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SmartCow: an integrated infrastructure for increased research capability and innovation in the European cattle sector

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SmartCow
an integrated infrastructure for increased research capability and innovation in the European cattle sector

EXECUTIVE SUMMARY

Background	Concerns regarding the greenhouse gas emissions of livestock production have been focused on methane emissions from ruminants, which account for 30 to 44% of global anthropogenic methane emissions (e.g. Arndt et al., 2022). Substantial research activity over the last 30 years, on a global scale, has focused on how dietary composition and other management strategies determine methane emissions, to predict emissions for specific production systems and develop mitigation strategies (Beauchemin et al., 2020). Respiration chambers have been used since the 1800s to measure gaseous emissions from cattle and when used correctly are considered a 'gold standard' measurement technique for methane emissions, but with recent research interest in methane, numerous new facilities have been established and there are considerable concerns regarding the precision and accuracy of the measurements being obtained at some facilities (Gardiner et al., 2015). One of the aims of SmartCow (https://www.smartcow.eu/) is to identify sources of variation in key 'gold standard' in vivo measurements of feed efficiency and greenhouse gas emissions, including methane emissions of cattle. This will enable the application of the 3-Rs by reducing variability, increasing precision and accuracy, and standardizing the methods and procedures used across research facilities within SmartCow installations and more widely. SmartCow WP5, working alongside WP3 for standardization issues, will evaluate and enhance the techniques being used, as well as contribute to the development of alternative approaches within WP6 through sample provision.
Objectives	The overall aim is to identify and address sources of variation in measurements of methane emissions by cattle using respiration chambers, thereby improving the measurements and unifying the approaches used across SmartCow installations, providing a global standard. The objectives were to (i) improve the accuracy and precision of measurements, and (ii) unify the methods used across SmartCow infrastructures.

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	Specific objectives were to identify sources of variation in measurements obtained using respiration chambers through a meta-analysis of historic data and a ring test of SmartCow facility respiration chamber methane gas recovery and develop optimised procedures for measuring whole animal methane emissions using respiration chambers.
Methods	We used 2 approaches: **Meta-analysis:** A meta-analysis of existing measurements of methane emission by lactating and growing cattle was conducted to determine the extent to which variation in methane measurements obtained using respiration chambers is attributable to research facility site and associated methodology. Briefly, a database containing 4329 individual cow measurements of methane emission carried out at 14 research sites was assembled. Sites included SmartCow facilities and collaborators within the Global Research Alliance. Individual cow measurements included dry matter intake (DMI), the chemical composition of ration components (CP, GE, EE, Ash, NDF and ADF concentration), resulting nutrient intakes, animal characteristics (e.g. breed, body weight), and production outputs including milk yield and composition for lactating cattle. Multivariable analysis was used to account for variation due to experiment within research site, research site, diet intake, diet composition, and animal characteristics to ascertain the extent to which location and methodology accounted for variation in measurements of methane emission. **Ring test of methane recovery:** The National Physical Laboratory (NPL; Teddington, England, UK) provided a ring-test of SmartCow respiration chamber facilities at INRAE, AU, FBN, WU, and URead, using procedures developed previously for a Defra funded methane inventory project in the UK (Gardiner et al., 2015). NPL staff visited each facility and delivered controlled releases of methane approximating cattle emissions on a steady state basis. Each facility measured and calculated the methane emission via the facility's normal in-house measurement and calculation procedures. Releases were carried out directly into the analysers, output ducting and the chambers themselves enabling facilities to be characterised in terms of analyser efficiency, ducting efficiency and isolated chamber efficiency. From the test data, it was also possible to determine a combined efficiency for each chamber (i.e. when the chamber, ducting and analyser were used together) and from these an overall facility efficiency for each site. Consequently, the facility efficiency values could be used to determine the comparability across the five facilities.

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Results & implications	**The results of the meta-analysis (MS5.2) and ring-test of chamber recoveries (MS5.3) were reported and discussed in detail at a workshop held virtually on 6 January 2022.** Minutes from the meeting and individual presentations were made available to SmartCow researchers on the SmartCow collaborative website (https://www.smartcow.eu/). These presentations and the discussions highlighted improvements made at each SmartCow installation, and identified key sources of variation and areas of focus for future improvement in the precision and accuracy of respiration chamber measurements of methane emission by cattle. A full report of the methodology used, results and discussion of the meta-analysis of variation in measurements of methane emission can be found in the full meta-analysis report (MS5.2; see below). Initial evaluation of the relationship between DMI and methane emission found there appeared to be less variation due to the research site when only measurements obtained using respiration chambers (versus GreenFeed and SF6 techniques) were included. After accounting for dietary effects and a significant effect of individual experiments within site (38 to 46%), the variation in methane emission attributable to research site was relatively small (7% to 11%). In addition, accounting for the number of days of measurement and experimental design did not improve predictions of emission. Similar results were found for methane yield (CH4/kg DMI). The results suggest that the comparability of measurements obtained using respiration chambers at the different research sites represented in the data base is reasonably good, but the analysis did not include measurements obtained using other methods. A full report of the methane recovery ring test performed by NPL (**MS5.3**) is included below. The recovery test was performed at 5 of the SmartCow facilities that use respiration chambers for measuring methane emission from cattle. For analyser efficiencies, it was found that all deviated from unity by no more than the 3rd decimal place, with one exception due to an issue with the in-house gas cylinder used for span calibration. The ducting efficiencies evidenced increased deviations from unity compared to the analyser efficiencies, which was expected due to the difficulty of calibrating flow measurements. Isolated chamber efficiencies demonstrated increased deviations compared to ducting efficiencies, possibly evidencing some element of inhomogeneous concentration mixing and/or flow profiles. Combined chamber efficiencies were improved compared to isolated chamber efficiencies, as in some cases analyser or ducting efficiencies had opposing effects on

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	recovery compared to the isolated chamber. These combined chamber recoveries were then used to determine an average facility efficiency which showed comparability across the five SmartCow facilities (6.2% ; k = 2, 95% confidence) that was markedly better than a similar UK ring-test (Gardiner et al., 2015) where the analogous value was 25.7% (k = 2, 95% confidence). These results suggest that as observed in the meta-analysis of variation due to research site, the variation in respiration chamber measurements of methane emissions by cattle at SmartCow installations is low compared to the variation due to individual experimental conditions and known effects of diet composition and intake and cattle characteristics such as gender and physiological state. Discussion of the results at the January 2022 workshop highlighted key practices to reduce variation and increase the precision and accuracy of the measurements. Some of these include routine checks for leaks, flow meter calibration, tests for adequate internal mixing of the incoming air and expired gases inside chambers, and the establishment of user groups to facilitate future ring-tests and sharing of precision standards and equipment for performing recovery tests. The SmartCow consortium Publisso publication 'Methods in cattle physiology and behaviour research – Recommendations from the SmartCow consortium' includes a chapter 'Respiration chamber facility' that describes recommended procedures for operating respiration chambers, including measurements of methane emission (DOI: 10.5680/mcpb011) and a chapter ''The gas recovery test of respiratory chambers' that describes recommended procedures for conducting routine methane and carbon dioxide recovery tests for respiration chambers to validate measurements (DOI: 10.5680/mcpb010). **The results of the joint research activities for WP5 highlight important aspects of the procedures described in the book of methods and potential sources of variation that warrant attention, but do not suggest that major revisions of the current chapter are required.** Specific sources of variation were identified at each SmartCow respiration chamber facility and it is recommended that specific attention should be paid to the following: a. leaks, including chambers themselves, ducting, or analyzer plumbing b. flow meter calibration, which should be performed regularly c. mixing of air in chambers and ducting - use of fans for mixing of air within chambers if needed d. timing of sample analysis when switching analyzer sample flow between chambers, taking into account analyzer response times e. the importance of routine recovery test for validation of the measurements and identification of measurement bias and drift.

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	One of the proposals at the SmartCow workshop was the formation of a user group for the sharing of best practices and equipment such as gas flow meters for the precision release of methane during recovery tests. Such a user group could also provide training opportunities for staff and students. The formation of such a user group would facilitate future ring-tests for validating local recovery measurements and periodic tests of chamber facility comparability. It is proposed that with the formation of the SmartCow European Research Group a respiration chamber user group should be formed as part of the Research Group activities.

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Table des matières

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1 Introduction

Concerns regarding the greenhouse gas emissions of livestock production have been focused on CH4 emissions from ruminants, which account for 30% of global anthropogenic CH4 emissions (Arndt et al., 2022). Substantial research activity over the last 30 years, on a global scale, has focused on how dietary composition and other management strategies determine CH4 emissions, in order to predict emissions for specific production systems and develop mitigation strategies (Beauchemin et al., 2020). Recent commitments at COP26 to reduce national CH4 inventories by 30% by 2030 relative to 2020 and other carbon reduction commitments by animal industry stakeholders and governments have led to a recent upsurge of interest in research on CH4 mitigation strategies. The availability of large amounts of individual observations of CH4 emission by cattle has led to a number of recent meta-analyses to determine dietary and other factors that determine CH4 emissions (e.g., Niu et al., 2018; van Lingen et al., 2019; Benaouda et al., 2019). These meta-analyses have included random effects of experiment to account for variation associated with specific experimental procedures and conditions to improve predictions of CH4 emission, but variation due to research site (location) and the methodology used has not been a focus.

Measurement of CH4 emissions by cattle using respiration chambers has been associated with measurements of energy balance of cattle since the 1800s and when used appropriately respiration chambers continue to be considered the 'gold standard' for measuring CH4 emissions (Hammond et al., 2016). However, it is known that such measurements can be subject to variation due to differences in the methods used and how they are implemented. For example, a ring test of methane recovery measurements for a project in the UK, based on the recovery of methane quantitatively released at strategic locations within the measurement system, found a large variation in methane recovery between research sites that was in part associated with measurement of air flow rate (Gardiner et al., 2015). Other specific sources of variation in CH4 emission measurements include system leaks, calibration or other analyzer errors, differences in the number of days of measurement, the methods used to determine feed dry matter intake (DMI), the experimental design used (change-over or continuous designs), or the type of measurement (respiration chamber, SF6 tracer, or GreenFeed; Hammond et al., 2016).

2 Objectives:

The overall objective of task 5.2 was to identify sources of variation in methane emission measurements using a meta-analysis of historical measurements of methane emission by individual cattle and a ring test of respiration chamber methane recovery at SmartCow facilities to optimise procedures for measuring whole animal methane emissions by growing and lactating cattle.

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3 Methods:

First, historic measurements of methane emission for cattle will be compared through a meta-analysis of existing data to determine the extent to which the 'location effect' introduces systemic variation after accounting for the effects of diet composition and methods on measured methane emission.

Second, measurements of methane recovery will be obtained for respiration chambers used for growing and lactating cattle based on measurements obtained using local procedures during precision gravimetric methane release, much as described by Gardiner et al., (2015). The comparability of measurements across research facilities (sites) will be determined along with local sources of error and standard protocols for calibration will be developed.

3.1) Meta-analysis of existing data: A database containing 4329 individual cow measurements of CH_4 emission from trials carried out at 14 research sites was assembled using data from SmartCow (https://www.smartcow.eu/) partners as well as the Feed and Nutrition Network of the Global Research Alliance (https://globalresearchalliance.org/research/livestock/networks/feed-nutrition-network/). Sites were located in North America (3) and Europe (11). The data set was comprised of 108 separate experiments with sites contributing data from 1 to 36 experiments (33 to 935 observations). Individual cow measurements included dry matter intake (DMI), the chemical composition of diets fed (CP, GE, EE, Ash, NDF and ADF content), resulting nutrient intakes, animal characteristics (e.g. breed, body weight), and production outputs including milk yield and composition for lactating cattle. Individual cow methane (CH_4) emissions were measured using respiration calorimetry chambers (Chamber), GreenFeed (CLock, USA) and the sulphur hexafluoride (SF6) tracer technique in the initial database assembled. Individual cows' records missing either CH_4 emissions or DMI were excluded (25 records). GreenFeed and SF6 measurements represented a small proportion of the overall data set and were excluded from the final data set. Sites contributing data from a single experiment only were also excluded to permit the fitting of site and experiment as random effects in the mixed models analysis. For the remaining 2863 observations, single variables were tested one by one with DMI in a series of mixed models where CH4 emission in g/d was the outcome variable. Experiment code nested within site was the random effect in a random intercept model. Variables were selected for further model development if their effect was significant or had a nonsignificant trend ($P < 0.10$). With CH4 emissions as the outcome variable, these were tested in linear mixed models with experiment nested within site as the random effect. An automated model build function based on lowest BIC while controlling for variance inflation factors was used to determine the 'best model' fit (van Lingen et al., 2019) using R version 4.0.3 (R Core Team, 2020). Using this approach, breed could not be included in the model fitting process due to being correlated with milk production (fat concentration and milk yield) and cow status, and was therefore not considered for covariate selection and model fitting. The sum of the variance associated with each random effect (site and experiment) and the residual variance were used to calculate a percentage variance associated with each.

