THE GLOBAL STANDARD
FOR LIVESTOCK DATA

Section 20 - Recording Dairy Cattle Methane Emission for Genetic Evaluation

Section 20 – Methane

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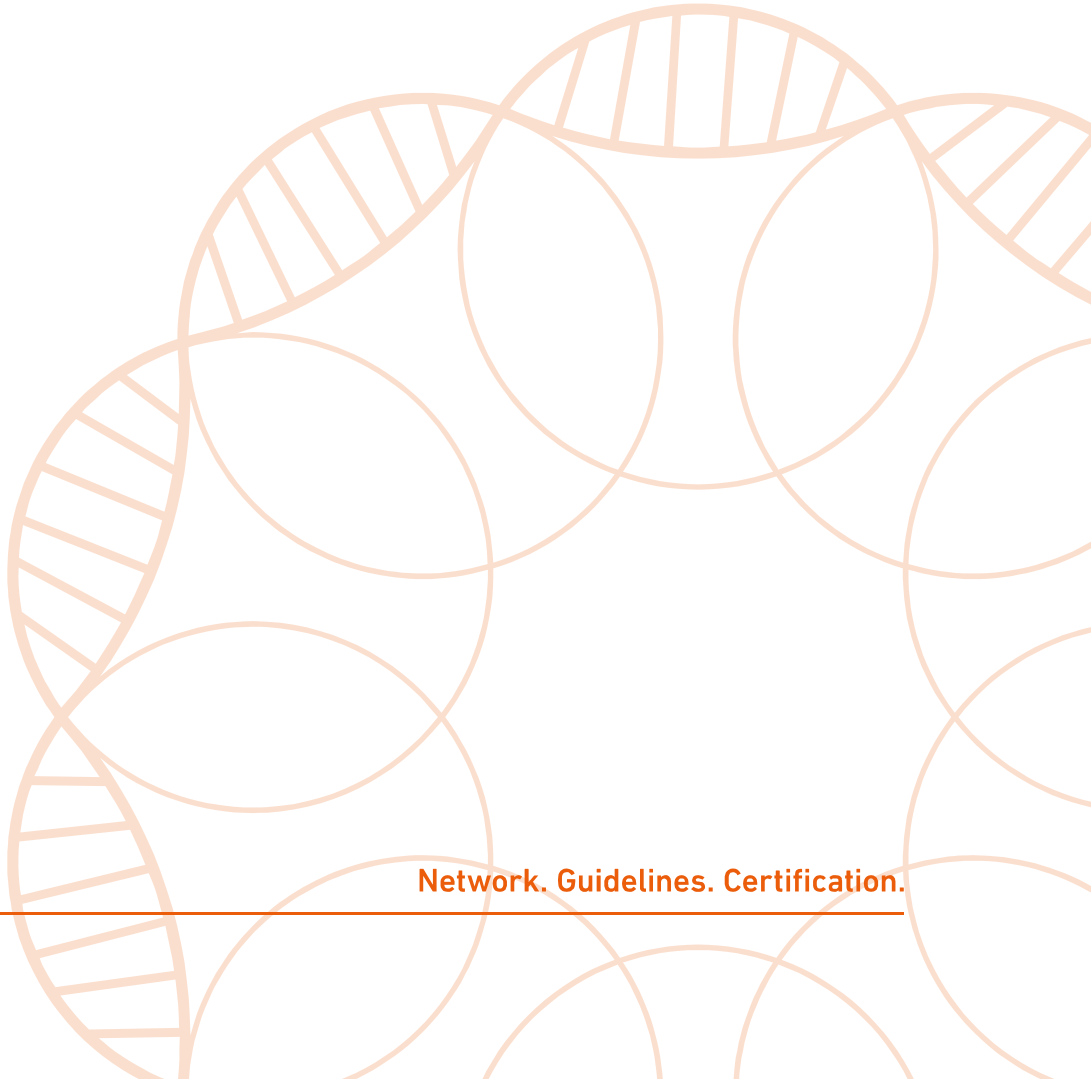

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Summary of Changes

Date of Change	Nature of Change
March 2020	Draft from Feed & Gas WG put into standard template for ICAR Guidelines. Separate out EDGP database to become as standalone appendix.
April 2020	Edits and acknowledgements added by Feed & Gas WG.
May 2020	Approved by ICAR Board on 26th May subject to addition of disclaimer. Disclaimer added as new chapter 2 - the fact specific device manufacturers are mentioned in these guidelines is in no way an endorsement of the devices or their accuracy by ICAR.

