	0.0099	0.069	0.126	153	0.096	0.0094	0.075	0.118	55	0.093	0.0096	0.074	0.117
	2.4	96	0.099	0.0121	0.074	0.132	151	0.096	0.0122	0.070	0.133	151	0.097	0.0123	0.057	0.138	53	0.092	0.0096	0.072	0.118
	2.5	96	0.095	0.0129	0.044	0.129	149	0.090	0.0141	0.061	0.135	149	0.091	0.0140	0.037	0.122	52	0.085	0.0146	0.053	0.130

[1] C.P: trial identified by chamber.pair, where there are two chambers and 5 cow pairs. Cow pairs were numbered within chamber, ie trials 1.1 and 2.1 utilised a different pair of cows.

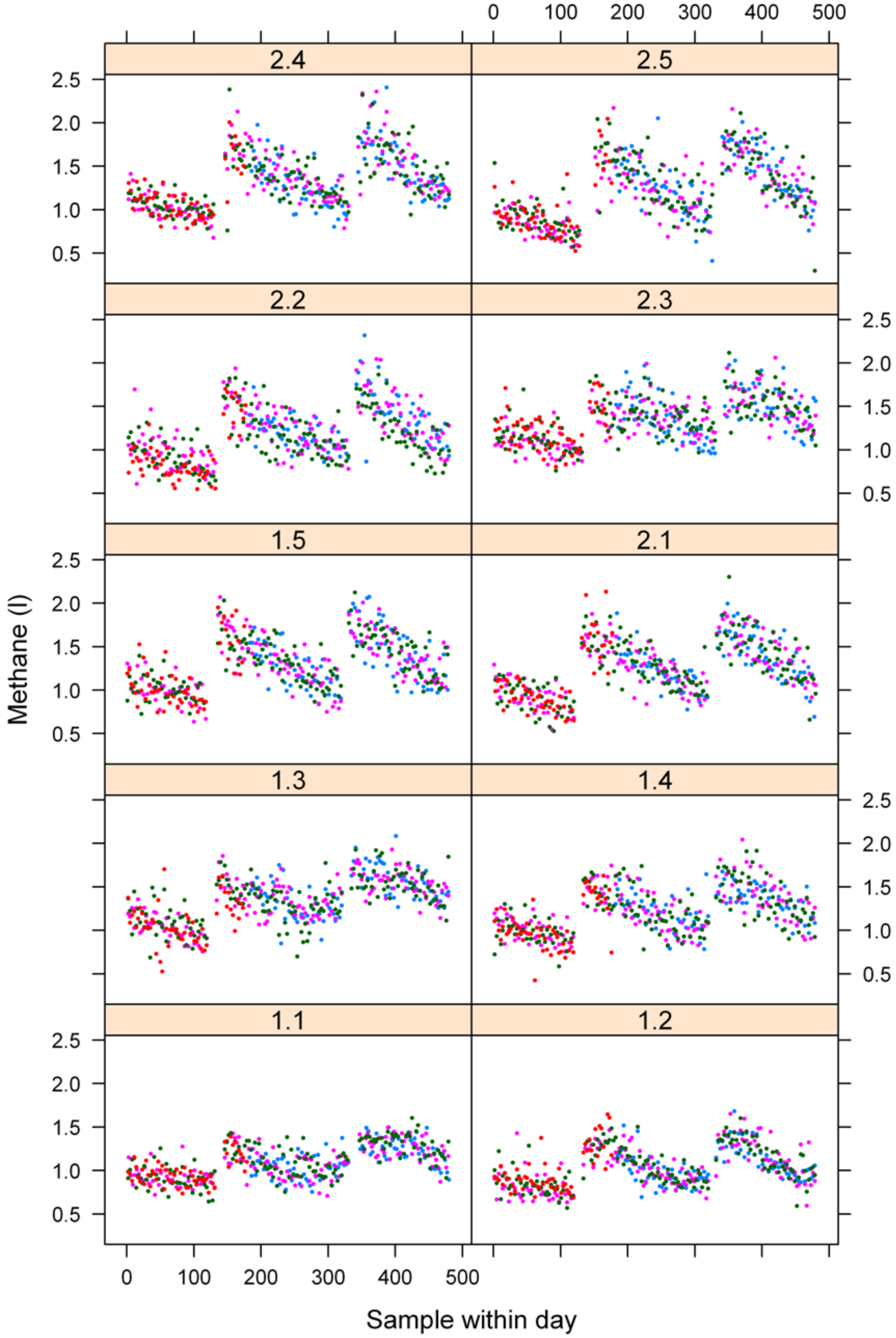


Figure 1 Three-minute methane yield against time of day within trial (identified as chamber.pair). Colours relate to observations on different days within the trial.