3.2) Ring test of methane recovery: The National Physical Laboratory (NPL; Teddington, England, UK) provided a ring-test of SmartCow respiration chamber facilities at INRAE, AU, FBN, WU, and URead, using procedures developed previously for a Defra funded methane inventory project in the UK (Gardiner et al., 2015). NPL staff visited each facility and delivered controlled releases of methane approximating cattle emissions on a steady state basis. Facility staff did not influence the methane releases carried out by NPL other than facilitating logistical arrangements. Similarly NPL staff did not influence the operation of the facilities and the measurements of methane release obtained. Testing was blind in that each facility did not know the quantities of methane that were released. For each

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release, each facility was requested to measure and calculate the methane emission via the facility's normal in-house measurement and calculation procedures. Releases were carried out directly into the analysers, output ducting and the chambers themselves enabling facilities to be characterised in terms of analyser efficiency, ducting efficiency and isolated chamber efficiency. From the test data, it was also possible to determine a combined efficiency for each chamber (i.e. when the chamber, ducting, and analyser were used together) and from these an overall facility efficiency for each site. Consequently, the facility efficiency values could be used to determine the comparability across the five facilities.

4 Expected outcomes:

Identification of the extent of variation in the accuracy of methane emission measurements within the SmartCow infrastructure along with recommended measurement protocols that improve the precision and accuracy of future measurements obtained.

5 Key Results: Brief synopsis of the outcomes of the joint research activities for WP5

The results of the meta-analysis of methane measurements (MS5.1 – appendix 1) and ring-test of methane recovery measurements (MS5.3 – appendix 2) were reported and discussed in detail at a workshop held virtually on 6 January 2022. Minutes from the workshop and individual presentations are available on the SmartCow collaborative website. These presentations and the minuted discussions highlighted key findings and observations at each SmartCow installation and identified key sources of variation and areas of focus for future improvement in the precision and accuracy of measurements of methane emission by cattle.

5.1) Meta-analysis of methane measurements: A full report of the methodology used, results, and discussion of the meta-analysis of variation in measurements of methane emission can be found in the full meta-analysis report (**MS5.2 – Appendix 1**). Initial evaluation of the relationship between DMI and methane emission found there appeared to be less variation due to the research site when measurements obtained using GreenFeed and SF6 techniques were excluded (Figure 1). As observed in previous meta-analyses (e.g., Niu et al., 2018), DMI was a major determinant of CH4 emission, with dietary forage and ether extract concentration also having significant positive and negative effects, respectively. There was also a positive association between milk fat concentration and CH4 emission, which may reflect dietary forage effects. In addition, there was an overall negative effect of feed additives for the studies included in the present data set. After accounting for these dietary effects and a significant effect of individual experiments within each site (38 to 46% in models without or with milk production related variables, respectively), the variation in CH4 emission using respiration chambers that was attributable to research site was relatively small (7% or 11%). In addition, accounting for the number of days of measurement and experimental design did not improve predictions of CH4 emission. Similar results were found for methane yield (g CH4/kg DMI), with 0 to 9% of variation due to site and 43 to 47% due to experiment for the data set including lactating cattle. For methane yield, milk fat content was positively related, and diet concentrations of ether extract and ADF had significant negative

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and positive effects, respectively. The results suggest that the comparability of measurements obtained using respiration chambers at the different research sites represented in the data base is reasonably good, but the analysis did not include measurements obtained using other methods such as the GreenFeed system or SF_6 tracer method.

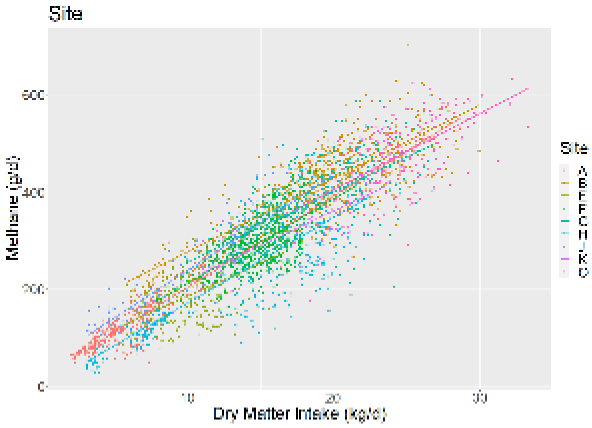

Figure 1. Relationship between dry matter intake and methane emission of cattle for each research site.

5.2) Ring test of methane recovery. A full report of the methane recovery ring test performed by NPL (**MS5.3**) is included below. The recovery test was performed at 5 of the SmartCow facilities that use respiration chamber facilities for measuring methane emission by cattle. As noted above, the tests were carried out to determine the efficiency of each chamber's analyser, ducting, and isolated chamber and these efficiencies were then used to calculate a combined chamber efficiency. For analyser efficiency, it was found that the deviation from unity was no more than the 3rd decimal place, with one exception. This exception was investigated by the associated facility and the cause was traced back to an issue with the in-house gas cylinder used for span calibration. The ducting efficiencies evidenced increased deviations from unity compared to the analyser efficiencies. This broadly correlates with expectations as there is generally less quality assurance / quality control (QA/QC) associated with the calibration of flow meters used for measuring chamber exhaust air flow rates. For example, daily or weekly in-situ calibrations are possible for analysers whereas flow sensors typically have to be returned to 3rd parties for calibration and this is only possible less frequently. Isolated chamber efficiencies (Figure 2) demonstrated increased deviations compared to ducting efficiencies, possibly evidencing some element of inhomogeneous concentration mixing and/or flow profiles. Combined chamber efficiencies (Figure 3) were improved compared to isolated chamber efficiencies, as in some cases analyser or ducting efficiencies had opposing effects on recovery compared to the isolated chamber. Combined chamber recoveries were used to determine an average facility efficiency (Table 1) and from the facility efficiencies comparability across the five facilities was determined (as 6.2% (k = 2, 95% confidence), which was markedly better than a similar UK ring-test from ~10 years prior (Gardiner et al., 2015) where the analogous value was 25.7% (k = 2, 95% confidence). Although, it was noted that the UK ring-test was not of the same facilities and that some of the facilities were designed for sheep, whereas all the facilities reported herein were designed for cattle. Lastly, it was shown that if each facility applied, the calibration function determined in the ring-test the comparability could potentially be improved to 5.4% (k = 2, 95% confidence).

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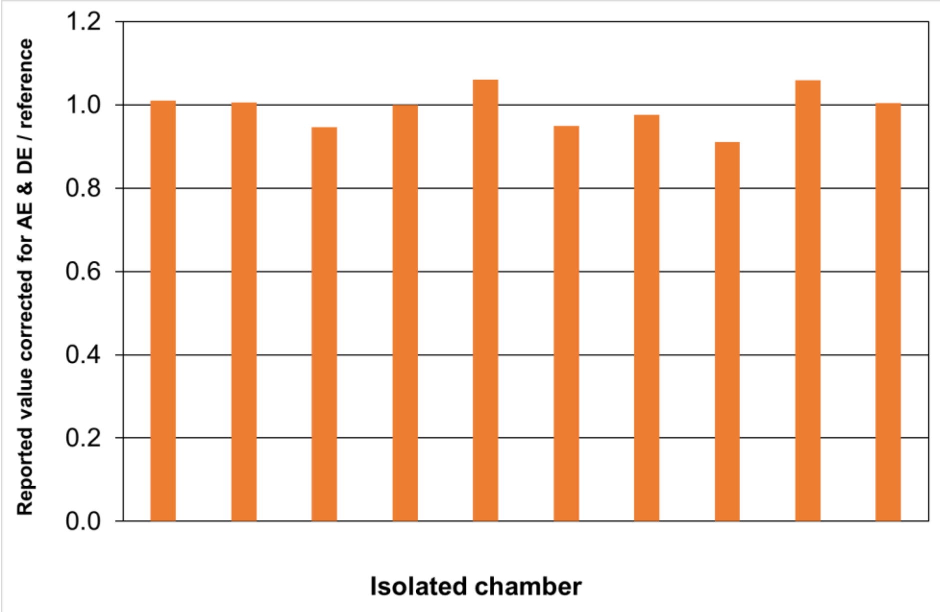

Figure 2. Anominized isolated respiration chamber methane recoveries for 10 chambers at 5 SmartCow research facilities (1.0 = 100%) after correction for analyzer and ducting efficiency. Note only chambers where ducting efficiency was determined are included.

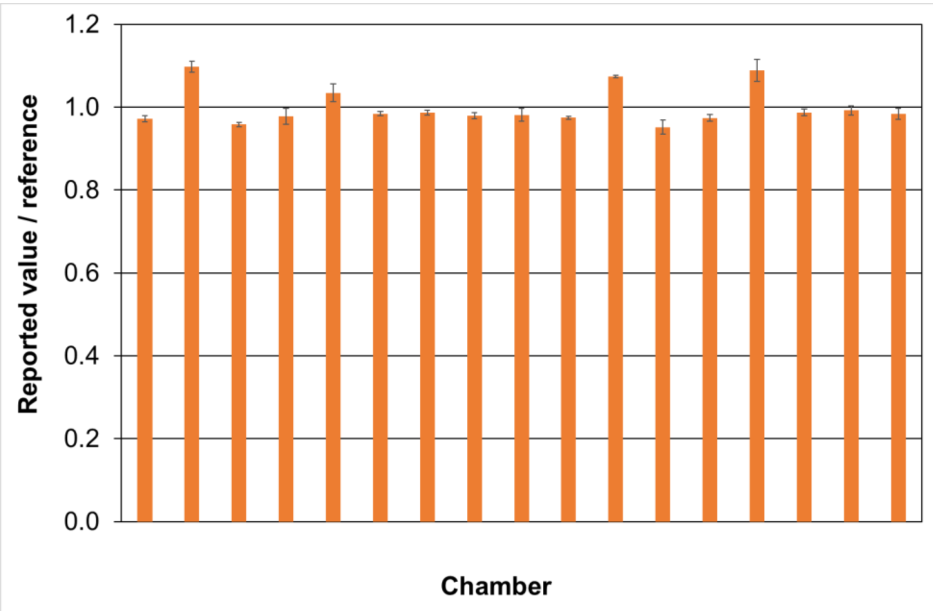

Figure 3. Anominized combined respiration chamber methane recoveries for 17 chambers at 5 SmartCow research facilities (1.0 = 100%).

These results suggest that as observed in the meta-analysis of variation due to research site, the variation in respiration chamber measurements of methane emissions by cattle at SmartCow installations is low compared to the variation due to individual experimental conditions and known effects of diet composition and intake and cattle characteristics such as gender and physiological state.

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Discussion of the results at the January 2022 workshop highlighted key practices to reduce variation and increase the precision and accuracy of the measurements, as noted in the minutes provided below (Appendix 1). Some of these include routine checks for leaks, flow meter calibration, tests for adequate internal mixing of the incoming air and expired gases inside chambers, and the establishment of user groups to facilitate future ring-tests and sharing of precision standards and equipment for performing recovery tests.

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7 Recommendations for optimised methane emission measurement protocols.

Collective recommendations for best practice procedures for the conduct of methane measurements using respiration chambers and gas recovery tests for respiration chambers were published by the SmartCow consortium in the Publisso publication 'Methods in cattle physiology and behaviour research – Recommendations from the SmartCow consortium'.

The chapter 'Respiration chamber facility' describes recommended procedures for operating respiration chambers, including measurements of methane emission:

Danesh Mesgaran S, Derno M, Kuhla B, Beauchemin K, Martin C, Hellwing AL, Lund P, Miller G, Humphries D, Heetkamp M. Respiratory chamber facility. In: Mesgaran SD, Baumont R, Munksgaard L, Humphries D, Kennedy E, Dijkstra J, Dewhurst R, Ferguson H, Terré M, Kuhla B, (editors). Methods in cattle physiology and behaviour – Recommendations from the SmartCow consortium. Cologne: PUBLISSO; 2020-. DOI: 10.5680/mcpb011 (https://books.publisso.de/en/publisso_gold/publishing/books/overview/53/194).