1 Introduction

Increases in milk production through management and genetics have substantially improved feed efficiency and decreased costs per unit of product over recent decades. However, dairy systems are also associated with environmental costs (Baskaran *et al.*, 2009), with methane (CH4) emissions associated with rumen microbial fermentation being both an important contributor to global greenhouse gas (GHG) emissions, as well as an avoidable loss of energy that could otherwise be directed into milk production. The livestock sector is responsible for 14.5% of the global GHG (Gerber *et al.*, 2013); dairy cattle account for 18.9% of these emissions, mainly in the form of enteric CH4 emissions (van Middelaar *et al.*, 2014).

Methane is a greenhouse gas with a global warming potential 28 times that of CO2 (Myhre *et al.*, 2013). Methane from ruminant livestock is generated during microbial fermentation in the rumen and hindgut (enteric CH4), and from decomposition of manure. Enteric CH4 contributes 80% of CH4 emissions by ruminants, and manure decomposition contributes 20%. Enteric CH4 accounts for 17% of global CH4 emissions and 3.3% of total global greenhouse gas emissions from human activities (Knapp *et al.*, 2014). There is, therefore, a significant research interest to find ways to reduce enteric CH4 emissions by ruminants.

Ruminant animals have a digestive system to digest plant materials efficiently. Like most mammals, ruminants lack the cellulase enzyme required to break the beta-glucose linkages in cellulose, but they play host to diverse populations of rumen microbes that can digest cellulose and other plant constituents. When rumen bacteria, protozoa and fungi ferment carbohydrates and proteins of plant materials, they produce volatile fatty acids, principally acetate, propionate and butyrate. High fibre diets favour acetate synthesis. Synthesis of acetate and butyrate are accompanied by release of metabolic hydrogen, which, if allowed to accumulate in rumen fluid, has negative effects on microbial growth, and feed digestibility (Janssen, 2010). Rumen Archaea are microorganisms that combine metabolic hydrogen with CO2 to produce CH4 and water. Archaea play a vital role, therefore, in protecting the rumen from excess metabolic hydrogen, and the CH4 they produce is an inevitable product of rumen fermentation.

A number of CH4 phenotypes have been defined (Hellwing *et al.*, 2012); the most widely used is CH4 production (MeP) in liters or grams per day.

The CH4 production trait is highly correlated with feed intake (Basarab *et al.*, 2013; De Haas *et al.*, 2017) and, thereby, with the ultimate breeding goal trait: milk production in dairy cattle. The economic value of daily dry matter intake and associated methane emissions in dairy cattle showed that increasing the feed performance estimated breeding value by one unit (i.e. 1 kg of more efficiently converted DMI during the cow's first lactation) translates to a total lifetime saving of 3.23 kg in DMI and 0.055 kg in methane (Richardson *et al.*, 2019). Feed Performance was defined as a 1 kg increase in more efficiently used feed in a first parity lactating cow. These results show not only the relation between DMI and CH4 production, but also the economic relationship between these traits. Persistency of lactation was found to be positively associated with increased feed efficiency and decreased methane production and intensity. Feed efficiency was associated with lower methane intensity. Feed efficiency and methane emissions can be improved by selecting for dairy cattle that are smaller and have increased persistency of lactation. Efficiency and methane emissions can be further improved by improved management of body condition score and by extending lactations beyond the conventional 305-day length (Seymour, 2019). According to Ellis *et al.* (2007), DMI predicted MeP with an R2 of 0.64, and ME intake (MJ/d) predicted MeP with an R2 of 0.53 for dairy cattle. AlternativePhenotype definitions include CH4 intensity (MeI), which is