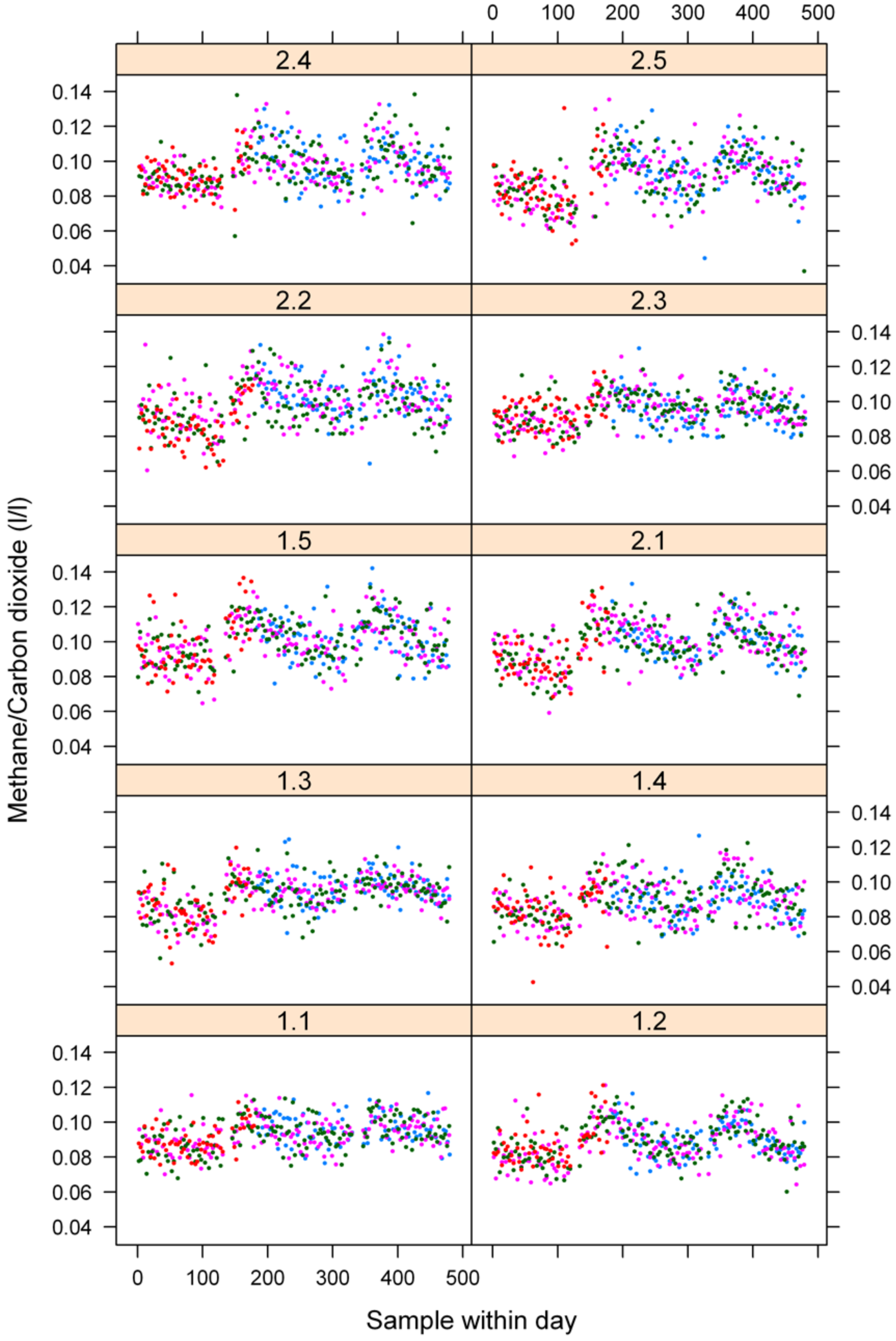


Figure 2 Ratio of three-minute methane and carbon dioxide yields against time of day within trial (identified as chamber.pair). Colours relate to observations on different days within the trial.

2.2 Analyses

2.2.1 *Fixed effects*

Various fixed effect models were considered via a stepwise regression procedure aimed at minimising Akaike's Information Criterion (AIC). Fixed effects considered were: number of samples since feeding/milking event (linear, quadratic and cubic regressions: SL, SQ and SC); whether the last event was in the morning or the afternoon (AMPM); the interactions of AMPM with SL, SQ and SC; and a linear regression on day of event.

The procedure was run for CH4 and CH4:CO2, and the same fixed effect model was selected for each trait: including AMPM and the interactions of AMPM with SL and SQ.

2.2.2 *Random regression*

Random regression was used to obtain estimates of cow variation for time since last event curves, these analyses quantify differences from the average methane production across the day and allow prediction of cow differences in total daily production via selection index theory.

Three univariate mixed models were fitted for either trait (CH_4, CH_4:CO_2), using the previously selected fixed effect model and sequentially adding in random cow terms (intercept, linear and quadratic regressions on time since last event) as described in Table 3. The fixed effect part of these models describes the average performance over the day, while the terms associated with cows relate to how cows vary from this average curve (in terms of intercept, linear and quadratic slopes).

Table 3 Univariate mixed models fitted

Model		Covariance components
1	$y_{ijk} = \mu + M_{ijk} + \beta_{s\|M_{ijk}} s_{ijk} + \beta_{ss\|M_{ijk}} s_{ijk}^2 + u_i + e_{ijk}$	σ_e^2, σ_u^2
2	$y_{ijk} = \mu + M_{ijk} + \beta_{s\|M_{ijk}} s_{ijk} + \beta_{ss\|M_{ijk}} s_{ijk}^2 + u_i + u_{s_i} s + e_{ijk}$	σ_e^2, σ_u^2, $\sigma_{u_s}^2$, σ_{uu_s}
3	$y_{ijk} = \mu + M_{ijk} + \beta_{s\|M_{ijk}} s_{ijk} + \beta_{ss\|M_{ijk}} s_{ijk}^2 + u_i + u_{s_i} s + u_{ss_i} s_{ij}^2$	σ_e^2, σ_u^2, $\sigma_{u_s}^2$, $\sigma_{u_{ss}}^2$, σ_{uu_s}, $\sigma_{uu_{ss}}$, $\sigma_{u_s u_{ss}}$

where: y_{ijk} was the k^{th} observation for the trait being analysed on cow-pair *i* on the j^{th} day of the trial;
μ was the overall mean;
M_{ijk} was the indicator of whether the last milking/feeding event was the morning or afternoon event;
s_{ijk} was the number of samples since the last milking/feeding event;
$\beta_{s|M_{ijk}}$ and $\beta_{ss|M_{ijk}}$ were fixed interactions of M_{ijk} with linear and quadratic regression coefficients on number of samples since the last event (ie slopes differ following morning and afternoon events);
u_i, u_{si} and u_{ssi} were random effects of the i^{th} pair of cows (overall and linear and quadratic effects of number of samples since the last event);
e_{ijk} was a random residual term, and
σ_e^2, σ_u^2, $\sigma_{u_s}^2$, $\sigma_{u_{ss}}^2$, σ_{uu_s}, $\sigma_{uu_{ss}}$, $\sigma_{u_s u_{ss}}$ are variances and covariances associated with the random residual (*e*) and cow (intercept, linear and quadratic: u, u_s and u_{ss}) effects.

The mixed models were fitted using an average information residual maximum likelihood procedure as implemented in the ASREML software.

The analysis of three-minute methane yields showed a significant improvement in the log-likelihood when a linear random regression effect on samples since the last event was included (compared to just having an intercept cow effect). When a quadratic random regression was also included, the variance associated with this was very low and was fixed by the software to keep the cow effects covariance matrix positive-semi definite. The improvement in the log-likelihood was significant with quadratic term and associated correlations included. Table 4 contains the results of univariate analyses of three-minute methane yields under models 2 and 3 (as defined in Table 3).

The univariate analyses of CH_4:CO_2 found a significant linear random regression terms, but not quadratic. These runs were carried out to assess the model to be used for CH_4:CO_2 in the bivariate analyses and to provide good starting values for these, but the results themselves are not of importance here and so are omitted.

Table 4 Results of univariate analysis of three-minute yields of CH_4

	Model 2[*]		**Model 3**		
Term[†]	s.d.	Correlation	s.d.	Correlation	
		u		*u*	u_s
e	0.174		0.172		
u	0.152		0.164		
u_s	6.94×10^{-4}	-0.772	1.78×10^{-3}	-0.633	
u_{ss}	-	-	4.86×10^{-6}	0.390	-0.952

[*] models as defined in Table 3. [†] residual (*e*) and cow intercept, linear and quadratic random regressions (u, u_s, u_{ss}), as in Table 3.

The estimated standard deviations for each random effect and the correlation between the different pair of cow effects are given in Table 4. Note that the data analysed was the methane production per cow, ie the average of the cow pair observation or half the observed value. The variance of a sample mean is $\mathrm{Var}(\bar{X}) = \frac{\sigma^2}{n} + \frac{n-1}{n}\rho\sigma^2$, with n being the number of observations in the mean and ρ the intra-class correlation. Assuming that the cows in a pair are independent ($\rho = 0$) then the cow variance would be twice the variance associated with the mean of the pair. For the purposes of this study, no rescaling of variances were performed before index calculations were carried out.

Bivariate random regression analysis with CH_4 and CH_4:CO_2 as dependent variables was performed with ASREML. It was not possible to run this analysis with the quadratic term for CH_4 included, therefore the bivariate analysis and the subsequent prediction of accuracies of total daily methane production was carried out using the model including random intercept and linear terms for time since last event, the quadratic terms in the fixed portion of the model were retained (Table 3, model 2).

Results of the bivariate analysis are given in Table 5 for cow level covariances. The residual standard deviations for CH_4 and CH_4:CO_2 were 0.174 and 0.0098, respectively, with a residual correlation of 0.794.

Table 5 Bivariate estimates of between cow covariances for intercept and linear time since last event effects. Standard deviation on diagonal, correlation below diagonal.