The chapter 'The gas recovery test of respiratory chambers' describes recommended procedures for conducting routine methane and carbon dioxide recovery tests for respiration chambers to validate measurements:

Danesh Mesgaran S, Frydendahl Hellwing AL, Lund P, Derno M, Kuhla B, Heetkamp M, Miller G, Humphries D, Anglard F, Rochette Y, Martin C, Gardiner T, Coleman M. The gas recovery test of respiratory chambers. In: Mesgaran SD, Baumont R, Munksgaard L, Humphries D, Kennedy E, Dijkstra J, Dewhurst R, Ferguson H, Terré M, Kuhla B, (editors). Methods in cattle physiology and behaviour – Recommendations from the SmartCow consortium. Cologne: PUBLISSO; 2020-. DOI: 10.5680/mcpb010

(https://books.publisso.de/en/publisso_gold/publishing/books/overview/53/193)

The results of the joint research activities for WP5 highlight key aspects of the procedures described and specific sources of variation that warrant close attention, but do not suggest that major revisions of the current chapters are required. The minutes of the workshop held in January highlight specific sources of variation that were identified at each SmartCow respiration chamber facility, such as specific leaks, flow meter calibration, use of fans for mixing of air within chambers, the timing of sample analysis when switching analyzer sample flow between chambers, and the importance of routine recovery test for validation of the measurements and identification of measurement bias and drift. When such differences occur the identification of the source of the error and corrective action is essential.

One of the proposals at the SmartCow workshop was the formation of a user group for the sharing of best practices and equipment such as gas flow meters for the precision release of methane during recovery tests (see the book of methods chapter figure 1). The formation of such a user group would facilitate future ring-tests for validating local recovery measurements and periodic tests of chamber facility comparability. It is proposed that with the formation of the SmartCow European Research Group that a respiration chamber user group be formed as part of the Research Group activities.

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8 Appendixes

8.1 Appendix 1: MS5.2 Evaluation of historical data for variation in methane emission.

Smartcow Summary of Results – Methane Meta-analysis

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Introduction

Concerns regarding the greenhouse gas emissions of livestock production have been focused on CH4 emissions from ruminants, which accounts for 30% of global anthropogenic CH4 emissions (Arndt et al., 2022). Substantial research over the last 30 years, on a global scale, has focused on how dietary composition and other management strategies determine CH4 emissions, to predict emissions for specific production systems and develop mitigation strategies (Beauchemin et al., 2020). Recent commitments at COP26 to reduce national CH4 inventories by 30% by 2030 relative to 2020 and other carbon reduction commitments by animal industry stakeholders and governments have led to a recent upsurge of interest in research on CH4 mitigation strategies. The availability of large amounts of individual observations of CH4 emission by cattle has led to several recent meta-analyses to determine dietary and other factors that determine CH4 emissions (e.g., Niu et al., 2018; van Lingen et al., 2019; Benaouda et al., 2019). These meta-analyses have included random effects of experiment to account for variation associated with specific experimental procedures and conditions to improve predictions of CH4 emission, but variation due to research site and the methodology used has not been a focus.

Measurement of CH4 emissions by cattle using respiration chambers has been associated with measurements of energy balance of cattle since the 1800s and respiration chambers continue to be considered the 'gold standard' for measuring CH4 emissions (Hammond et al., 2016). However, it is known that such measurements can be subject to variation due to differences in the methods used and how they are implemented. For example, a ring test of methane recovery measurements for a project in the UK, based on the recovery of methane quantitatively released at strategic locations within the measurement system, found a large variation in methane recovery between research sites that was mainly associated with measurement of air flow rate (Gardiner et al., 2015). Other specific sources of variation in CH4 emission measurements include differences in the number of days of measurement, the methods used to determine feed dry matter intake (DMI), the experimental design used (change-over or continuous designs), or the type of measurement (respiration chamber, SF6 tracer, or GreenFeed; Hammond et al., 2016).

Therefore, our objective was to assemble a data base of CH4 emission measurements from cattle and associated diet and production variables and conduct a meta-analysis to determine the extent to which variation in measurements is attributable to research location and the methods used, after accounting for variation due to feed DMI and composition and other animal characteristics that may affect CH4 emissions. This will provide an evidence base for recommendations of 'best practice' at SmartCow

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research facilities that increase precision and accuracy, and minimize animal numbers required for 'in vivo' experiments.

Methods

Database

A database containing 4329 individual cow measurements of CH_4 emission from trials carried out at 14 research sites associated with 12 different research institutions was assembled using data from SmartCow (https://www.smartcow.eu/) partners as well as the Feed and Nutrition Network of the Global Research Alliance (https://globalresearchalliance.org/research/livestock/networks/feed-nutrition-network/). Sites were located in North America (3) and Europe (11). The data set was comprised of 108 separate experiments with sites contributing data from 1 to 36 experiments (33 to 935 observations). Individual cow measurements included dry matter intake (DMI), the chemical composition of ration components (CP, GE, EE, Ash, NDF and ADF concentration), resulting nutrient intakes, animal characteristics (e.g. breed, body weight), and production outputs including milk yield and composition for lactating cattle (Tables 1 and 2). Individual cow methane (CH_4) emissions were measured using respiration calorimetry chambers (Chamber), GreenFeed (CLock, USA) and the sulphur hexafluoride (SF6) tracer technique in the initial database assembled. Individual cows' records missing either CH_4 emissions or DMI were excluded (25 records). GreenFeed and SF6 measurements represented a small proportion of the overall data set and were excluded from the final modelling data set. Finally, sites contributing data from a single experiment only were excluded to permit the fitting of site and experiment as random effects in the mixed models analyses conducted. The data exclusions and processing are summarised in Figure 1. For categorical variables missing data were coded as 'unknown' to maintain the maximal number of observations in the data set. For numerical variables missing data were classed as such and termed NA (not available) in R.

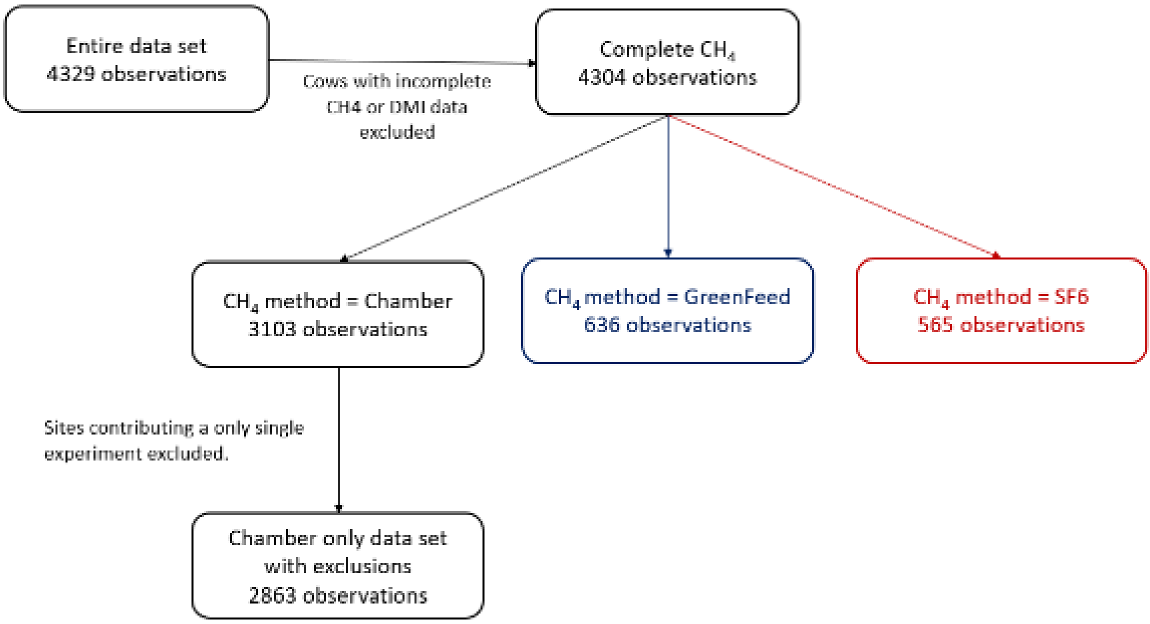

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Figure 1 – Summary data sets considered in the modelling processes with details of data exclusions made.

Model development

Variables with a high proportion of missing observations (>60%) were excluded from further analyses (i.e., average daily gain (meat animals), forage and concentrate DMIs, Lignin and starch concentrations, Rumen pH, NH_3 and VFA compositions, Milk GE, crude protein, lactose and the composition of milk fatty acids, animal age and body condition score). A correlation matrix was produced for numerical variables to identify highly correlated variables (MS Excel, Appendix 1). Due to the confounding of DMI with nutrient intakes, DMI, diet nutrient concentration and corresponding nutrient intakes were highly correlated. Therefore, only DMI and diet compositions (gross energy [GE], ether extract [EE], ash, neutral detergent fibre [NDF] and acid detergent fibre [ADF] concentration) were taken forward for the multivariable model development. Single variables were tested one by one along with DMI in a series of mixed models where CH_4 emission in g/d was the outcome variable. Experiment code nested within site was the random effect in a random intercept model. Variables were selected for further model development if their effect was significant or had a nonsignificant trend ($P < 0.10$). The resulting short list of variables for multivariable modelling included: DMI, cow status, experimental design, days in milk at the start of the trial, the inclusion of additives, percentage of forage in the ration, DMI measurement method, diet CP, EE, ADF, NDF concentration, milk production, percentage milk fat, breed and bodyweight (table 3).

Multivariable model development

With CH_4 emissions or yield as the outcome variable the above short list of variables were tested in linear mixed models with experiment nested within site as the random effect. An automated model build function based on the lowest BIC while controlling for variance inflation factors was used to determine the 'best model' fit (van Lingen et al., 2019) using R version 4.0.3 (R Core Team, 2020). Using this approach breed could not be included in the modelling fitting process due to being correlated with milk production variables (fat and yield) and cow status, so was not considered for covariate selection and model fitting. The sum of the variance associated with each random effect (site and experiment) and the residual variance were used to calculate a percentage variance associated with each.

Results

Descriptive summaries

The complete data set includes lactating beef cattle, growing cattle and dry dairy cattle but experiments with lactating dairy cattle dominate the data set and as a result, the majority of animals are multiparous, Holstein X Friesian, females (table 1). Longer durations of methane collection were associated with the SF6 and GreenFeed measurement methods compared to respiration chambers. The longest duration for chamber measurements was 5 days with the highest frequency reported for 3 days. Manual weighing was the most frequent method used for dry matter intake measurements, with some measurements obtained using electronic weighing of feed mangers in respiration chambers. Back calculations and herbage loss estimates of DMI were associated with trials undertaken at pasture and therefore restricted to non-chamber methane measurements (table 1).

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Table 1. Descriptive frequency data from animals and methodological data from the entire and respiration chamber only data sets

Categories	Entire data set n (%)	Chamber only* n (%)
Animals		
Cow Status		
Lactating dairy	2840 (65.6)	2110 (73.7)
Lactating beef	99 (2.3)	48 (1.7)
Dry dairy	240 (5.5)	127 (4.4)
Dry beef	318 (7.3)	291 (10.2)
Growing	832 (19.2)	287 (10.0)
Breed		
Holstein Friesian	2278 (52.6)	1390 (48.6)
Jersey	83 (1.9)	82 (2.9)
Ayrshire	204 (4.7)	195 (6.8)
Brown Swiss	136 (3.1)	48 (1.7)
Angus	85 (2.0)	85 (3.0)
Hereford x AA	196 (4.5)	193 (6.7)
Other beef/ beef X	578 (13.4)	253 (8.8)
Unknown	769 (17.8)	617 (21.6)
Sex		
Male	470 (10.9)	237 (8.3)
Female	3606 (83.3)	2376 (83.0)
Unknown	253 (5.8)	250 (8.7)
Lactation category		
Multiparous	1184 (27.4)	734 (25.6)
Not-applicable	833 (19.2)	288 (10.1)
Primiparous	458 (10.6)	288 (10.1)
Unknown	1854 (42.8)	1553 (54.2)
Methodologies		
Type of experiment		
Change over	2177 (50.3)	1300 (45.4)
Randomised trial	1113 (25.7)	760 (26.5)
Unknown	1039 (24.0)	803 (28.0)
Duration of collection		
1 day	127 (2.9)	127 (4.4)
2 days	745 (17.2)	657 (22.9)
3 days	1093 (25.2)	847 (29.6)
4 days	615 (14.2)	364 (12.7)
5 days	463 (10.7)	405 (14.1)
6 days	168 (3.9)	0 (0.0)
7 days	135 (3.1)	0 (0.0)
8 days	33 (0.8)	0 (0.0)
28 days or more	200 (4.6)	0 (0.0)
Unknown	750 (17.3)	463 (16.2)

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Intake method		
Weighed manually	2305 (53.2)	1958 (68.4)
Weighed electronically	277 (6.4)	241 (8.4)
Back calculation	84 (1.9)	0 (0.0)
Herbage loss	271 (6.3)	0 (0.0)
Unknown	1392 (32.2)	664 (23.2)
Additives in Ration		
Additives	442 (10.2)	258 (9.0)
No additives	3887 (89.8)	2605 (91.0)
CH4 measurement method		
Chamber	3117 (72.0)	2863 (100.0)
GreenFeed	640 (14.8)	0 (0.0)
SF6	572 (13.2)	0 (0.0)

* Chamber only data set with sites contributing only one experiment excluded (n= 2863)

Variations in individual variables including mean, standard deviation and minimum and maximum values related to diet composition, nutrient intake, milk production and CH_4 emission are presented in table 2. As the aim of this meta-analysis was to consider sources of variation in the measurement of methane production of cattle, all data were retained unless there was a known error.