defined as liters or grams of CH_4 per kg of milk, and CH_4 yield (MeY), which is defined as liters or grams of CH_4 per kg of dry matter intake (DMI) (Moate *et al.*, 2016). Residual CH_4 production (RMP) is calculated as observed minus predicted CH_4 production (Herd *et al.*, 2014, Berry *et al.*, 2015), with predicted values based on factors such as milk production, body weight and feed intake. At the moment, it is not obvious which of these phenotypes to use; but, it is important to monitor associations between the chosen CH_4 phenotype and the other important traits in the breeding goal (e.g. production, fertility, longevity) to avoid unfavorable consequences. Berry and Crowley (2012) describe advantages and limitations of ration traits. For example, because feed efficiency traits are a linear combination of other traits it is not recommended to include them in an overall total merit index, which is a clear limitation. For all applications it is necessary to measure the CH_4 emission of each animal individually. These guidelines are intended to make the right choices for this.

Whilst diet changes and feed additives can be effective mitigation strategies for CH_4 emissions (Beauchemin *et al.*, 2009; Martin *et al.*, 2010; Hristov *et al.*, 2013), their effects depend on the continued use of a particular diet or additive and there have been issues with the rumen microbiomes adapting to additives. Rumen bacterial communities are highly dynamic after a diet switch and did not stabilize within 5 wk of cows grazing pasture (Bainbridge *et al.*, 2016). In contrast, breeding for reduced CH_4 emissions should result in a permanent and cumulative reduction of emissions (Wall *et al.*, 2010). Several studies have shown that CH_4 emissions by ruminants have a genetic component, with heritability in the range 0.20 – 0.30 (de Haas *et al.*, 2011; Donoghue *et al.*, 2013; Pinares-Patiño *et al.*, 2013, Kandel *et al.*, 2014A, B; Lassen and Lovendahl, 2016; López-Paredes *et al.* 2020). Breeding for reduced CH_4 emissions, alone or together with other mitigation strategies, could therefore be effective in reducing the environmental impact of cattle farming and, possibly, also in increasing feed efficiency. Such a breeding scheme would require, as a fundamental starting point, accurate measures of individual CH_4 emissions on a large scale.

Several techniques have been developed for the measurement of CH_4 emissions from ruminants, with varying degrees of accuracy (see reviews by Cassandro *et al.*, 2013 and Hammond *et al.*, 2016A), but routine individual measurements on a large scale (a requisite for genetic selection) have proven to be difficult to obtain and expensive to measure (Pickering *et al.*, 2015; Negussie *et al.*, 2016). Therefore, identifying proxies (i.e. indicators or indirect traits) that are correlated to CH_4 emissions, but which are easy and relatively low-cost to record on a large scale, would be a welcome alternative. Proxies might be less accurate but could be measured repeatedly to reduce random noise and in much larger populations.

These guidelines are highly indebted to Garnsworthy *et al.* (2019). In this paper the methods to measure CH_4 are compared with special emphasis to the genetic evaluation of dairy cattle.

2 Disclaimer

The fact that specific device manufacturers are mentioned in these guidelines is in no way an endorsement of the devices or their accuracy by ICAR.

3 Definitions and Terminology

Table 1 contains a list of important definitions for terms and abbreviations used in these guidlelines.

Table 1. Definitions of Terms used in these guidelines.