		CH_4		**CH_4:CO_2**	
		u	u_s	*U*	u_s
CH_4	*u*	1.52×10^{-1}			
	u_s	-0.772	6.94×10^{-4}		
CH_4:CO_2	*u*	0.623	-0.703	5.06×10^{-3}	
	u_s	-0.511	0.899	-0.601	1.76×10^{-5}

2.3 Index predictions

A phenotypic variance-covariance matrix for a full day's samples can be constructed from the random regression results: $\mathbf{V}_d=\mathbf{ZVZ}'+\mathbf{I}\sigma_e^2,=\mathbf{V}_{dc}+\mathbf{I}\sigma_e^2$, where **Z** is a matrix with three columns containing 1, s_i and s_i^2 for the i^{th} sample within a day (ie 480 rows), **V** is the estimated variance-covariance matrix between cow effects and **I** is a 480x480 identity matrix. The matrix $\mathbf{V}_{dc}$ is the covariance matrix between cow

effects at each sampling point in the day. The variance of the total daily methane production (ie the sum of 480 sequential sample yields) is equal to the sum of all of the elements of $\mathbf{V}_d$.

When setting up matrix **Z** the positions of the first sample after the morning and afternoon events were taken to be samples 141 and 341 (just after 7am and 5pm).

Because the two events within a day are treated as being equal, the possibility of repeated records on the same event exists. That is, there may be two observations in a day which are *n* samples after an event – one *n* samples after the morning event and one *n* samples after the afternoon event. Therefore, an index of two samples may be two observations on one trait, and an index of three samples may be an index on two traits with one of these having two observations, etc. These repeated records have to be accounted for in the variances used.

The accuracy of prediction of an index is $r_{IH} = \mathbf{b}'\mathbf{G}/\sqrt{\mathbf{b}'\mathbf{P}\mathbf{b}V_H}$, where V_H is the variance of daily methane production, **P** is the phenotypic covariance matrix between traits (samples), **G** is the covariance between the observed traits and the daily methane production and **b** is a vector of index weights (calculated as $\mathbf{b} = \mathbf{P}^{-1}\mathbf{G}$). Diagonal elements of **P** were calculated as $p_{ii} = (1 + (n-1)r_i/n)\sigma^2_{p_i}$, where n was the number of observations on that trait (trait defined in terms of time since event), r_i was the repeatability of the trait (total cow pair variance divided by phenotypic variance for that trait) and $\sigma^2_{p_i}$ was the phenotypic variance of trait *i*.

The total daily methane production can be predicted using either three-minute methane yields, where **P** and **G** were based on the across day covariance matrix between these yields, or using CH_4:CO_2, where **P** was based on across day covariance matrix among these observations and **G** was based on the covariances between CH_4:CO_2 samples and the total daily methane production.

The accuracy of predicting total daily methane production by CH_4 or CH_4:CO_2 under six recording scenarios were considered using index methodology.

2.3.1 Two measurements per day of 15 minutes duration when in milking parlour.

The fifteen minute recording period was taken as five consecutive three-minute samples as per the current data. In the current data analysis the observations cease for a period during the feeding/milking event. Not knowing the methane production profile during these events, the index calculations were performed using the 15 minute periods at either 8am and 8pm or 9am and 9pm. The accuracy of prediction of total daily methane production was 0.85 (8am and 8pm) or 0.87 (9am and 9pm) based on CH_4 observations and 0.31 or 0.32 using CH_4:CO_2 observations.

2.3.2 Five measurements per day during 6 minutes with 4h interval (and 8h during night) – at 6am, 10am, 2pm, 6pm, 10pm (equivalent to measuring in concentrate feeder).

The six minute recording period was considered as two consecutive three-minute observations. Accuracy of prediction of total daily methane production was 0.89 when predicting from CH_4 observations, and 0.33 when predicting from CH_4:CO_2.

2.3.3 One measurement of four consecutive hours, with one measurement every 9 minutes (equivalent to measuring in cubicle).

The four hour window was considered 21 times, beginning on the hour from midnight through to 8pm.

Accuracy of prediction of total daily methane production ranged from 0.73 to 0.96 when predicting from CH_4 observations, with the maximum corresponding to the four hour period starting at 11am. For prediction from CH_4:CO_2 the accuracy ranged from 0.35 to 0.39 with the maximum from the four hour period starting at 1pm.

2.3.4 Two measurements of 2 hours with 8 hours in between, with 1 measurement every 9 minutes (equivalent to measuring in cubicle).

The 12 hour (2 hours recording, 8 hour interval, 2 hours recording) was considered 12 times, beginning on the hour between midnight and noon.

Accuracy of prediction of total daily methane production ranged from 0.85 to 0.96 when predicting from CH_4 observations, with the maximum when the first recording period started at midnight. For prediction from CH_4:CO_2 the accuracy ranged from 0.34 to 0.39 with the maximum from the four hour period starting at 2am.

2.3.5 Completely random sampling of 10 – 15 – 20 measurements per day (equivalent to measuring in cubicle).

For each sample size, sampling was repeated 5000 times. Minimum, maximum and mean of the 5000 replicates are given in Table 6 for prediction by either CH_4 or CH_4:CO_2. The densities of the accuracy distributions sampled are plotted in Figures 3 and 4 for CH_4 and CH_4:CO_2, respectively.

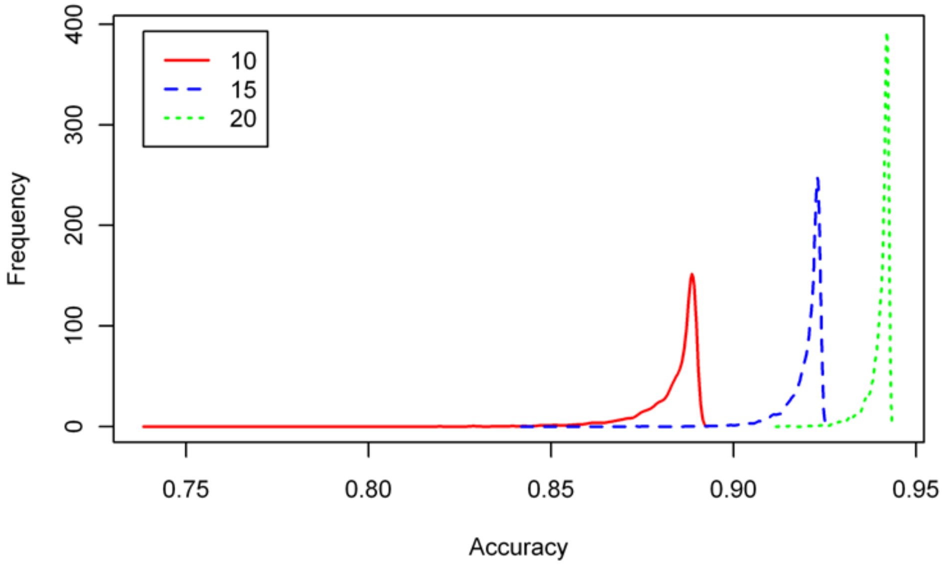


Figure 3 Distribution of accuracy of prediction of total daily methane production from 10, 15 or 20 randomly placed CH_4 samples throughout the day