Table 2. Descriptive statistics of diet composition, nutrient intake, and milk production for the entire data set (4329 observations).

	Mean	SD	Min	Max	CV	n
Diet Composition[1]						
CP concentration (g/kg DM)	164	32.0	44	435	0.20	4132
GE concentration (MJ/kg DM)	19	0.8	14	21	0.04	3246
EE concentration (g/kg DM)	38	13.0	1.2	129	0.34	3289
ASH concentration (g/kg DM)	80	29.7	9	193	0.37	4152
NDF concentration (g/kg DM)	392	115.1	175	779	0.29	2920
ADF concentration (g/kg DM)	228	78.7	75	522	0.35	2704
Nutrient intakes						
DM intake (kg/d)	15.6	6.7	2.1	38	0.43	4309
Forage proportion (% diet DM)	63.0	25.0	4.7	100	0.43	3690
CP intake (g/d)	2568	1223.1	271	6212	0.48	4126
N intake (g/d)	411	195.7	43	994	0.48	4126
GE intake (MJ/d)	265	103.5	40	556	0.39	3241
EE intake (g/d)	630	405.1	19	2192	0.64	3251
ASH intake (g/d)	1190	509.1	91	3102	0.43	4146
NDF intake (g/d)	5335	2381.9	421	13892	0.45	2878
ADF intake (g/d)	3144	1420.0	189	7483	0.45	2698
Output data						
Milk production (kg)	28.5	10.38	0.57	65.73	0.36	2857
Milk fat (%)	4.2	0.70	1.47	9.01	0.17	2796
Milk protein %	3.2	0.40	2.06	5.39	0.12	2801
CH4 (g/d)	302.4	125.46	27.5	728.6	0.41	4309

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Body weight (kg)	560.2	130.00	129	1010	0.23	4272

[1]Diet composition reported individually but likely measured on fewer sample numbers.

Site-level variation is depicted in a scatter plot of the full data set (figure 2a). However, when these data are plotted for each CH4 measurement method, some of the variation due to site appears to be associated with experiments using the GreenFeed and SF6 methods, which were excluded from mixed model analysis as noted above (figure 2b).

Table 3. Summary of outcomes from modelling methane emission (g/d) with a single predictive variable tested with dry matter intake (DMI, kg/d) only. Variables with BIC less than DMI alone (29460) or with P-value <0.1 were used in further modelling indicated by a tick (✓)

	Variable	Categories	Coefficient	SE	P value	Used in modelling
DMI			***15.9***	***0.3***	***<0.001***	
DMI +	Cow Status	lactating beef	-30.3	19.1	0.116	
	(ref: lactating dairy)	dry dairy	-33.9	**6.7**	**<0.001**	✓
		dry beef	-65.2	**17.6**	**<0.001**	
		growing	-50.8	**15.9**	**0.002**	
DMI +	Additives (ref: additive)	no additive	16.8	**5.2**	**0.002**	✓
DMI +	experimental design	randomised	12.6	11.6	0.208	×
	(ref: cross over)	unknown	-19.8	15.3	0.202	
DMI +	duration CH4 measurement	2d	-2.7	13.6	0.841	
	(ref: 1d)	3d	-2.4	15.1	0.876	×
		4d	-2.4	16.6	0.887	
		5d	-5.6	16.7	0.738	
		unknown	-55.9	**28.8**	**0.055**	
DMI +	parity (ref: multiparous)	not applicable	-44.4	**15.9**	**0.006**	
		primiparous	-9.0	6.8	0.184	✓
		unknown	-24.0	**9.8**	**0.016**	
DMI +	DIM start		0.07	**0.01**	**<0.001**	✓
DMI +	forage %		0.80	**0.08**	**<0.001**	✓
DMI +	DMI measurment	weighed electronically	41.5	**19.0**	**0.034**	✓
	(ref: weighed manually)	unknown	-15.2	14.7	0.303	
DMI +	GE concentration		-2.4	2.2	0.286	×
DMI +	CP concentration		-0.2	**0.04**	**<0.001**	✓
DMI +	EE concentration		-1.1	**0.10**	**<0.001**	✓
DMI +	Ash concentration		-0.04	0.07	0.517	×
DMI +	ADF concentration		0.30	**0.03**	**<0.001**	✓
DMI +	NDF concentration		0.19	**0.02**	**<0.001**	✓
DMI +	Milk production		-1.4	**0.19**	**<0.001**	✓
DMI +	Milk fat %		20.6	**1.5**	**<0.001**	✓

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DMI +	Milk protein %		14.8	**3.0**	**<0.001**	✓
DMI +	Breed (ref: HF)	Jersey	15.2	**6.3**	**0.016**	
		Ayrshire	29.5	14.6	0.469	
		Angus	-9.4	7.6	0.217	
		Herefors x Angus	-41.4	**21.3**	**0.059**	✓
		Brown swiss	20.2	**9.1**	**0.027**	
		Other beef	-12.3	10.3	0.231	
		unknown	-9.4	13.0	0.474	
DMI +	Sex (ref: male)	female	28.1	19.3	0.148	✗
		unknown	-3.8	21.7	0.860	
DMI +	Body weight		0.10	**0.01**	**<0.001**	✓
DMI +	Feed restricted (ref: restricted)	Ad libitum	13.5	24.6	0.593	✗

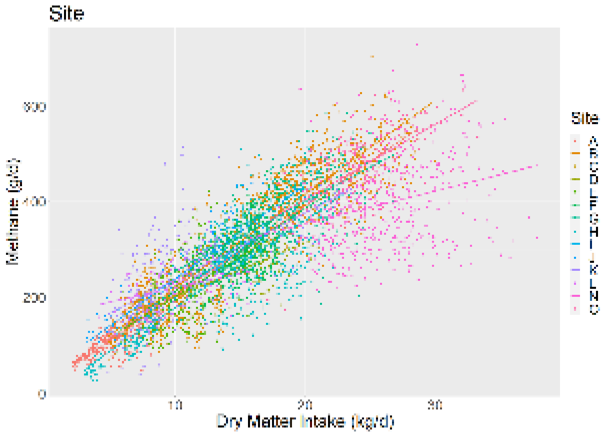

Figure 2a. Scatter plots of methane (g/d) and DMI (kg/d) with regression lines illustrate site-level variation.

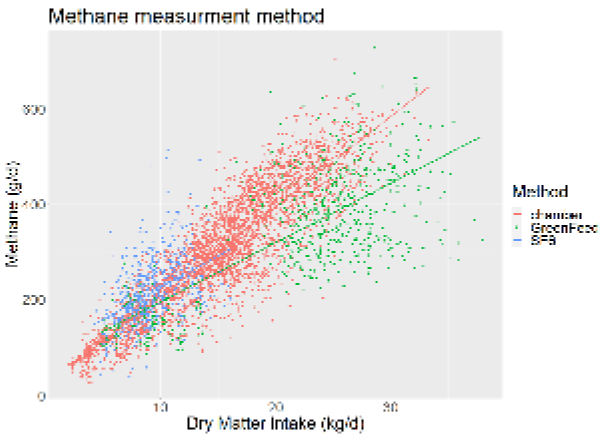

Figure 2b. Scatter plots for each methane measurement technique (chamber, GreenFeed and SF6) for methane (g/d) and DMI (kg/d) illustrate site-level variation.

The variation associated with cow status and measurement methodology is summarised in figures 3a-d for the chamber only data set.

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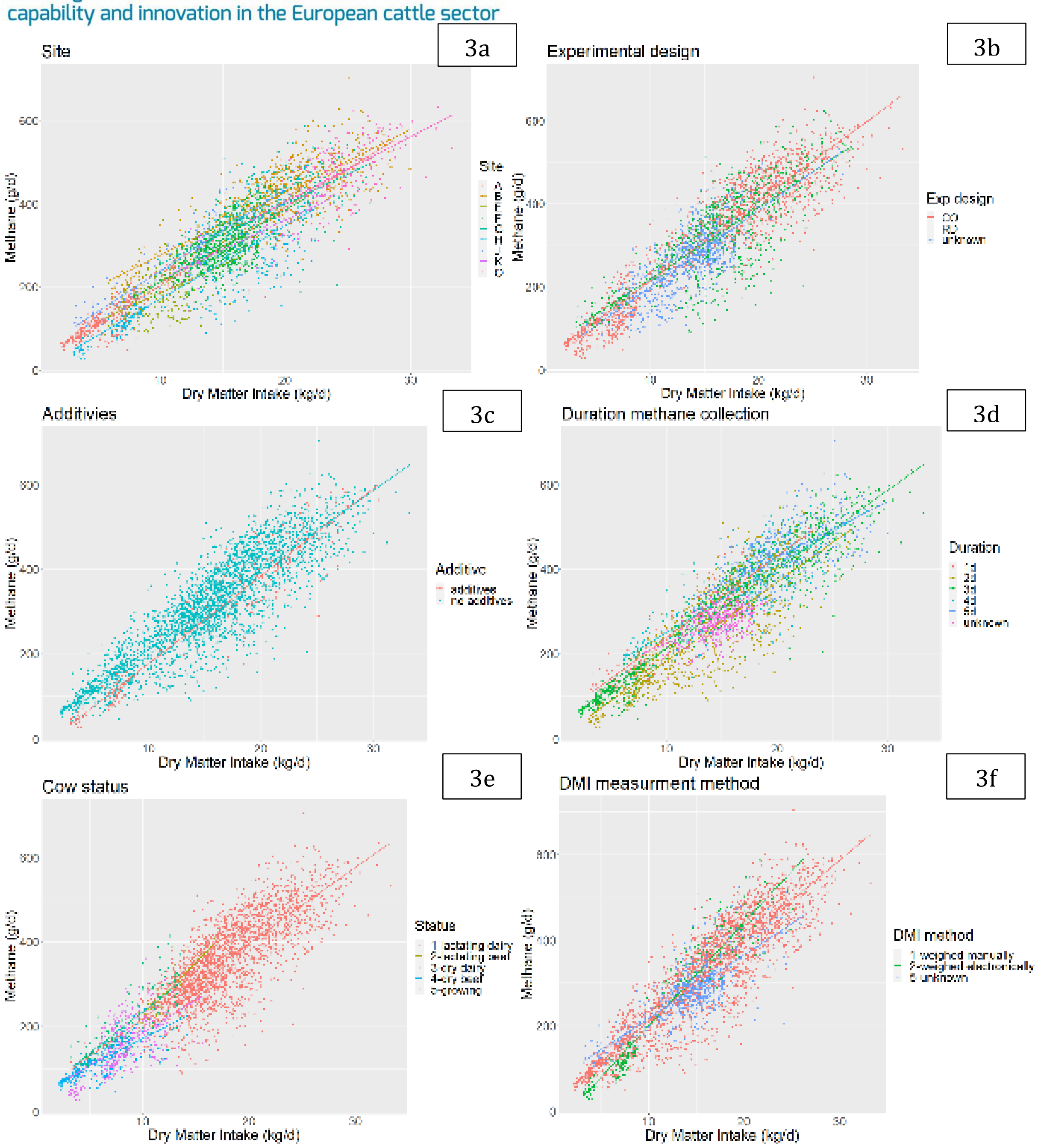

Figures 3a-d. Scatter plots of methane (g/d) and DMI (kg/d) with regression lines for site (a), experimental design (change-over [CO], continuous design [CD]) (b), additive inclusion (c), duration of methane collection (d) cow status (e), and DMI measurement method (f) for a data base of 3103 individual cow observations from respiration chambers.

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Multivariable model

The best linear mixed effects models for CH4 emissions (g/d) based on goodness of fit criteria with the inclusion or exclusion of variables related specifically to milk production are presented in tables 4 and 5.