Term	Definition
ADF	Acid detergent fibre
ADL	Lignin
BCS	Body condition score
CH_4	Methane
CV	Coefficient of variation
DIM	Days in milk
DMI	Dry matter intake
DMPR	Daily methane production rate
EE	Ether extract
Enteric methane	Methane from ruminant livestock generated during microbial fermentation in the rumen and hindgut
EOBC	Essential oils and their bioactive compounds
FTIR	Fourier-transform infrared
GE	gross energy intake
GHG	Greenhouse gas
LMD	laser methane detector
ME	Metabolizable energy
MeI	CH_4 intensity
MeP	CH_4 production (liters or grams per day)
MeY	CH_4 yield
MIR	milk mid-infrared spectroscopy
NDF	Neutral detergent fibre
NDIR	Nondispersive Infrared
PAC	Portable accumulation chambers
PAIR	photoacoustic infrared
Proxy	Not methane itself, but a substance enabling to measure methane levels indirectly – easy, cheap, accurate, quantitative
PY	protein yield
RMP	Residual CH_4 production
RMPR	Residual methane production rate
RMSPE	Root mean square prediction error
SF6	SF6 tracer gas technique

Term	Definition
TMR	Total mixed ration
VFA	Volatile Fatty Acid
Ym	Methane conversion rate

Appendix 1 to this guideline (here) contains information about the EDGP database including examples of the data storage structure.

4 Scope

A variety of technologies are being developed and employed to measure CH4 emissions of individual dairy cattle under various environmental conditions, as is evidenced by frequent reviews (Storm *et al.*, 2012; Cassandro *et al.*, 2013; Hammond *et al.*, 2016A; de Haas *et al.*, 2017). The first objective of the current guidelines is to review and compare the suitability of methods for large-scale measurements of CH4 output of individual animals, which may be combined with other databases for genetic evaluations. Comparisons include assessing the accuracy, precision and correlation between methods. Combining datasets from different countries and research centres could be a successful strategy for making genetic progress in this difficult to measure trait if the methods are correlated (de Haas *et al.*, 2017). Accuracy and precision of methods are important. Data from different sources need to be appropriately weighted or adjusted when combined, so any methods can be combined if they are suitably correlated with the 'true' value. The second objective of the current guidelines, therefore, is to examine correlations among results obtained by different methods, ultimately leading to an estimate of confidence limits for selecting individual animals that are high or low emitters (see also Garnsworthy *et al.*, 2019).

5 Methane determining factors

5.1 Diet and rumen microbiota

Table 2 contains a list of dietary or microbiota factors that determine CH4 production.

Table 2. Methane determining factors related to diet and rumen microbiota.

Factors	Reference
The main determinants of daily methane production are dry matter intake and diet composition: the more feed consumed, and/or the greater the fibre content of the diet, the more methane is produced per day. However, per unit of DMI, and per unit of fat+protein yield the grass diet produced less enteric CH_4 per cow than the TMR diet. Nutritional approaches for methane mitigation include reducing the forage to concentrate ratio of diets, increasing dietary oil content, and dietary inclusion of rumen modifiers and methane inhibitors.	Beauchemin *et al.*, 2009; Cottle *et al.*, 2011; Knapp *et al.*, 2014; O'Neill *et al.*, 2011; Sauvant *et al.*, 2011
Methane output per kg of product is affected mainly by cow milk yield or growth rate, and by herd-level factors, such as fertility, disease incidence and replacement rate.	Garnsworthy, 2004