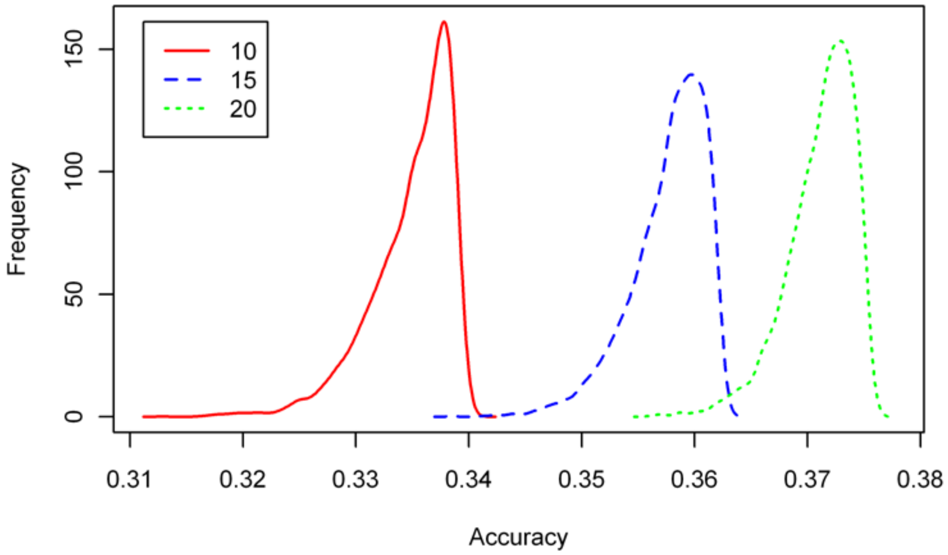


Figure 4 Distribution of accuracy of prediction of total daily methane prediction from 10, 15 or 20 randomly placed $CH_4:CO_2$ samples throughout the day

Table 6 Accuracy of prediction of total daily methane production of 5000 random samples of N observations.

N	**Observations on:**					
	CH_4			**$CH_4:CO_2$**		
	Min	**Max**	**Mean**	**Min**	**Max**	**Mean**
10	0.74	0.89	0.88	0.31	0.34	0.33
15	0.84	0.92	0.92	0.34	0.36	0.36
20	0.91	0.94	0.94	0.36	0.38	0.37

In addition, the number of random observations was increased from 5 to 100 in increments of 5 and for each level 1000 replicates were assessed and the mean accuracy calculated. These results are plotted in Figure 5 and show that there is little gain in accuracy of prediction beyond around 40 observations on either trait.

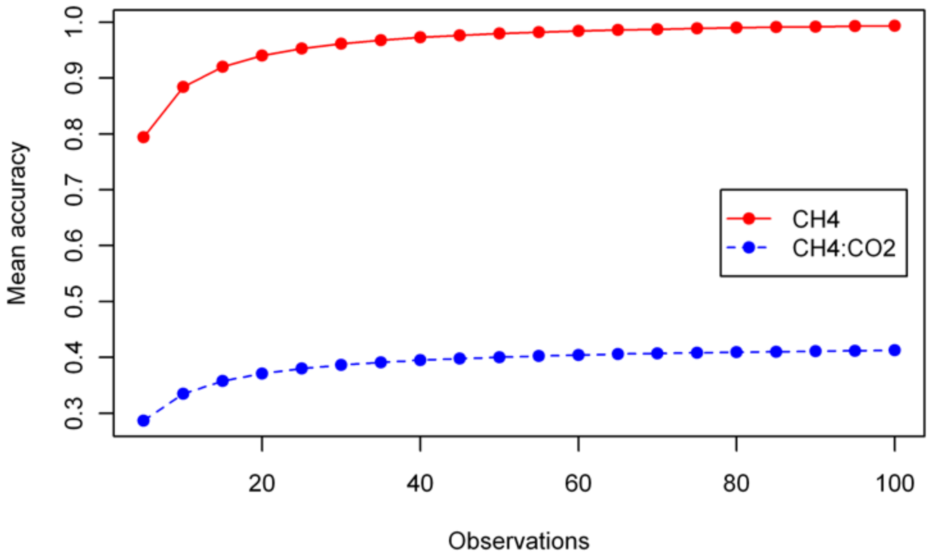


Figure 5 Mean accuracy of prediction of total daily methane production for a range of numbers of observations

2.3.6 Sampling of one measurement every hour, or every two hours (equivalent to measuring in cubicle).

Repeated on the hour (or two-hour) across the full 24 hour period from midnight, i.e. either 24 or 12 equally-spaced observations per day.

Accuracy of prediction of total daily methane production ranged was 0.95 (1 hour spacing) or 0.90 (2 hour spacing) when predicting from CH_4 observations. For prediction from CH_4:CO_2 the accuracy 0.38 (1 hour spacing) or 0.35 (2 hour spacing).

2.4 Discussion

The REML analyses showed how patterns of daily methane production can be modeled, both to give an average profile and to detect and model differences from this average profile for individual cows.

Limitations of the data prevented the models from being as sophisticated as might be desirable – given a sufficient number of cows there may be estimable cow variation for the quadratic effect of samples since last event. However, our data structure is somewhat idealised having come from respiration chambers rather than in-herd testing. In a commercial dairy herd, the pattern of methane production is unlikely to follow that seen in Figure 1, rather frequency and duration of feeding of events would be expected to vary between cows resulting in a more ‘choppy’ profile of methane production, with less extreme peaks and troughs (except possibly overnight).

Total daily methane production can be predicted from individual methane production samples or CH_4:CO_2 observations. The accuracy of prediction from methane yields is considerably higher, as would be expected.

A number of recording scenarios were considered. However, given the unrealistic data structure, the random samples are probably the most useful in representing what may be viewed on the farm. A cow being recorded twenty times a day would have an accuracy of prediction of 0.94 if CH_4 was recorded or 0.37 if was CH_4:CO_2 recorded. The accuracy increases asymptotically, with little benefit to more than 40 observations whichever trait is being used for prediction.

The equipment used to record in the field will differ greatly from the respiration chambers, and the traits observed may vary considerably from those in these data.

While this study has considered daily methane production, the main interest for breeding purposes is to predict methane production across the lactation, from which the accumulated emission of methane during a lactation cycle can be derived. The accuracy to estimate this parameter depends on one hand on the accuracy to estimate daily emission, as dealt with in this analysis, and on the other hand on the variation between days in the lactation cycle and the applied sampling frequency of days during the lactation. However, the three-day trial periods in this analysis made consideration of changes across days impractical (day was considered in early fixed effect analyses with no systematic effect found). Datasets from respiration chamber research that provide information of individual methane emissions representing the long term fluctuations in the lactation cycle are not available. It remains therefore unclear how accurate accumulated methane yields can be estimated by long term monitoring of the individual methane emission or the CH_4:CO_2 ratio.

3 Set-up of measuring equipment

3.1 Methane conversion and selection of monitoring parameters

A cost affordable large scale approach for the measurement of the conversion of feed into methane (CH_4) by individual dairy cows, comprising a few thousand animals, can only be undertaken if the measurements can be integrated in the management of dairy farms in practice. In such an undertaking the choice of the actual monitoring parameter is an important issue. In our preceding internal study the use of measurements of CH_4:CO_2 ratio in exhaled air, instead of the volume of CH_4 emitted, was proposed as an attractive technical option (Ogink et al., 2010). The reason is that with the ratio method only the gas concentrations in the animal's breath have to be measured, whereas for emitted CH_4 volume both air volumes and CH_4 concentration have to be recorded. The latter is a far more invasive and complicated task. It was argued that the CH_4:CO_2 ratio directly reflects the conversion of nonutilized feed C into emitted CO_2 and CH_4, and as such the avoidance of the extra greenhouse effect caused by the conversion of nonutilized C into CH_4 instead of CO_2. For dairy cows the conversion of feed to CH_4 can be defined as the ratio of the accumulated CH_4 production and the total milk production during a lactation cycle. Because feed intake, CO_2 production and milk production are strongly correlated, the CH_4:CO_2 ratio reflects this conversion to CH_4 per unit of feed intake or per unit of milk. However, variation in CH_4:CO_2 ratio may include various aspects of the cows metabolism. If we assume that the quantity and quality of offered feed is controlled and that it can be considered a fixed factor, we surmise that genetically based differences in the conversion of feed to CH_4 may be caused by a combination of mechanisms that all may be present in the current dairy cow population:

1. A lower production of CH_4 in the rumen (and large intestine) per unit of feed intake, at a given level of feed intake, quality of feed and efficiency of feed conversion into milk. This effect can both be monitored by directly measuring the conversion of feed to CH_4 and milk, i.e. recording CH_4 emission and milk production, or by recording the CH_4:CO_2 ratio as an indication of CH_4 emission per unit of milk produced.
2. A higher overall efficiency of feed converted into milk (total milk over total feed intake during a full lactation cycle) at a given level of feed intake and quality of feed; this lowers the amount of CH_4 produced per unit of milk because more milk is produced, whereas CH_4 and CO_2 are not changing per unit of feed intake. This mechanism can only be identified by directly monitoring the conversion of feed into CH_4 and milk. It does not have to be expressed by the CH_4:CO_2 ratio when it is unrelated to a lower amount of rumen degraded feed being converted into CH_4. It may be reflected by CH_4:CO_2 ratio when an improved efficiency is associated or caused by improved rumen degradation of feed which may increase CH_4:CO_2 ratio.
3. An improved conversion of feed to milk as a result of a higher *ad lib* feed intake and milk yield (dilution of feed requirements for maintenance per unit of milk produced), without changes in feed quality. This mechanism can only be identified by directly monitoring the conversion of feed into CH_4 and milk. Because a higher intake may affect both CH_4 and CO_2 and it is uncertain how CH_4:CO_2 ratio would be affected.
4. An improved conversion of feed to milk as a result of a higher *ad lib* feed intake and milk yield, achieved by a change in diet quality (for example more concentrates and less roughage intake). This effect can both be monitored by measuring the conversion of feed into CH_4 and milk and by measuring the CH_4:CO_2 ratio. An improved diet quality is likely to decrease CH_4:CO_2 ratio.

The described mechanisms 3 and 4 are based on higher feed intakes and higher cow productivity. It can be expected that they are closely correlated with selection for a higher milk production, and as such are already included in the standard breeding goals for high milk yields. However, the first two mechanisms may represent potential genetic variation sources that are not yet included in current breeding strategies and that may be utilized in lowering the conversion of feed into CH_4 and/or milk, i.e. a lower amount of CH_4 produced and/or a lower amount of feed required per unit of milk. It is not known if and how strong these two mechanisms act in current dairy cow populations, neither is it known which one is the most dominant.

One of the central questions in designing a set-up for the measurement equipment is whether we can rely solely on the gas ratio as the single monitoring parameter or that individual CH_4 emission has to be considered as well. From the discussion on potential mechanisms it follows that by measuring only gas ratios there is a chance that the potential effects of improved conversion of feed into milk on the conversion of feed into CH_4 will be ignored. This would mean that potentially a part of the genotypic variation in methane conversion is not identified, variation that is of extra interest because it represents an economical benefit as it is related to higher feed efficiencies. We therefore prefer at this stage to elaborate two approaches, one based on measuring CH_4:CO_2 ratio as the single monitoring parameter (approach A), and a second one based on a combination of monitoring both the CH_4:CO_2 ratio and the CH_4 emission (approach B). Two options are elaborated for approach A, because one of the two options can be easily combined or integrated with approach B, as will be later explained.

3.2 Choices in the location of sampling equipment

The following criteria for the installation of sampling equipment can be defined:

- The design of the sampling equipment should allow a straightforward integration of the installation in a normal dairy farm in practice, without requiring large investments in constructions or demanding barn modifications.
- During operation the sampling installation should not disturb normal farm management, nor affect cow behaviour. Forced routings and handling of cows have to be avoided.
- Installations should be sufficient robust (cow proof) to operate for a year and longer.
- Operation should be fully automatic, and not require extra attention from the farmer.
- Sampling during feeding intervals are preferably avoided as long as the direct effect of feed intake on the instantaneous methane emission is not known.
- Recording performance, investment costs and operation costs have to be in balance.

Given these criteria two potential sampling locations that comply with requirements can be identified:

- Sampling of respirational air in a milking robot.
- Sampling of air at the head end of cubicles

Because we do not know if and how feed intake affects methane emission patterns during feeding, the optional location of sampling in concentrate feeders is not considered. With regard to monitoring respirational air during milking we assume that enough sampling time is left during which the animal is not consuming concentrates. The sampling in a milking robot is preferred over sampling in multi-stand milking parlours because the simultaneous analysis of respirational air from 8 to 16 cows during a 15 minutes milking period requires a multitude of analyzers, whereas one analyzer would be sufficient in case of a milking robot.

3.3 Set-ups for sampling and measurement equipment

In this section we will describe layouts for both monitoring approaches A (CH_4-CO_2 ratio) and B (both ratio and CH_4-emission). For A we will work out two options based on different locations of sampling points.

3.3.1 Set-up based on CH_4-CO_2 ratio measurements in a milking robot (A1)

The integration of a respirational air sampler in the milking robot offers the following advantages:

- The cow's heads are in a relatively fixed position during their stay in the robot.
- Cow identification is already integrated in the milking robot.
- Cows will enter the robot at various times during the day (2-3 times), which allows for random sampling throughout the day.
- Because cows are entering one by one, all concentration measurements can be performed by a single analyzer. Normally a milking robot serves about 50-60 cows. Despite the relatively short staying times of cows, two milking robots could be measured as well by one analyzer if sampling switch devices are applied.

Sampling here can be organized by integrating a small sampling tube near the head of the cow. The construction of a sampling hood just above the animal that draws representative air from the front position of the animal could be considered, especially when the cow's head is moving around. Sampling should be organized in such way that sampling during the consumption of concentrates is excluded or separated from the non-feeding part. Both the exhaled air of the cows and the background air must be sampled and analyzed. Madsen et al. recently developed an operational system in a milking robot and reported first results (Madsen et al., 2010).

A lay-out of the sampling set-up and the required equipment is given in Figure 6a. Hardware specifications, investments and operational costs will be worked out in section 4.2

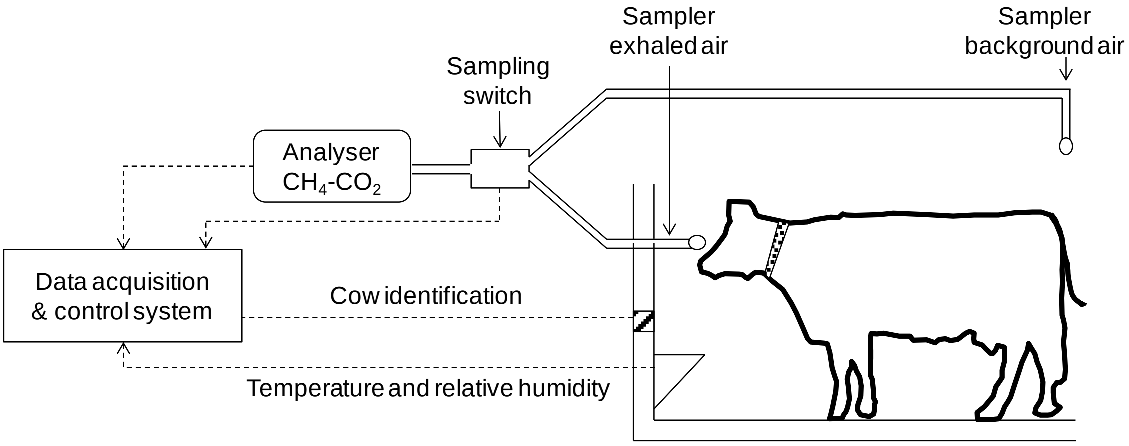


Figure 6a Side view set-up A1 (sampler, tubes, sampling switch, multi-gas analyzer, data acquisition and control system, cow registration, CO_2, CH_4, temperature, relative humidity)