Table 4. Best linear mixed effects model for CH_4 emission (g/d) including milk production related variables – model 1

		Coefficient	SE	df	T-value	P value
Intercept		11.25	19.3	57.6	0.582	0.562
DMI intake		15.9	0.4	1212.1	37.053	<0.001
Cow status	Ref: Lactating Dairy					
	Lactating beef	-37.7	39.4	35.2	-0.955	0.345
Percent forage		0.3	0.1	1158.3	2.842	0.005
EE concentration		-1.1	0.1	1371.0	-10.044	<0.001
Percent milk fat		19.7	1.7	1356.3	11.871	<0.001
Inclusion of additives	Ref: additives					
	No additives	15.9	8.4	1076.3	1.900	0.058

No. observations = 1374, No. of experiments = 36, No. of Sites = 7, random effect = site|experiment, Model variance: experiment = 45.7%, site = 7.3%, residual 47.0%.
DMI = Dry matter intake, kg/d; EE = Ether extract, g/kg DM.

Table 5. Best linear mixed effects model for CH_4 emission (g/d) excluding milk production related variables – model 2

		Coefficient	SE	df	T-value	P value
Intercept		41.8	15.5	66.7	2.701	0.009
DMI intake		13.2	0.4	1874.1	32.992	<0.001
Cow status	Ref: Lactating Dairy					
	Lactating beef	-45.3	19.0	42.0	-2.375	0.022
	Dry dairy	-53.2	8.6	1881.7	-6.207	<0.001
	Dry beef	-79.8	18.0	33.1	-4.435	<0.001
	Growing	-35.4	18.5	33.8	-1.919	0.064
Percent forage		0.8	0.1	1695.4	10.150	<0.001
EE concentration		-1.1	0.10	1939.5	-10.509	<0.001
Inclusion of additives	Ref: additives					
	No additives	18.4	7.4	1255.1	2.491	0.013
Body weight (kg)		0.1	0.01	1930.8	9.049	<0.001

No. observations = 1945, No. of experiments = 50, No. of Sites = 8, random effect = site|experiment, Model variance: experiment = 37.9%, site = 11.7%, residual 50.4%.
DMI = Dry matter intake, kg/d; EE = Ether extract, g/kg DM.

Table 6. Best linear mixed effects model for CH_4 yield (g/kg DMI) including milk production related variables – model 3

	Coefficient	SE	df	t value	P value
Intercept	11.65	0.97	620.1	12.028	0.001

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EE content	-0.042	0.008	912.9	-5.534	0.001
ADF content	0.027	0.004	833.2	7.568	0.001
Milk fat percentage	1.212	0.110	923.4	11.013	0.001

No. observations = 938, No. of experiments = 35, No. of Sites = 8, random effect = site|experiment, Model variance: experiment = 43%, site = 0%, residual 57%.
EE = Ether extract, ADF = Acid detergent fibre.

Table 7. Best linear mixed effects model for CH_4 yield (g/kg DMI) excluding milk production related variables – model 4.

	Coefficient	SE	df	t value	P value
(Intercept)	16.45	0.88	38.9	18.64	<0.001
EE concentration	-0.052	0.010	1477	-5.25	<0.001
ADF concentration	0.031	0.003	1216	11.99	<0.001

No. observations = 1511, No. of experiments = 48, No. of Sites = 9, random effect = site|experiment, Model variance: experiment = 47%, site = 4%, residual 49%.
EE = Ether extract, ADF = Acid detergent fibre.

Discussion

The experiment site was not a major source of variation in methane emission measurement in the final models, accounting for 7% (model accounting for milk production variables) or 12% (model excluding milk production variables) and for methane yield, 0% (model accounting for milk production variables) or 4% (model excluding milk production variables) of variation in observations from respiration chamber measurements across seven or eight research sites respectively in Europe and North America. In comparison, variation associated with individual experiment accounted for more than 46%, 38%, 43% and 47%, respectively. These individual experiments varied by methodology, animal type and status, and dietary compositions (tables 1 and 2).

In the final models accounting for variation in CH_4 emission due to DMI and diet composition, variables associated with methodologies utilized for the respiration chamber studies were not included. These were potential variation due to the method of DMI measurement, experimental design, and days of measurement. For the model including milk production parameters (model 1) the production status of the animal (i.e., lactating dairy vs lactating beef) improved the model fit, with beef cattle having a lower CH_4 emission; however, the effect was not significant (P = 0.333). However, for model 2 which excluded milk production parameters to permit inclusion of a wider range of production status (i.e. lactating dairy, lactating beef dry dairy, dry beef and growing animals) CH_4 emissions were significantly lower for lactating beef (P<0.05) dry dairy (P<0.001), dry beef (P<0.001) and growing animals (P<0.05) compared with lactating dairy. Except for production status, the variables in models 1 and 2 and their effect sizes were consistent. The exclusion of milk fat percent results in the inclusion of body weight in model 2. Increased bodyweight is associated with significantly increased CH_4 emissions probably related to increased DMI in the larger animals. In the final models accounting for variation in CH_4 yield variables related to the methodology were also not retained. As with methane emissions, methane yield was

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associated with a reduction in EE concentration and an increase in ADF concentration (models 3 & 4). Similarly methane yield was associated with increased milk fat percentage.

Increased DMI is widely acknowledged as an important driver of CH_4 emission (e.g., Niu et al., 2018) as observed in this meta-analysis. Increasing the percentage of forage in the diet was associated with an increase in methane emissions which agrees with the findings of (Aguerre et al., 2011) and results from other meta-analyses, as higher forage diets favour acetate production and the release of H_2 which is utilized for CH_4 production (Niu et al., 2018). Decreasing diet forage concentration is often associated with a decrease in milk fat concentration, and milk fat concentration had a significant positive association with CH_4 emission in the present analysis. A positive association between milk fat concentration and CH_4 emission of lactating dairy cows was reported previously (Niu et al., 2018). Increased ether extract concentration in the diet was associated with a significant reduction in CH_4 which is in agreement with other meta-analyses (Doreau et al., 2011; Eugène et al., 2008). Dietary lipids provide a source of dietary energy which is not fermented in the rumen and therefore reduces H_2 and CH_4 production (Martin et al., 2021).

There is a non-significant trend for an increase in CH_4 production with trials not using dietary additives in model 1 and a significant increase in CH_4 emissions with not using dietary additives in model 2, suggesting that trials with non-lactating animals that would be excluded from model 1 are exerting an effect on this finding. While there are several additives specifically aimed at methane reduction which have demonstrated effects (Hegarty et al., 2021; Honan et al., 2021), in the data base evaluated the type of additive was not considered and the number of observations from trials including dietary additives was relatively small (10%; Table 1).

Similar results were found for methane yield (CH_4/kg DMI), with 9% of variation due to site and 42% due to experiment for the data set excluding lactating cattle. For methane yield, diet concentrations of ether extract, forage, and ADF all had significant effects (Table 6). For the data set including lactating dairy cattle, no variation was attributable to research site, and ether extract, ADF concentration, and milk fat concentration had significant effects.

Conclusion

As observed in previous meta-analyses (e.g., Niu et al., 2018), DMI was a major determinant of CH_4 emission, with dietary forage and ether extract concentration also having positive and negative effects, respectively. There was also a positive association between milk fat concentration and CH_4 emission, which may reflect dietary forage effects. In addition, there was an overall negative effect of feed additives for the studies included in the present data set. After accounting for dietary effects and a significant effect of experiment, the variation in CH4 emission using respiration chambers that was attributable to the research site was relatively small (7% or 12%). In addition, accounting for the number of days of measurement and experimental design did not improve predictions of CH4 emission. The relatively low variation due to research site suggest that the comparability of measurements obtained using respiration chambers at the different research sites represented is reasonably good, but the present analysis did not include measurements obtained using other methods such as the GreenFeed system or SF6 tracer method.

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8.2 Appendix 2. MS5.3 Results of methane recovery tests for SmartCow respiration chambers

SMARTCOW WP5: RING-TEST OF RESPIRATION CHAMBER FACILITIES

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ABSTRACT

We report results from a ring-test carried out under the SmartCow project across five European respiration chamber facilities. The National Physical Laboratory (NPL) performed the role of ring-test provider visiting each facility and delivering controlled releases of methane approximating cattle emissions if considered on a steady state basis. Facility staff had no influence on the methane releases carried out by NPL other than facilitating logistical arrangements, similarly NPL staff had no influence on the operation of the facilities. Testing was blind in that each facility had no knowledge of the quantities of methane that would be released. For each release each facility was requested to measure and calculate the methane emission via the facility's normal in-house measurement and calculation procedures. Releases were carried out directly into the analysers, output ductings and the chambers themselves enabling facilities to be characterised in terms of analyser efficiency, ducting efficiency and isolated chamber efficiency. From the test data it was also possible to determine a combined efficiency for each chamber (i.e. when the chamber, ducting and analyser were used together) and from these an overall facility efficiency for each site. Consequently, the facility efficiency values could be used to determine the comparability across the five facilities.

With respect to analyser efficiencies, it was found that all deviated from unity by no more than the 3rd decimal place, with one exception. This exception was investigated by the associated facility and the cause traced back to an issue with the in-house gas cylinder used for span calibration. The ducting efficiencies evidenced increased deviations from unity compared to the analyser efficiencies. This broadly correlating with expectations as there is generally less quality assurance / quality control (QA/QC) associated with the former, e.g. daily/weekly in-situ calibrations are possible for analysers whereas flow sensors have to be despatched to 3rd parties for calibration and this is only possible less frequently. Isolated chamber efficiencies demonstrated increased deviations compared to ducting efficiencies, possibly evidencing some element of inhomogeneous concentration mixing and/or flow profiles. From the facility efficiencies a comparability across the five facilities was determined as 6.2% (k = 2, 95% confidence), markedly better than a similar UK ring-test from ~10 years prior where the analogous value was 25.7% (k = 2, 95% confidence). Although, it was noted that the UK ring-test was not of the same facilities and that some of the facilities were designed sheep, whereas all the facilities reported herein were designed for cattle. Lastly, it was shown that if each facility applied the calibration function determined in the ring-test the comparability could potentially be improved to 5.4% (k = 2, 95% confidence).

SmartCow: an integrated infrastructure for increased research capability and innovation in the European cattle sector

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SmartCow

an integrated infrastructure for increased research capability and innovation in the European cattle sector

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Approved on behalf of NPL by
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CONTENTS

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INTRODUCTION

The ring-test sits under WP5 of the SmartCow project. WP5 is concerned with identifying and addressing variation in key *in vivo* measurements of dietary nutrient use efficiency and associated methane emissions of cattle to improve measurements. The objectives of WP5 are to (a) improve the accuracy and precision of measurements, and (b) unify the methods used across SmartCow infrastructures.

As one of the key outputs of WP5, herein is reported the results of a ring-test across five respiration chamber facilities. We report details of the methodology of the ring-test, provide test data from the three key components common to all facility designs (analyser, ducting, chamber), discuss issues of accuracy and precision, and from the ring-test data determine the comparability across the five facilities.

EXPERIMENTAL

EXPERIMENTAL APPROACH

The respiration chamber facilities at Reading, Wageningen, Saint-Genès-Champanelle, Dummerstorf and Tjele were each considered to comprise of 3 key components (analyser, ducting, chamber), where several chambers and associated ductings are connected to a single analyser (Fig. 1). Whilst all facilities involved in the ring-test measured concentration at some location in the extract duct, this was not true for flow with one of the facilities measuring flow within the input duct to each chamber. This facility operated with pre-chamber pumping ('push' type facility) rather than post-pumping, hence the air pressures in the chambers were super- rather than sub-atmospheric.

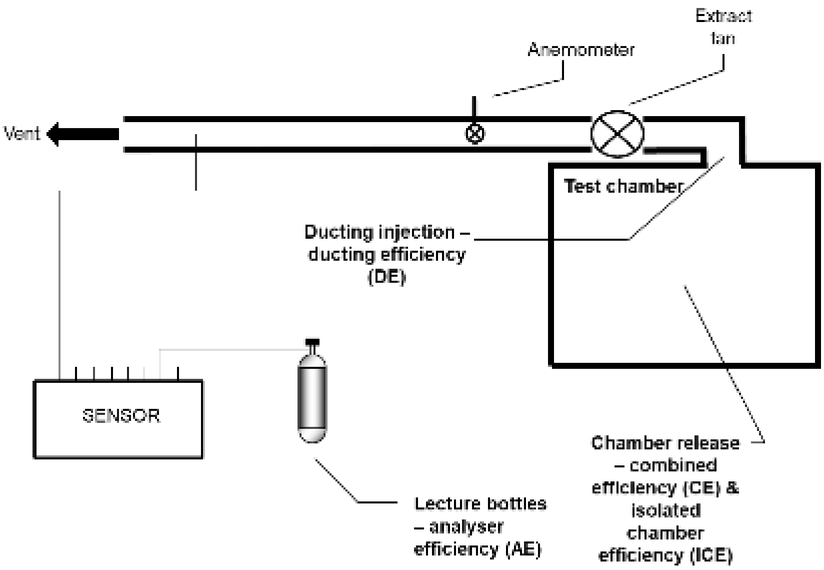

Figure 1 Schematic of a typical respiration chamber facility detailing release points for test gases during the SmartCow ring-test.