Factors	Reference
Methane output varies considerably between individual animals. For animals fed the same feed, the between-animal coefficient of variation (CV) in methane was 8.1%.	Blaxter and Clapperton, 1965
The amount of digestible nutrients consumed especially of the carbohydrate fraction (starch, sugar, N-free residuals) is reliable to estimate CH_4 release with high precision. Furthermore, diets rich in fat reduced CH_4 formation in the rumen.	Jentsch *et al.*, 2007
DMI was also the most important determining factor, but there were different regression lines for maize silage and dried grass as the main roughage component: CH_4 (g)=93+16.8×DMI(kg) and CH_4 (g)=81+14.0×DMI(kg), respectively. Methane release was particularly dependent on the intake of crude fiber (CF) and ether extract (EE): CH_4 (g)=63+80xCF (kg)+11xNFE (kg)+19xCP(kg)-195xEE (kg).	Kirchgessner *et al.*, 1991
Methane linearly increased with NDF intake (CH_4 (L)=59.4×NDF[kg]+ 64.6) for cows together with their calves independent of the breed.	Estermann *et al.*, 2002
Enteric CH_4 could be predicted with the equation: CH_4 (g/d)=84+47×cellulose(kg/d)+32×starch(kg/d)+62×sugars (kg/d).	Hindrichsen *et al.*, 2005
The higher the percentage concentrate the lower Ym.	Zeitz *et al.*, 2012
Additives can sometimes have a methane reducing effect: higher dosages mitigate methane more. Saponins mitigate methanogenesis by reducing the number of protozoa, whereas condensed tannins act both by reducing the number of protozoa and by a direct toxic effect on methanogens.	Beauchemin *et al.*, 2008; Jayanegara *et al.*, 2012; Zmora *et al.*, 2012; Cieslak *et al.*, 2013; Guyader *et al.*, 2014
Plant essential oils have been shown as promising feed additives to mitigate CH_4 and ammonia emission, but results were inconsistent.	Cobellis *et al.*, 2016; Moate *et al.*, 2011
Nitrate and sulphate addition decreased the enteric methane emissions negatively affecting diet digestibility and milk production. The effects of the salts are additive.	van Zijderveld *et al.*, 2010; van Zijderveld *et al.*, 2011
The methanogenesis in the rumen of calves is associated with the development of the ruminal protozoa population. The absence of protozoa in the rumen reduced both the CH_4 production and the digestibility of carbohydrates.	Schönhusen *et al.*, 2003
Implementing good grazing management reduced gross energy intake loss as CH_4 by 14%.	Wims *et al.*, 2010

5.2 Host genetics, physiology and environment

A low-moderate proportion of variation in CH4 emissions among ruminants is under genetic control. Heritability coefficients of MeY and RMPR were h^2=0.22 and 0.19 respectively in a

population of 1,043 Angus growing steers and heifers measured during 2 days in RC (Donoghue *et al.*, 2016). The heritability coefficient of MeY was h^2=0.13 in a population of 1,225 dual-purpose growing sheep measured during 2 days in RC (Pinares-Patino *et al.*, 2013). Table 3 contains information of heritability of traits related to CH4 production.

Table 3. Heritability information of methane-related traits and measurements.

Factors	**Reference**
List with several h^2	Pickering *et al.*, 2015
List with several h^2	MPWG White paper Dec 18
Methane emissions from individual cows during milking varied between individuals with the same milk yield and fed the same diet. Between-cow variation in MERm is greater than within-cow variation and ranking of cows for CH_4 emissions is consistent across time. Variation related to body weight, milk yield, parity, and week of lactation/days in milk. The monitored variation might offer opportunities for genetic selection.	Garnsworthy *et al.*, 2011A; Garnsworthy *et al.*, 2011B
Mechanistic modelling approach: potential for dietary intervention as a means of substantially reducing CH_4 emissions without adverse effects on dietary energy supply.	Mills *et al.*, 2001
The CH_4-to-CO_2 ratio measured using the non-invasive portable air sampler and analyzer unit based on Fourier transform infrared (FTIR) detection method is an asset of the individual cow and may be useful in both management and genetic evaluations.	Lassen *et al.*, 2012
The estimated heritability for CH_4 g/day and CH_4 g/kg of FPCM were lower than common production traits but would still be useful in breeding programs	Kandel *et al.*, 2013
Genetic correlation between CH_4 intensity and milk yield (MY) was -0.67 and with milk protein yield (PY) was -0.46 in Holstein cows.	Kandel *et al.*, 2014A, B
Milk production and CH_4 emissions of dairy cows seemed to be influenced by the temperature humidity index.	Vanrobays *et al.*, 2013A
Estimate the heritability of the estimated methane emissions from 485 Polish Holstein-Friesian dairy cows at 2 commercial farms using FTIR spectroscopy during milking in an automated milking system by implementing the random regression method. The heritability level fluctuated over the course of lactation, starting at 0.23 (SE 0.12) and then increasing to its maximum value of 0.3 (SE 0.08) at 212 DIM and ending at the level of 0.27 ± 0.12. Average heritability was 0.27 ± 0.09.	Pszczola *et al.*, 2017
CH_4 measured with a portable air-sampler FTIR detection method on 3,121 Holstein dairy cows from 20 herds using automatic milking systems. The heritability of CH_4_MILK was 0.21 ± 0.06. It was concluded that a high genetic potential for milk production will also mean a high genetic potential for CH4 production. The results suggested that CH_4 emission is partly under genetic control, that it is	Lassen and Løvendahl, 2016