An important item in this set-up is the overall measurement error. It should be small enough to reveal the animal variation. The measurement error is composed of the sampling error and instrumental error. With regard to the sampling error it is of interest to evaluate whether the relatively limited sampling intervals (2 or 3 periods of about 10 minutes, excluding time needed for feed intake) is sufficient to predict the daily mean CH_4-CO_2 ratio. From the first sampling scenario (2.3.1) in the data analysis it can be learned that sampling 2 times over a short interval of 15 minutes results in a correlation between true daily methane production and the sampled methane yield of 0.85. Because within-day variation of the gas ratio of cow pairs is about half of the variation of methane emission (variation coefficients 12 and 23% respectively), the accuracy of predicting the true daily gas ratio from sampled gas ratios may expected to be higher than for daily methane production. It should be realized that the variation patterns in the dataset are based on cow pairs, and that individual variations will be larger. Furthermore we may expect that as a result of more variation in feeding times and activity, cows in a cubicle house will show more variation in methane emission and gas ratios compared to the studied patterns in the respiration chambers. Stronger variations within a day means that predictor correlations will be lower than estimated for the experimentally stabilized situation in the dataset.

3.3.2 Set-up based on CH_4-CO_2 ratio measurements in cubicles (A2)

In this set-up sampling inlets are constructed at the cubicle’s end close to the head of lying cows. The advantages of this approach are:

- The cow’s heads are in a relatively stable position during their stay in the cubicle. Lying in cubicles is part of their behaviour and does not require extra handling.
- Cows will be lying in cubicles for extended periods that may take several hours, this will create the opportunity of relatively long sampling periods that are distributed over day and night.

Sampling here can be organized by locating a small sampling tube near the head of the cow. The construction of a sampling hood just above the animal that draws representative air from the front position of the animal could be considered, especially when practice shows that the cow’s head is moving around. In this approach all cubicles in one row can be equipped with sampling tubes. By means of sampling switches one analyzer may serve all tubes consecutively during fixed sampling periods. A number of background sampling points has to be included in the sampling scheme. Precautions have to be taken that the exchange of air between cubicles’ ends is prevented. Each sampling cubicle has to be equipped with a cow identification sensor.

A lay-out of the sampling set-up and the required equipment is given in Figure 6b. Hardware specifications, investments and operational costs will be worked out in section 4.2 In terms of investments, costs will be higher than for A1 because extra cow identification units have to be installed.

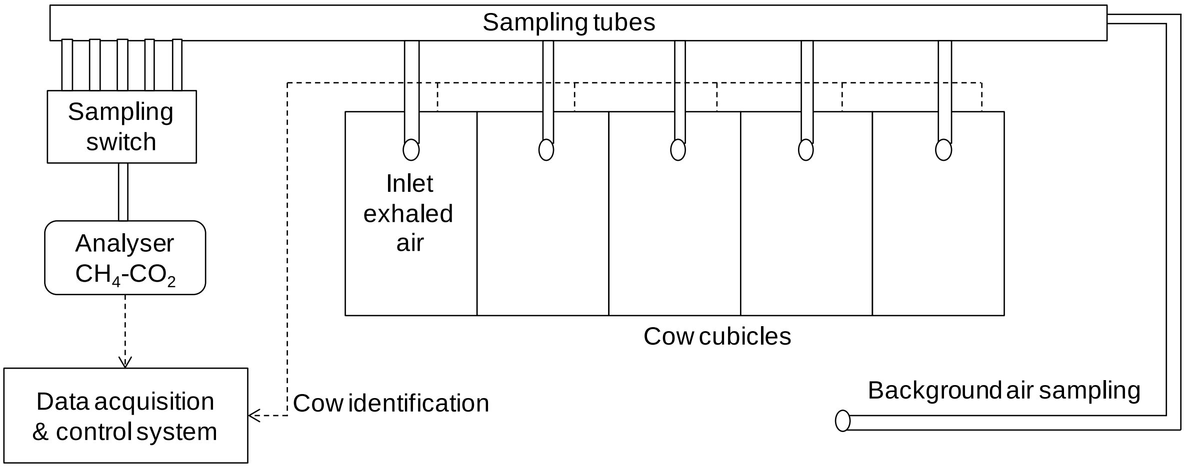


Figure 6b Top view set-up A2 (sampling locations, tubes, sampling switch, multi-gas analyzer, data acquisition and control system, cow registration, CO_2, CH_4, temperature, relative humidity)

Compared to A1 the benefits are that one animal can be sampled more frequently and randomly across the day. There is no risk of potential side effect of direct feed consumption on the recorded gas ratios. As can be learned from the analysis, frequent and randomly sampling will improve the correlation between the true daily ratio mean and the sampled ratio mean.

3.3.3 Set-up based on CH_4 emission measurement hoods placed over cubicles (B)

- In this set-up individual CH_4 emissions are measured by construction of sampling hoods that are placed as ceilings over separated cubicles (Figure 6c). The hoods are equipped with a ventilation fan to provide a fixed known air flux through the outlet of the hood. The design should allow free unhampered access for cows and the cubicle should despite the additional equipment be attractive enough for lying. Robustness is another major design item. The underpressure by forced ventilation should be high enough to collect all cow emitted methane at the outlet of the hood, whereas air flow should be low enough to create a high concentration gradient between the background concentrations and the concentration of methane at the outlet of the hood. Background concentrations have to be measured with high accuracy to avoid problems with the emission of nearby standing or passing animals. Similarly as for measuring concentration ratio’s in cubicles the following aspects are in favour of placing sampling devices over the cubicles:

- The cows are lying in a stable position during their stay in the cubicle. Lying in cubicles is part of their behaviour and does not require extra handling.
- Cows will be lying in cubicles for extended periods that may take several hours, this will create the opportunity of relatively long sampling periods that are distributed over day and night.

A lay-out of the sampling set-up and the required equipment is given in Figure 6c. Hardware specifications, investments and operational costs are worked out in section 4.2. In terms of investments, costs will be higher compared to the previous options because extra cow identification units have to be installed and sampling hoods have to be developed, tested and installed.