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All facilities operated on the principle of incoming air from an input duct (not shown in Fig. 1) passing through the chamber and mixing with emission from the ruminant under test prior to venting to atmosphere via an output duct. An analyser via a pre-determined switching regime sequentially samples and provides measurements of methane concentration from the sequence of output ducts and ambient air, the latter showing the background methane present before mixing with the ruminant emission. The concentration measurements are combined with temperature, humidity, pressure and flow measurements and via in-house calculation an emission is determined. As the flow measurement is typically volume based many facilities report measurements in units of $L.day^{-1}$, however, others prefer to convert into units of mass and so it is also common to see emissions reported in units of $g.day^{-1}$.

Testing was blind in that facilities were not informed of the amount of methane released in each experiment carried out during the ring-test. Facility staff were present throughout all testing and were asked to operate their facilities following their normal in-house procedures. Similarly, after each ring-test visit, the facility was asked to determine the amount of methane they believed to have been released in each test via the facility's normal in-house measurement and calculation procedures, and if reporting in units if $L.day^{-1}$ to state the temperature and pressure reference values used for correction.

TYPES OF TESTS

The ring-test at each facility involved carrying out three different methane release experiments: injection at the analyser, upstream release in the extract ducting, release in the chamber. This enabled performance to be expressed in terms of: analyser efficiency, ducting efficiency, isolated chamber efficiency, combined efficiency, and lastly an overall facility efficiency.

Analyser testing

2.5L reference gas bottles were acquired from BOC Gases of nominally 10, 25, 50, 100, 150, 200, 250, 300, 500, 750 and 1000 $\mu mol.mol^{-1}$ methane in synthetic air (N_2+O_2 only). The gas bottle concentrations were certified by NPL to an uncertainty of 0.75% (k = 2, 95% confidence) in accordance with procedures for which NPL holds ISO/IEC 17025 accreditation [i]. For each analyser a sub-set of four of the gas bottles was selected to span the range of concentrations typically observed in the course of routine ruminant emission measurements in addition to zero gas (BOC Gases, ≥99.998% purity nitrogen). Test gas was delivered based on an overflow arrangement where ¼" stainless steel Swagelok fittings were used to split test gas via a T-piece between the analyser and a rotameter venting to atmosphere (Fig. 2). Overflow via the rotameter ensured the measurement cell in the analyser was not over-pressurised and maintained at normal operating pressure. Each gas bottle was connected to the overflow assembly via a stainless steel two-stage regulator (BOC Gases). Prior to testing the overflow assembly was pressurised and tested for leaks with Snoop solution.

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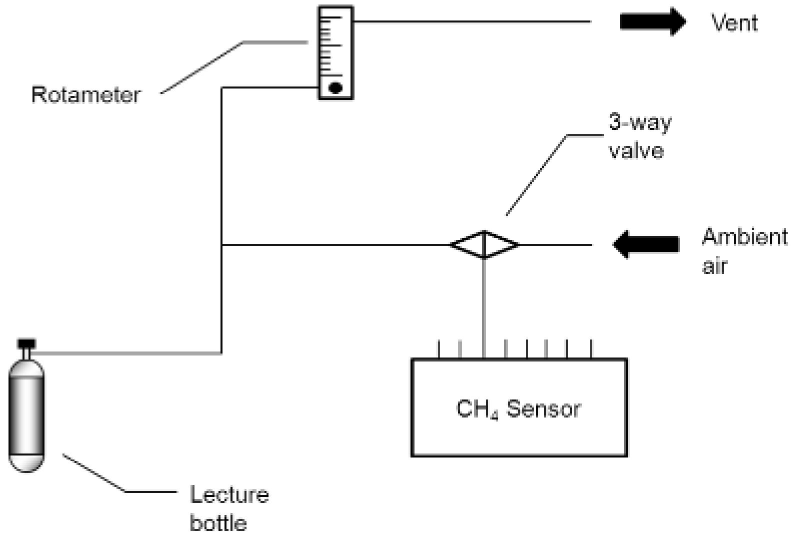

Figure 2 Schematic of overflow assembly for analyser testing.

Sequentially introducing each of the five test gases enabled a linear functioned to be determined relating the reported concentration to the certified reference concentration. The analyser efficiency was calculated from this as described below in 2.3. The extent of linearity was determined as the R^2 value returned from the Excel analysis of variance (ANOVA) regression function, whilst the T90 response time was determined via an instantaneous switch using a 3-way valve (not shown in Fig. 2) between zero gas and the highest concentration test gas selected for the given facility. T90 response time was defined as the time taken to reach 90% of the final stable reading when there is a change between two different concentration levels.

Extract duct releases

Test gas was generated by dynamically mixing methane (BOC Gases, ≥99.95% purity) and nitrogen (BOC Gases, ≥99.998% purity) using a bespoke blender based on Aera FC-7000 series mass flow controllers. The mass flow controllers were calibrated using a BIOS flowmeter with a certified uncertainty (*k* = 2, 95% confidence) of 0.18%, the certification being carried out by an ISO/IEC 17025 accredited provider. For each release into extract ducting (or indeed in a chamber) the methane was delivered at a fixed flow and in many of the tests (but not all) the rate was ~0.5 $L.min^{-1}$, this being selected as it was equivalent to the emission rate of a dairy cow if averaged over 24 h. The nitrogen flow rate in nearly all tests was ~4.5 $L.min^{-1}$. The nitrogen was only used for health and safety reasons and had no impact on the amount of methane delivered, so for most facilities was irrelevant. The exception was for the facility where the flow sensors were positioned in the input ducts, as the additional flow added by the nitrogen was not captured. Hence, the facility was provided with the amount of nitrogen released for each test, so they were able to complete their in-house calculation and report the methane flux. The outputs from the mass flow controllers were combined using ¼" stainless steel Swagelok fittings. ¼" perfluoroalkoxy (PFA) tubing was then used to transport the test gas from the bespoke blender to a 'ducting diffuser' comprising of a series of ¼" Swagelok T-pieces

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providing 6 outputs dispersing the test gas in multiple directions. This was not used at two of the facilities due to the limited diameter of the extract ducting, hence, here test gas was delivered directly from the ¼" PFA tubing.

Ducting efficiency testing wasn't possible at one of the five facilities due to facility design. Across the remaining four facilities it was possible to carry out releases in 10 out of 14 extract ducts within the time constraints of the ring-test.

Chamber releases

The same apparatus and approach was used for releasing methane in the chambers as for the extract ducting, with the exception of replacing the ducting diffuser with a 'chamber diffuser' comprising of a greater number of ¼" stainless steel Swagelok T-pieces resulting in 18 outputs delivering test gas in multiple directions over a volume ~4300 cm^3. It was possible to use the chamber diffuser at all facilities and the diffuser was positioned after consultation with facility staff at a location approximating the common position for the ruminant's head.

Across the five facilities it was possible to carry out releases in 16 out of 22 chambers within the time constraints of the ring-test. Hence, with one chamber having been tested twice 17 combined efficiencies were determined. Also, with ducting efficiencies having been determined for 10 of the chambers it was possible to determine 10 isolated chamber efficiencies. Lastly, as it was not possible to carry out a release in every chamber at every facility, some facility efficiencies were determined without combined efficiencies for some of facility's chambers, i.e. it was assumed that the chambers that were tested were representative of all the chambers at these facilities.

CALCULATING EFFICIENCIES OF FACILITY & COMPONENTS.

Each facility was characterised from the acquired experimental data in terms of analyser efficiency, ducting efficiency, isolated chamber efficiency, combined efficiency (Fig. 3) and facility efficiency. These were defined as follows:

- **Analyser efficiency (AE):** normalising the reported concentration to the reported concentration post correction by the respective linear function. Carried out at the highest concentration test level.
- **Ducting efficiency (DE):** normalising the reported methane flux after correction for analyser efficiency to the reference flux.
- **Isolated chamber efficiency (ICE):** normalising the reported flux after correction for both analyser efficiency and ducting efficiency to the reference flux.
- **Combined efficiency (CE):** normalising the reported flux (without any corrections) to the reference flux.
- **Facility efficiency:** weighted mean of the combined efficiencies of the given facility weighted by the inverse of the associated uncertainties (i.e. so the greatest weighting was applied to the combined efficiency with the lowest uncertainty).

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$$Combined\ efficiency\ (CE) = \frac{facility\ reported\ flux}{reference\ flux}$$

$$Analyser\ efficiency\ (AE) = \frac{reported\ conc.}{corrected\ reported\ conc.}$$

$$Ducting\ efficiency\ (DE) = \frac{facility\ reported\ flux/_{AE}}{reference\ flux}$$

$$Isolated\ chamber\ efficiency\ (ICE) = \frac{facility\ reported\ flux/_{AE.DE}}{reference\ flux}$$

Figure 3 Efficiency equations used to characterise each respiration chamber facility.

NOTE: An internal leak was found within an analyser at one of the facilities. The leak rate was determined before starting the ring-test and all concentration data were corrected in-line with this. Consequently, all the data presented in this report are post this leak being taken in account. Hence, all efficiencies herein are the efficiencies expected for the facility with the analyser operating within normal parameters (i.e. in the absence of a leak).

RESULTS & DISCUSSION

ANALYSER EFFICIENCIES, T90 RESPONSE TIMES AND LINEARITIES

With respect to analyser efficiencies, it is seen (Fig. 4 & Table 1) that four of the five analysers have an efficiency very close to unity with the 3rd analyser showing a negative deviation (n.b. the order of the bars and rows in Fig. 4 and Table 1 correlate). For each facility the analyser efficiency is determined at the highest concentration test level, which approximately correlates with the maximum of the range of concentrations typically observed by the facility in the course of measuring ruminant emissions. Clearly, the emissions of cattle are not steady state and account is made of this by testing the linearity of the analysers across the respective typically observed concentration ranges. As none of the analysers deviate from unity by more than the fourth decimal place (Table 1) this provides confidence that the analyser efficiencies are maintained across the concentration ranges the analysers are typically required to measure.

There are a number of rationales that can lead to a bias in analyser efficiency, these include:

- Certification: Span calibration cylinders should not be used beyond the concentration certification expiry date. Also, it is strongly recommended that a supplier is used that holds ISO/IEC 17025 accreditation for such certifications. This ensures that there has been independent verification that the supplier’s technical procedure for certification is fit and proper.

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- Cylinder mixing: Methane in synthetic air ($O_2 + N_2$) or nitrogen is a relatively stable mixture so stratification within the cylinder would not normally be expected.

- Linearity: If the concentration of the span cylinder deviates significantly from the concentrations typically seen in experimental work, then any lack of linearity is potentially amplified as a function of the deviation.

- Low pressure: When a cylinder is close to empty components / contaminants can start to desorb from the internal walls and this can impact the composition. Methane is not expected to interact with the internal walls, but this is possible for CO_2. When the cylinder is initially filled some CO_2 can disappear to the internal walls, so the post-filling certification applies to all the free CO_2 in the gas phase. Once the cylinder reaches very low pressures this CO_2 can desorb increasing the CO_2 concentration and diluting the concentration of the other components (e.g. methane) causing deviation from the certificated values. Some gas providers will give information on the minimum pressure validity of the certification.

As noted above, of the five analysers it is only the 3rd that shows a significant deviation from unity. With an R^2 value of 0.9999 (Table 1) non-linearity is eliminated as a possible cause. Further investigation carried out by the facility identified the most likely cause as cylinder mixing. Whilst, as mentioned above, stratification of methane in synthetic air is not normally expected, in this instance there were contributory factors in terms of the cylinder composition containing additional components and the storage arrangements.