Factors	Reference
possible to decrease CH_4 emission from dairy cattle through selection, and that selection for higher milk yield will lead to higher genetic merit for CH4 emission/cow per day.	
CH_4 production was measured of 184 Holstein-Friesian cows in. the milking robot with a in total 2,456 observations for CH_4 production. Heritability for CH_4 production ranged from 0.12 ± 0.16 to 0.45 ± 0.11, and genetic correlations with MY ranged from 0.49 ± 0.12 to 0.54 ± 0.26. The positive genetic correlation between CH_4 production and milk yield indicates that care needs to be taken when genetically selecting for lower CH4 production, to avoid a decrease in MY at the animal level. However, this study shows that CH_4 production is moderately heritable and therefore progress through genetic selection is possible.	Breider *et al.*, 2019
CH4 concentration was measured with NDIR, and CH4 production was estimated from CH4 concentration and body weight. Heritability for CH4 concentration was 0.11 ± 0.03 and for CH4 production 0.12 ± 0.04. Positive genetic correlation was observed with MY (0.17-0.21), PY (0.22-0.31) and FY (0.27-0.29). Other type traits showed positive correlation with methane production (chest width=0.26, angularity =0.19, stature =0.43 and capacity =0.31) possibly associated to higher milk feed intake from these animals. Rumination time was negatively correlated to CH4 production (-0.24) and CH4 concentration (-0.43). However, larger CH4 production and CH4 concentration was associated with shorter days open.	López-Paredes *et al.* (2020)
Genetic parameters of CH4 emissions predicted from milk fatty acid profile (FA) and those of their predictors in 1,091 Brown Swiss cows reared on 85 farms showed that enteric CH4 emissions of dairy cows can be estimated on the basis of milk fatty acid profile. Additive genetic variation of CH4 traits was shown which could be exploited in breeding programmes.	Bittante and Cecchinato, 2020
A total of 670 test day records were recorded on lactating Holstein Friesian cows reared in 10 commercial dairy herds. Predicted methane production (PMP) was estimated to be 15.33±1.52 MJ/d in dairy cows with 23.53±6.81 kg/d of milk yeild (MY) and 3.57±0.68% of fat content (FC). Heritability of MY was 0.09 with a posterior probability for values of h2 greater than 0.10 of 44%. Estimates of heritability for FC and protein content (PC) were 0.17 and 0.34, respectively, with a posterior probability for values of h2 greater than 0.10 of 77% and 99%. For somatic cell score (SCS), heritability was 0.13 with a posterior probability for values of h2 greater than 0.10 of 67%. Heritability for the trait PMP was moderate to low (0.12); however, posterior probability for values of h2 greater than 0.10 was 60%. Medians of the posterior distributions of genetic correlations between PMP and milk production traits were: 0.92, 0.67, 0.14, and 0.14 between PMP and MY, PMP and FC, PMP and PC, and PMP and SCS,	Cassandro *et al.*, 2010