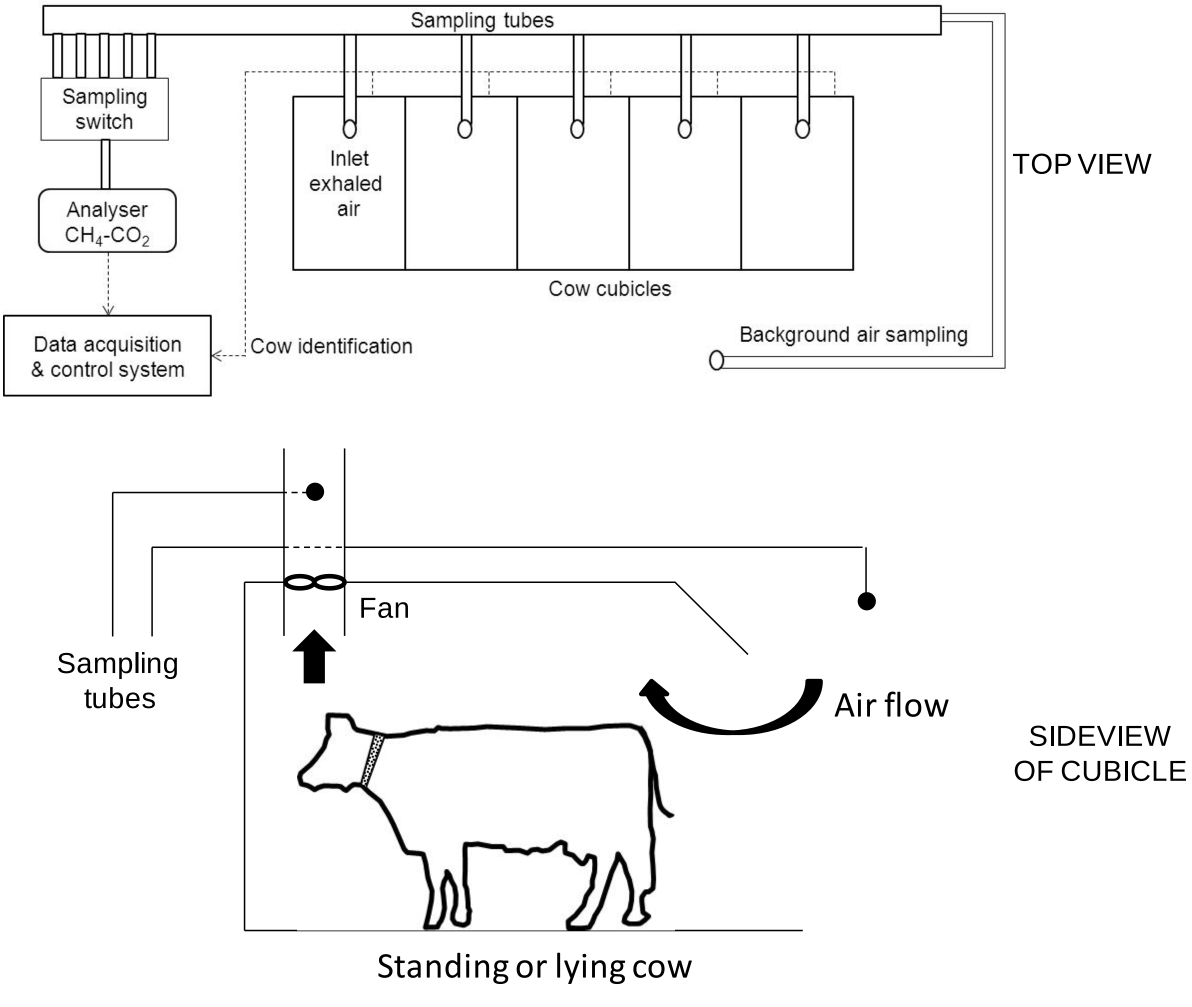


Figure 6c Top and side view set-up B (sampling locations, tubes, switch, multi-gas analyzer, data acquisition and control system, cow registration, CO_2, CH_4, temperature, relative humidity)

The data analysis showed that all sampling strategies designed for use in cubicles produce high correlations between true daily methane emission and the sampled methane yields.

4 Future research options and costs

4.1 Future research options

A number of issues have to be researched and solved before a large study at farm scale can be set up in which individual methane emissions of dairy cows can be determined for a large number of animals. These items will be discussed below. The research that is still needed -following from these items- are integrated in a proposed research scheme that eventually will lead to operational measurement methods that can be used in large scale farm monitoring studies.

Development of sampling and measurement equipment
In case of the gas concentration methods (methods A1 and A2) essential issues are the efficiency of sampling animal breath at as high as possible concentrations in exhaled gases, the elimination of other disturbing sources (animals) and a proper monitoring of background concentrations. Developing of prototypes and testing of their performance in experimental and real cow situations is required.

Selection of key monitoring parameters
From the analysis and scenarios elaborated in chapter 2 and the technical criteria and limitations outlined and discussed in chapter 3, two basic approaches were developed. One is based on measuring CH_4:CO_2 ratio as the single monitoring parameter (approach A) and the second on a combination of monitoring the individual methane emission and the CH_4:CO_2 ratio (approach B). Although option A looks to be the most attractive in terms of costs and its potential to be relatively quickly developed as an automated system in normal farm practice, there are risks that part of the variation in methane efficiency may be overlooked by monitoring the CH_4:CO_2 ratio only. Because this specific type of variation in methane efficiency may be linked with a higher feed efficiency, an economically interesting trait, it is important that the size of this type of variation as a proportion of the total variation in methane efficiency is studied in a representative cattle herd. This implies that a comparative research effort is required in which both approaches are compared in a farm herd, before sampling efforts are scaled up to multi-farm level for large number studies. The outcome of this comparative research should determine which approach has to be applied at multi-farm level.

Effect of feed intake in case of monitoring gas ratios
Within approach A two methods are proposed that differ with regard to sampling location. The location of sampling tubes in a milking robot has a number of clear benefits in terms of consecutive measurements of single cows, cow fixation and cow identification. However, it is not clear how the CH_4:CO_2 ratio during a milking robot interval is affected by the physical effects of concentrate intake on the direct release of methane from the rumen and indirect release through the lungs. We do not know to what extent measured gas concentrations may be blurred by feed intake effects, and how much inaccuracy is introduced by this process. For that reason we did not opt for using concentrate feeders as sampling locations. Although we expect that in case of sampling in a milking robot the potential impact of this effect is smaller, it cannot be fully excluded. We believe that a comparison is required of sampling concentrations in the milking robot and sampling in cubicles. Although sampling in cubicles is associated with higher costs (extra cow identification equipment) it may well be that these higher initial costs are outweighed by more representative and precise sampling of gas concentrations.

Required sampling frequency across lactation cycles
As discussed in chapter 2.4, an important element in the overall accuracy of a sampling scheme for methane conversion is the variability of monitored parameters across the lactation cycle. Earlier we indicated that the actual target parameter is defined as the overall methane conversion over the complete lactation cycle. For this parameter in principle a poor or moderate accuracy of measurements at day level can be easily compensated for by frequent samplings across the lactation cycle. One may compare this with the effect of increasing the sample size from a standard normal

distribution on the standard error of the estimated mean of this distribution. Variability across the lactation cycle could not be distracted from the respirational dataset in this study, and as far as we know, no information from research literature is available. The size of this variability and the knowledge of systematic patterns are important elements in designing an optimal sampling scheme for large scale applications.

Optimising the accuracy-cost relationship
From the results of the analysis in chapter 2 we expect that for both key monitoring parameters (emission and ratio) an acceptable minimum daily accuracy can be reached. Where the daily accuracy is related to estimating the true daily mean. We realize that this analysis gave a somewhat biased picture because of the smooth emission patterns and high precision equipment in the respiration chambers, but think that in technical terms enough options are available to ensure a basic accuracy level at daily level. At this stage it is however not possible to estimate the overall accuracy of different sampling and measuring scenarios when related to the methane conversion over the full lactation cycle. Estimates of both the accuracy of the single daily measurements, and the variability and number of measurements across the lactation cycle are needed. A poor or moderate accuracy of the daily values can be compensated for by a higher frequency of measurements in the lactation cycle, and vice versa. It is not *a priori* required that the most accurate method for daily sampling is the most optimal one in the total scheme. Similarly as for the other items, information on how to optimize a large scale method can be derived by starting to measure with different methods at high frequency in a cow herd.