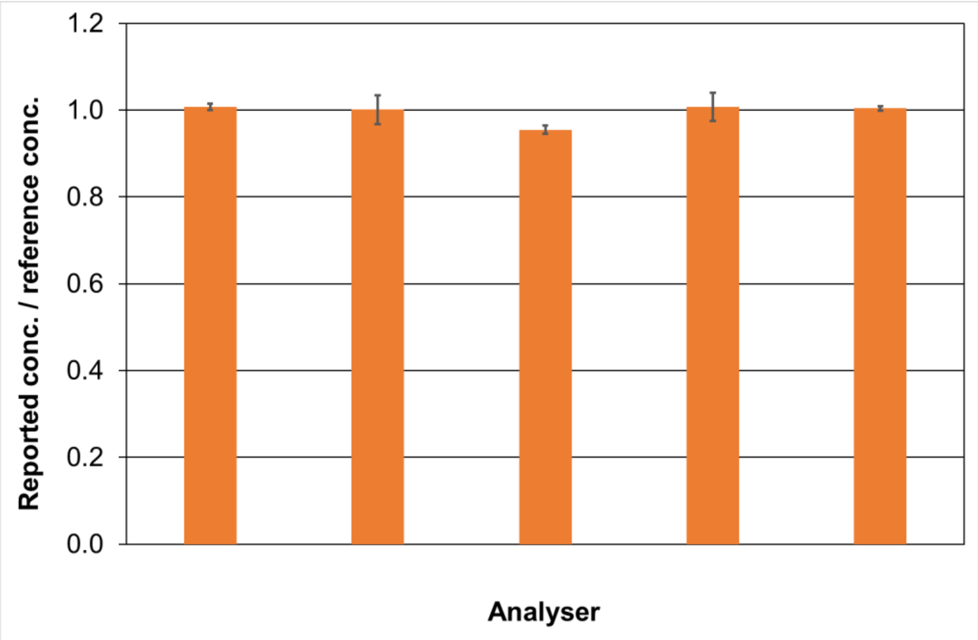

Figure 4 Analyser efficiencies.

At facilities it is common for an analyser to sequentially measure the concentration of more than one respiration chamber: and that is indeed the case for the five facilities discussed here. Consequently, it is critical to understand the time it takes for an analyser to respond when switching from one chamber to the next - or indeed from / to ambient air – in order to ensure the applicable concentration values are assigned to the correct chamber and ambient air

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measurements. In-line with instrument type approval test conventions, it can be assumed that after a time interval of 3 x T90 has passed that the sample the analyser is currently measuring is influenced by ≤0.1 % from the last. Therefore, it is recommended that when an analyser is switched to measuring the next chamber (or ambient air) data are only used after a period of 3 x T90 has elapsed (Fig. 5). Whilst, to protect anonymity, the analyser switching regimes of the facilities are not reported, what can be reported is that two out of the five facilities employ a switching regime where data are used within 3 x T90. However, in both cases data are not used within 2 x T90, so the level of influence of the previous measurement on the current is ≤1.0%.

Table 1 Analyser efficiencies, expanded uncertainties, linearities and T90 response times

Analyser efficiency	Analyser efficiency uncertainty (*k*=2, 95% confidence)	Analyser linearity / R^2	Analyser T90 response time / s
1.007	± 0.0067	1.0000	64.7
1.001	± 0.0334	0.9999	29.6
0.955	± 0.0092	0.9999	29.6
1.007	± 0.0327	0.9997	39.9
1.004	± 0.0059	1.0000	21.5

It might be considered that if the experiment being conducted is aimed at measuring the effect of some input quantity on the ruminants collectively (rather than differentiating between individual ruminants), and that each ruminant spends the exact same time in each chamber comprising a facility, that such cross-measurement biases would be equal and opposing, i.e. there would be no overall bias. However, generally this hypothesis will not hold true. For example, for a four-chamber facility the analyser switching regime will often consist of measuring, Chamber 1 / Chamber 2 / Chamber 3 / Chamber 4 / ambient air, and then repeat. When the analyser switches from ambient air to Chamber 1, the Chamber 1 measurement will be negatively biased (as there will be significantly less methane in ambient air than Chamber 1 decreasing the concentration measurement). However, Chambers 2, 3 and 4 will not be affected the same way as prior to each switch the analyser will not have been measuring ambient air. Consequently, the bias on Chamber 1 will not be the same as for the other Chambers. Hence, it's likely that an experiment will be subject to bias if data is used prior to 3 x T90.

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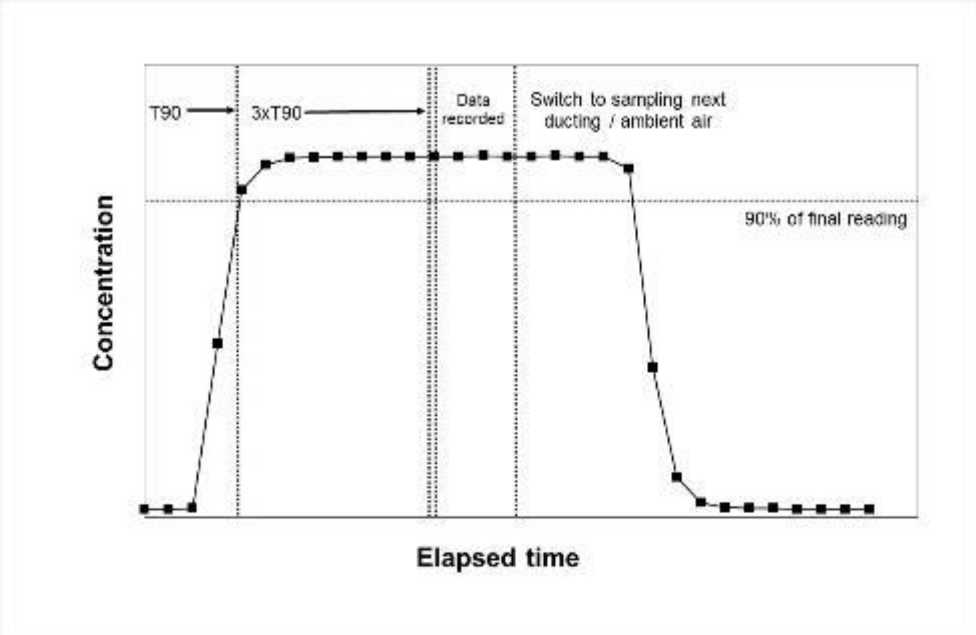

Figure 5 Representation of idealised analyser switching regime.

DUCTING EFFICIENCY

Greater deviation from unity is seen in the ducting efficiencies (Fig. 6 and Table 2) than for the analyser efficiencies. Frequently, the flow measurement is the dominant cause of bias in the ducting as generally the flow sensor is not subject to the same level of quality assurance / quality control (QA/QC) as, for example, the concentration sensor (analyser). Typically, analysers are easy to access, calibration artefacts (i.e. CH_4 gas cylinders) are readily available, zero and span calibrations can be carried out frequently (daily / weekly) as there is little time penalty, and near infra-red absorption (the most common analyser technology) is a relatively stable technique. In contrast, flow sensors are more difficult to access, exposed to more contaminants, and calibration artefacts are not readily available, consequently, sensors have to be despatched to 3rd parties resulting in calibration being infrequent and not conducted under normal operating conditions. Hence, as flow sensors are not subject to the same level of QA/QC as analysers, the ducting efficiencies evidencing a more marked deviation from unity is consistent with the above rationale.

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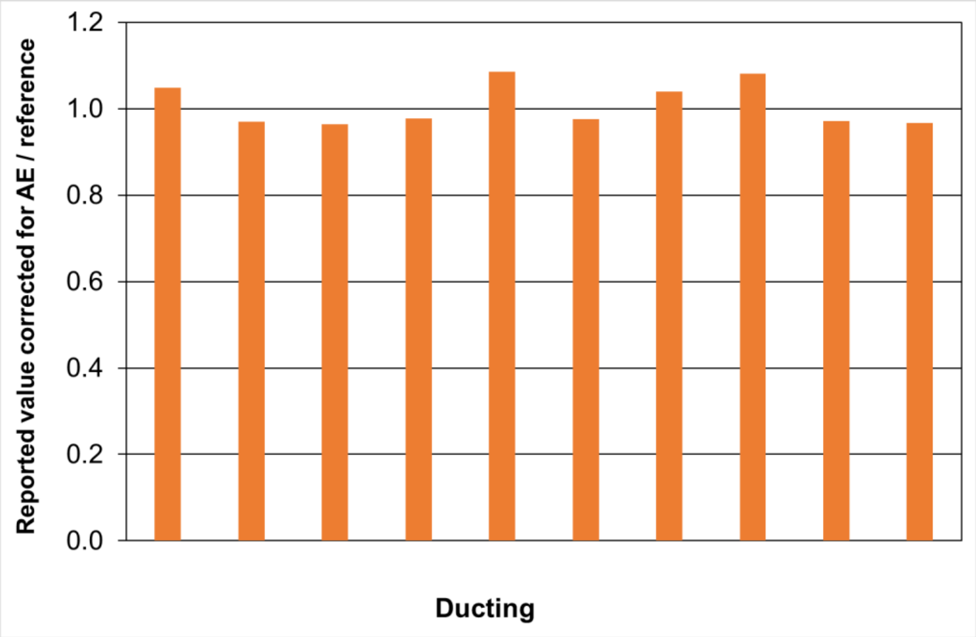

Figure 6 Ducting efficiencies.

Whilst the most frequent cause of bias in the ducting component of a facility is the flow sensor, there are other sources that should also be considered. Leaks are an obvious source of bias and in facilities that operate at sub-atmospheric pressures (post-chamber pump, “pull” type facilities) ambient air can ingress leading to ducting efficiencies below unity. Resulting in an artificially low emission being reported for the ruminant.

In contrast, at facilities operated at super-atmospheric pressures (pre-chamber pump, “push” type facilities) gas could leak out. However, as the flow sensor is expected to be installed pre-chamber in this scenario the leak is post-sensor and therefore is not directly measured. Consequently, as the flow measured is greater than the actual flow this results in an artificially high emission being reported for the ruminant.

Table 2 Ducting efficiencies

Fig. 6 bar#	Ducting efficiency
1	1.049
2	0.969
3	0.964
4	0.977
5	1.085
6	0.975
7	1.039
8	1.081
9	0.972
10	0.967

Inhomogeneous concentration mixing is a further possible source of bias in the ducting component of facilities. If the chamber gas is not homogeneously mixed as it enters the extract

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duct, then at the point when the analyser extracts sample there can be a concentration gradient across the diameter. Generally, gas is sampled from the extract duct via pipework suspended at a fixed-point, hence, this can lead to either an artificially high or low concentration measurement depending on the sampling point location across the gradient. However, there is a scenario where bias might be avoided as some facilities correct experimental data to the results of gas recovery tests. In this case, if the inhomogeneous concentration mixing is temporally independent and therefore reproducible (there would also need to be no temperature dependence given that gas emitted from a ruminant is likely to be at a different temperature to that from a cylinder), and inhomogeneous concentration mixing is the same for a gas recovery test as for ruminant emissions, then the correction will compensate for the bias. The level of correction correlating with how well the above assumptions hold. However, successfully demonstrating the above would not be trivial.

Similar to the inhomogeneous concentration mixing, there can also be flow inhomogeneity. For example, swirl can occur as gas passes though elbows in duct work. If the flow is not homogeneous then as a function the diameter there can be various off-axis flow components of various magnitudes. Hence, if the flow sensor is point-based then the extent of bias will be dependent on its location across the diameter, or if the sensor fills the diameter (e.g. vane-based) then the bias will correlate with the off-axis element of the flow profile. In terms of avoiding such issues, for large scale industrial ducts EN 15259 [[ii]] considers that homogeneous flow conditions will usually be encountered if there are at least five hydraulic diameters of straight duct upstream of the measurement plane and at least two downstream.

ISOLATED CHAMBER EFFICIENCIES

Isolated chamber efficiencies (Fig. 7 and Table 3) show deviations from unity slightly increased compared to the ducting efficiencies. In terms of phenomena causing biases then, as for ducting, leaks and inhomogeneous concentration mixing are both possible sources. With respect to leaks, in addition to what was discussed above back diffusion should also be considered. This especially applies to facilities of a design where there is a gap below the chamber door (or walls) where intake air is drawn into the chamber. Depending on the location and velocity of the emission, it is possible for gas to back-diffuse out under the chamber door (or wall). With respect to inhomogeneous concentration mixing, if there is room for the ruminant to move around in the chamber then there is the possibility that the nature of the inhomogeneity will be dependent on ruminant location. For example, at one of the facilities included in this study, identical releases were carried out in two different locations within a chamber and the isolated chamber efficiency was found to change by 6%, indicating inhomogeneous concentration mixing with source location dependency. Also, with this design the location of the ambient air measurement is important since the background methane in the intake air will be influenced if other ruminants are present in the barn.