Factors	Reference
respectively. Reduction of PMP seems to be viable through selection strategies without affecting udder health and PC.	
GWAS to study the genetic architecture of CH_4 production and detected genomic regions affecting CH_4 production. Detected regions explained only a small proportion of the heritable variance. Potential QTL regions affecting CH_4 production were located within QTLs related to feed efficiency, milk-related traits, body size and health status. Five candidate genes were found: CYP51A1 on BTA 4, PPP1R16B on BTA 13, and NTHL1, TSC2, and PKD1 on BTA 25. These candidate genes were involved in a number of metabolic processes that are possibly related to CH4 production. One of the most promising candidate genes (PKD1) was related to the development of the digestive tract. The results indicate that CH_4 production is a highly polygenic trait.	Pszczola *et al.*, 2018
A 1000-cow study across European countries revealed that the ruminant microbiomes can be controlled by the host animal. A 39-member subset of the core microbiome formed hubs in co-occurrence networks linking microbiome structure to host genetics and phenotype (CH_4 emissions, rumen and blood metabolites, and milk production efficiency).	Wallace *et al.*, 2019

6 Methane measurements methods

Several factors influence the choice of measurement method such as cost, level of accuracy, precision, scope of application, and scale, which vary across disciplines (Cassandro *et al.*, 2013; Hammond *et al.*, 2016A; Garnsworthy *et al.*, 2019). For instance, genetic selection programs require CH4 measurements on thousands of related individuals under the environmental conditions in which the animals are expected to perform (Falconer and Mackay, 1996). This can be challenging because dairy cattle perform in a wide range of conditions (e.g. grazing vs indoor housing).

There are a number of different measurement methods currently being employed, each with advantages and disadvantages in terms of the factors listed above. The currently accepted and widely used measurement methods are listed and described below.

The main features of methods for measuring CH4 output by individual animals are summarised in Table 4. Values for each feature are based on experience of experts in METHAGENE WG2 who have used the methods. All values are relative, and somewhat subjective, because absolute values will depend on installation and implementation of each method at different research centres. It should be noted that the measuring methods can be divided in two major sections: methods that measure the concentration and flux of CH4 (e.g. the respiration chamber), and methods that measure the flux of CH4 through the device (e.g. GreenFeed). This affects the useability of the methods for answering research questions – please see also the recommendations at the end of these guidelines.

6.1 Respiration chambers

Respiration chambers are calibrated to be accurate and precise, and are the gold standard for benchmarking new methods. Only respiration chambers measure total emissions from the

animal via the oral, nasal and anal routes; all other methods ignore emissions via the anus and only measure CH4 emitted in breath. Breath measurements are justified because 99% of CH4 is emitted from the mouth and nostrils, and only 1% via the anus (Murray *et al.*, 1976).

A single animal (or occasionally more) is confined in a chamber for between 2 and 7 days. Concentration of CH4 (and other gases if required) is measured at the air inlet and outlet vents of the chamber. The difference between outlet and inlet concentrations is multiplied by airflow to indicate CH4 emissions fluxes. In most installations, a single gas analyser is used to measure both inlet and outlet concentrations, often for two or more chambers. This involves switching the analyser between sampling points at set intervals, so concentrations are actually measured for only a fraction of the day. If the sampling points acquisition frequency is high it enables to draw the diurnal pattern of methane emission, comparable to the GreenFeed system.

Respiration chambers vary in construction materials, size of chamber, gas analysis equipment and airflow rate, all of which can influence results. Validation of 22 chambers at six UK research sites revealed an uncertainty of 25.7% between facilities, which was reduced to 2.1% when correction factors were applied to trace each facility to the international standard CH4 (Gardiner *et al.*, 2015). The main sources of uncertainty were stability and measurement of airflow, which are crucial for measuring CH4 emission rate. The authors concluded, however, that chambers were accurate for comparing animals measured at the same site. This is an added challenge to benchmarking alternative methods with respiration chambers if respiration chambers themselves have not been benchmarked with respiration chambers at other facilities. It should be noted that substantial errors can occur if appropriate calibration procedures are not followed (Gardiner *et al.*, 2015).