Research phases
Based on the research issues to be solved, as outlined before, the following phases in research are distinguished:

- Phase 1: developing, testing and validating measurement prototypes for A1, A2 and B. The required output of this phase are proper working sampling and measurement systems, that are robust and reliable.
- Phase 2: applying methods A and B simultaneously in a cow herd for a 1 year period on a research farm. This monitoring period will give insight into the performances of the different methods and the variability in methane conversion across lactation cycles. The results allow the design of an optimized sampling and measurement system to be used on large scale.
- Phase 3: large scale sampling on practical farms to identify markers that are best related to methane conversion.

4.2 Costs related to sampling and measurement installations

Two categories of costs are distinguished here:

- Prototype development: costs of developing and testing effective sampling hoods for the approaches A1, A2 and B.
- Installation costs on farm scale for measurement campaigns

Prototype development involves different consecutive cycles of construction of prototypes, testing prototypes in cow barns and using the test results for improving the prototype construction. This development and test work is laborious and could be carried out as part of a PhD study. In Table 7 prototype development costs are estimated for the different approaches. For personnel costs, the ratio of required labour hours provided by PhD researcher and post-doc researchers is 80:20. Developing a tested prototype for A1, A2 and B requires approximately; 6, 12 and 12-16 months.

Table 7 Costs (x € 1000) of prototype development of sampling and measurement installations for different approaches

Measurement approach	Cost prototypes sampling hoods: used materials, construction work	Measurement equipment: use of multigas analyzer, dataloggers	Personnel costs for designing and testing	Total costs
A1	10	5	20	35
A2	15	10	30	55
B	25	10	45	80

The costs of installation and operation of sampling and measuring equipment on farm scale are included in Table 8. The set-ups of A2 and B are based on sampling 20 cubicles. Estimated costs of using measurement equipment (analyzers and dataloggers) and personnel costs for regular data checks, troubleshooting and storage of raw data are based on a monitoring time of 1 year. Data analysis and reporting are not included.

Table 8 Costs (x € 1000) of installation sampling and measuring equipment on farm scale for different approaches

Measurement approach	Number of sampling points	Investment in sampling device, tubing	Investment cow identification	Measurement equipment use: 1 year period	Personnel costs, 1 year period: check and storage raw data	Total costs
A1	2	5	0	5	20	30
A2	20	15	25	10	20	70
B	20	35	25	10	20	90

5 Conclusions

The analyses of data from respiration chamber research showed that total daily methane emission can be predicted from individual methane emission samples or CH_4:CO_2 observations. Accuracies of a number of investigated recording scenarios differ. Highest accuracies are reached in scenarios based on recording methane emissions. The random sampling scenarios are probably the most useful in representing what may be viewed on the farm.

Assuming that there are no disturbing effects of feed intake at the moment of feed intake, the recording scenarios showed that measuring methane (or ratio CH_4:CO_2) in the milking parlour or concentrate feeders will provide predictions of the full day production that are close to the accuracy reached with scenarios based on sampling in cubicles. However, the assumption that there are no feed intake effects cannot be verified, and it is has to be realized that under real farm conditions methane emission patterns may be more variable.

It remains unclear how accurate overall methane conversion over the full lactation cycle can be estimated by long term monitoring of the individual methane emission or the CH_4:CO_2 ratio, because the dataset gave no insight in the variability of methane conversion across the lactation cycle. There is no information available from research literature on this point.

Monitoring individual methane conversion in cows by using the parameter CH_4:CO_2 ratio in exhaled air, instead of the volume of CH_4 emitted, is in technical terms an attractive option. The reason is that with the ratio method only the gas concentrations in the animal’s breath have to be measured, whereas for the emitted CH_4 volume both air volumes and CH_4 concentration have to be recorded. The CH_4:CO_2 ratio directly reflects the conversion of nonutilized feed C into emitted CO_2 and CH_4, and as such the avoidance of the extra greenhouse effect caused by the conversion of nonretained C into CH_4 instead of CO_2.

A number of potential mechanisms that may be at the basis of genetic differences in methane conversion between dairy cows were discussed in chapter 3. It was concluded that by measuring only CH_4:CO_2 ratios there is a chance that the potential effects of improved conversion of feed into milk that will lead to a lower methane conversion will be ignored. This would mean that potentially a part of the genotypic variation in methane conversion is not identified by this ratio parameter, variation that is of extra interest because it represents an economical benefit as it is related to higher feed efficiencies. We therefore elaborated two approaches, one based on measuring CH_4:CO_2 ratio as the single monitoring parameter (approach A), and a second one based on a combination of monitoring both the CH_4:CO_2 ratio and the CH_4 emission (approach B).

Two methods are elaborated for measuring the CH_4:CO_2 ratio, one based on sampling in milking robots (A1) and one on sampling in cubicles (A2). One method is elaborated for measuring individual methane emissions (B) by sampling exhaled air in modified cubicles.

A number of research issues has to solved before a method for large scale recording on farms can be accomplished. These issues relate to the development of operational sampling devices, choice of the most effective monitoring parameter (gas ratio sampling versus emission), short terms effects of feed intake on methane release, and variability of methane conversion across the lactation cycle. To solve these issues the following phases in future research are proposed:

- Phase 1: development, testing and validating measurement prototypes for A1, A2 and B. The required output of this phase are proper working sampling and measurement systems, that are robust and reliable.
- Phase 2: applying methods A and B simultaneously in a cow herd for 1 year period on a research farm. This monitoring period will give insight into the performances of the different

methods and the variability in methane conversion across lactation cycles. The results allow the design of an optimized sampling and measurement system to be used on large scale.

- Phase 3: large scale sampling on practical farms to identify markers that are best related to methane conversion.

Chapter 4.2 provides indicative costs of developing operational sampling and measurements systems for methods A1, A2 and B and estimated investment and operational costs of monitoring at farm scale during 1 year periods. Both development costs and monitoring costs are lowest for the method based on sampling in the milking robot (A1) and highest for measuring methane emissions in cubicles (B). However, next to these costs the measurement accuracies that can be reached by these systems will determine which system is most appropriate for large scale application.

Literature

Grainger et al., (2007). Methane emissions from dairy cows measured using the sulfur hexafluoride (SF6) tracer and chamber techniques Journal of Dairy Science 90: 2755-2766.

Madsen et al., (2010). Methane and carbon dioxide ratio in excreted air for quantification of the methane production from ruminants. Livestock Science 129: 223-227.

Ogink et al., (2010) (intern rapport Wageningen UR Livestock Research)

Waghorn et al., (2006). Ruminant contributions to methane and global warming - A New Zealand perspective. In: Climate Change and Managed Ecosystems LA English DT Article CT International Conference on Science of Changing Climates - Impacts on Agricultures, Forestry and Wetlands.

Zijderveld et al., (2011). Dietary inclusion of diallyl disulfide, yucca powder, calcium fumarate, an extruded linseed product, or medium-chain fatty acids does not affect methane production in lactating dairy cows. Journal of Dairy Science 94 :3094–3104.

Wageningen UR Livestock Research

Edelhertweg 15, 8219 PH Lelystad T 0320 238238 F 0320 238050

E info.livestockresearch@wur.nl I www.livestockresearch.wur.nl