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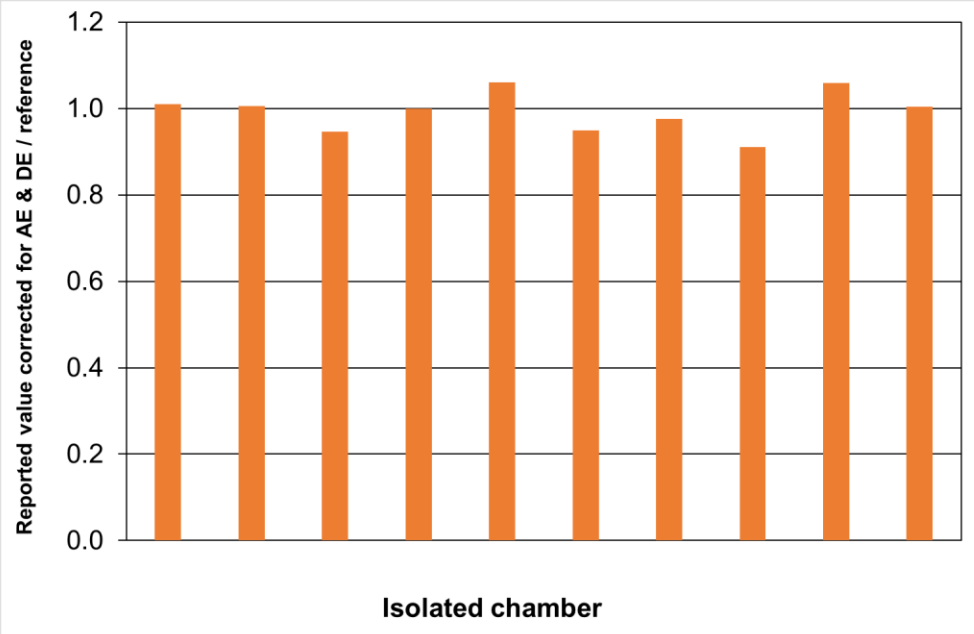

Figure 7 Isolated chamber efficiencies.

Also, although not tested in this study, it is also noted that chamber T90 response time is another possible source of bias. As noted above, the chamber T90 response time is a measure of the speed of response of the combined chamber, ducting, and analyser to a change in the chamber. The main consideration here (for similar rationale as already presented with respect to analyser T90 times) is to avoid using data until a period of 3 x T90 has elapsed when a chamber is closed after some type of outside intervention, e.g. a technician attending to animal welfare, exchanging one ruminant for another.

Table 3 Isolated chamber efficiencies

Fig. 7 bar#	Isolated chamber efficiency
1	1.010
2	1.006
3	0.945
4	1.000
5	1.060
6	0.949
7	0.975
8	0.910
9	1.059
10	1.004

COMBINED EFFICIENCIES

It is seen that the combined efficiencies (Fig. 8 and Table 4) deviate from unity to a lesser extent than the ducting efficiencies and isolated chamber efficiencies. This shows that some of the ducting and isolated chamber efficiencies are opposing resulting in an improved situation when the system is considered overall. It is worth emphasising the importance of this point,

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since whilst efficiencies of the components (analyser, ducting, chamber) are useful in terms of understanding where the greatest deviations reside, what ultimately determines the capability of a facility is the performance where all the components are used together. However, it should also be recognised that if a change is made improving one of the components this can, unfortunately, in some cases lead to a worsening of the combined efficiency.

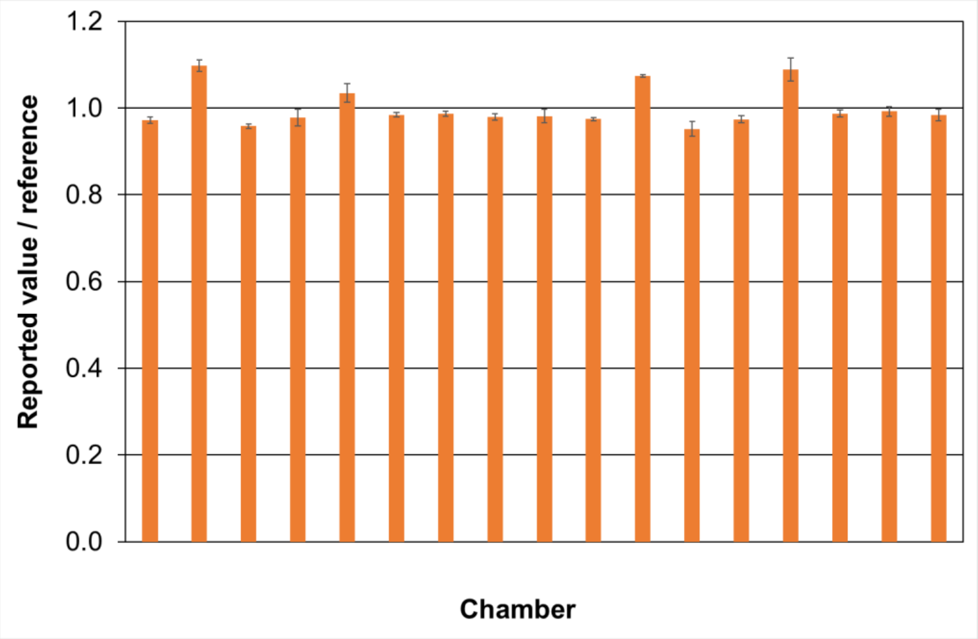

Figure 8 Combined efficiencies.

Table 4 Combined efficiencies

Fig. 8 bar#	Combined efficiency	Uncertainty (*k*=2, 95% confidence)
1	0.972	± 0.0072
2	1.097	± 0.0136
3	0.958	± 0.0055
4	0.978	± 0.0187
5	1.035	± 0.0219
6	0.984	± 0.0050
7	0.986	± 0.0062
8	0.979	± 0.0075
9	0.981	± 0.0152
10	0.975	± 0.0035
11	1.074	± 0.0033
12	0.952	± 0.0175
13	0.974	± 0.0084
14	1.089	± 0.0267
15	0.987	± 0.0086
16	0.992	± 0.0113
17	0.984	± 0.0130

RING-TEST VS RECOVERY TEST

It is also worth discussing the above results with reference to the Guideline document for performing Gas Recovery Tests of respiration chambers recently published in [iii]. It should be

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noted that a recovery test is not the same as the ring-test reported herein. The ring-test is an independent inter-laboratory comparison (ILC) and a calibration. The ILC involves: the same gas delivery apparatus; the same gas standards; same staff having had the same training; following the same procedure. In contrast, all of the above are typically variables in recovery tests. Although, with the publication of the Guideline greater future harmonisation should be achieved in terms of the procedure for recovery tests. With respect to calibration, the ring-test provides a calibration function to each facility (facility efficiency, see below) that when applied enables facility bias to, in principle, be largely removed. Consequently, if each facility bias is corrected in accordance with the supplied function, then what remains is the non-bias uncertainty sources of the facilities (i.e. precision), and the bias and precision uncertainty sources from the ring-test provider (NPL). However, a caveat to this is that some facilities correct experimental data to the results of recovery tests, so in this instance the recovery test is providing a form of calibration. Although, even if all five facilities involved in this study recovery test corrected experimental data this would unlikely provide the same level of inter-facility comparability as the ring-test. This because greater variability would be expected, due to: different gas delivery apparatus, different gas standards, different staff, potentially different procedures for data correction (the Guideline allows correction but does not stipulate the method).

So how do the ring-test and recovery test fit together? It is recommended that the recovery test is used after the ring-test calibration function (facility efficiency) has been applied. Facilities are expected to drift post application of the ring-test calibration function resulting in bias uncertainties increasingly affecting experimental data. The recovery test should be used as the ongoing QA/QC to ensure the ring-test calibration remains valid. However, clearly the ring-test calibration function will not remain valid indefinitely and routine repetitions of the ring-test are not pragmatic for the community. Consequently, it is recommended that in the future a two-tier hierarchy is adopted, where the community produces (via a users' group) a Guideline for local (facility level) calibration that, for example, is carried out every X years (or after a change has been made to the facility), with the Guideline for recovery tests carried out before and after each experiment.

FACILITY EFFICIENCIES

Facility efficiencies (Table 5) are calculated by determining the mean of the applicable combined efficiencies weighted by the inverse of the associated uncertainties (i.e. so the greatest weighting is applied to the efficiency with the lowest uncertainty). Determining 2x the standard deviation across the facility efficiencies provides an estimate of the comparability across the facilities. Following this calculation, it is seen (Table 6) that the comparability across the five SmartCow facilities is markedly better than that found in the UK based ring-test carried out approximately a decade ago [[iv]]. It should be noted that the two ring-tests are comprised of two different sets of facilities (although one facility participated in both, in the intervening period all the chambers were replaced so in effect this is not the same facility), and some of the UK ring-test facilities were designed for sheep rather than cattle. However, noting these caveats, and the lack (to the best of our knowledge) of any other applicable ring-tests of this nature, this is the most valid comparison possible to give some context to the reported data.

Table 5 Facility efficiencies

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Facility efficiency	Uncertainty (k=2, 95% confidence)
0.978	±0.006
0.979	±0.031
1.046	±0.040
1.017	±0.009
0.978	±0.016

If every facility in the SmartCow ring-test applied the applicable calibration function (facility efficiency) the comparability would improve to 5.4% (k = 2, 95% confidence). This is higher than 2.1% (k = 2, 95% confidence) reported for the UK ring-test, however, these two values are not calculated on the same basis. In the UK ring-test the precision of each facility was determined by carrying out a steady state release in each chamber and extrapolating the noise of the reported facility emission to 24 h. In SmartCow the precision of each facility is estimated by determining the individual precision of each component within the facility prior to combining these into the facility precision. Specifically, for a given facility the precision of the measurement of concentration, flow, temperature, pressure, are all individually determined, prior to taking the square root quadrature summation of these values with the uncertainty (bias and precision) attributable to the ring-test provider (NPL). This provides a more robust estimate of precision, moreover, due to funding limitations it wasn't possible to test every chamber at every facility and this is also a better approach from this perspective.

Table 6 Pre-calibration comparability across facilities in UK ring-test and SmartCow ring-test.

Ring-test	Comparability / % (k=2, 95% confidence)
UK	25.7
SmartCow	6.2

CONCLUSIONS

A successful methane emissions ring-test has been completed covering seventeen respiration chambers across five facilities. In characterising five methane analysers at the five respiration chamber facilities it was found that one showed an analyser efficiency deviating from unity more markedly than the remaining four. Subsequent investigation by the associated facility traced the issue back to the gas cylinder used for span calibration. All five analysers showed good linearity with none deviating from unity by more than the fourth decimal place of the R^2 values (returned from the Excel analysis of variance regression function). In addition, with respect to analyser T90 response times it was found that three of the five facilities excluded data within a period of 3xT90 from any switch from one measurand to the next - consistent with an idealised analyser switching regime - whereas two for the five excluded data from within a period of 2xT90 from any switch, risking the previous measurand influencing the current by ≤1.0%. Hence, it was recommended that these facilities considered adjusting their analyser switching regimes.

With respect to the ducting efficiencies, these evidenced an increased deviation from unity than was found for the analyser efficiencies. This broadly correlated with expectations as the latter are generally subject to greater QA/QC than the former, e.g. daily/weekly in-situ calibrations are possible for analysers whereas flow sensors have to be despatched to 3rd parties for calibration and this is only possible less frequently. Moving to isolated chamber efficiencies, again a further increase in the deviation from unity was found. Possible biases due to leaks and inhomogeneous mixing were discussed, and the issues of location dependency was demonstrated from data showing that at one of the facilities changing the release point location

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altered the emissions reported by the facility by 6%. Considering the combined efficiencies these were found to deviate from unity to a lesser extent that the ducting efficiencies and isolated chamber efficiencies. i.e. when the measurement system was in 'normal use' (since to carry out a measurement the analyser, ducting and chamber would all be used together) the situation is improved.

Calculating facility efficiencies from respective combined efficiencies it was possible to determine (k = 2, 95% confidence) facility comparability and this was found to be 6.2%. This was markedly better than the comparability found from a UK ring-test carried out ~10 year prior [iv] where a value of 25.7% (k = 2, 95% confidence) was returned. However, it was noted that only one facility from the UK ring-test was a participant in the SmartCow ring-test, and in the intervening period all the chambers at this facility had been replaced, so in effect it was not the same facility. Also, some the UK ring-test facilities were designed for sheep, whereas all the SmartCow ring-test facilities were designed for cattle. However, acknowledging these caveats, and the lack (to the best of our knowledge) of any other similar ring-tests this was the most valid comparison for the reported comparability. Lastly, it was shown that if each facility applied the calibration function determined in the ring-test this would in principle improve the comparability to 5.4% (k = 2, 95% confidence).

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