For large-scale evaluation of CH4 emissions by individual animals, respiration chambers are challenging with only a single study in growing Angus steers and heifers exceeding 1000 animals and finding CH4 production to be moderately heritable h2 = 0.27 ± 0.07 (Donoghue *et al.*, 2016). Installation and running costs are high, as only one animal is normally measured at once. If we assume that the monitoring time is three days per animal, and chambers are run continuously, then maximum throughput would be approximately 100 animals per chamber per year. In practice, throughput is likely to be 30 to 50 animals per year. Cows are social animals and confinement in a chamber may ultimately influence their feeding behaviour resulting in less feed consumed and in a different meal pattern compared with farm conditions. Altered feeding pattern or level is not a problem for metabolic studies evaluating feeds but can be a problem when evaluating individual animals. Furthermore, the representativeness of respiration chambers to grazing systems has been called into question (Pinares-Patiño *et al.*, 2013). However, promising developments have led to more animal friendly respiration chambers constructed from cheaper, transparent materials. These lower the cost and reduce the stress of confinement with minimal disruptions to accuracy, precision and no drop in feed intake of the cows (Hellwing *et al.*, 2012).

Where an alternative method may be cheaper, less invasive, easier to implement, or have a wider scope of application, it is of value to assess the relative accuracy, precision and correlation with the gold standard to assess the relative worth of the alternative method (Barnhart *et al.*, 2007). All methods measure CH4 with some level of error, so the 'true value' of an individual is not known. However, when the level of measurement error increases, so too does the imprecision. When comparing two methods where one or both methods has high imprecision a phenomenon known as 'attenuation of errors' occurs (Spearman, 1904). The increased measurement error biases the correlation between the two methods downwards and reduces the efficacy of detecting significant differences in accuracy (Adolph and Hardin,

2007). Or in terms of linear regression terms, when the observed CV of an alternative method is higher than that of the gold standard method, the slope of regression between the methods is decreased and the intercept is biased upwards.

Table 4. Summary of the main features of methods for measuring CH4 output by individual animals[1].

Method	Purchase cost[2]	Running costs[2]	Labour[2]	Repeatability	Behaviour alteration[3]	Through -put
Respiration chamber	High	High	High	High	High	Low
SF_6 technique	Medium	High	High	Medium	Medium	Medium
Breath sampling during milking and feeding	Low[4]	Low	Low	Medium	None	High
GreenFeed	Medium	Medium	Medium	Medium	Medium	Medium
Laser methane detector	Low	Low	High	Low	Low-Medium	Medium

6.2 Portable Accumulation Chambers

In Australia and New Zealand an alternative method was developed for the short-term measurement of Methane Production Rate (MPR) of sheep using Portable Accumulation Chambers (PAC) during 1 hour without leading discomfort to the animals. Similarly to RC, CH4 emissions recorded in PAC include gases from flatulence in addition to eructed and expired CH4, but only during 1 hour. For a detailed comparison of the PAC and respiration chamber methods see Jonker *et al.* (2018).

6.3 SF6

The SF6 technique samples breath over 24 hours, whereas other techniques use spot samples of breath over periods of minutes throughout the day, so diurnal variation has to be considered. The majority of CH4 (87-99%) is released by eructation (Blaxter and Joyce, 1963; Murray *et al.*, 1976), which provides a clear signal for sample processing. Please note that the

[1] Consensus views based on experiences of METHAGENE WG2 members (www.methagene.eu).

[2] Per measuring unit or group of animals.

[3] Compared to no methane recording: low = measuring in situ; medium = some handling, training or change in routine; high = confinement.

[4] Medium if using FTIR analyser.

tracheostomy used in Murray *et al.* (1976) may have resulted in a higher percentage, but in both publications, it is clear that the majority of the CH4 is released via eructation.

The SF6 tracer gas technique was developed in an attempt to measure CH4 emissions by animals without confinement in respiration chambers (Johnson *et al.*, 1994). Air is sampled near the animal's nostrils through a tube attached to a halter and connected to an evacuated canister worn around the animal's neck or on its back. A capillary tube or orifice plate is used to restrict airflow through the tube so that the canister is between 50 and 70% full in approximately 24 hours. A permeation tube containing SF6 is placed into the rumen of each animal. The pre-determined release rate of SF6 is multiplied by the ratio of CH4 to SF6 concentrations in the canister to calculate CH4 emission rate.

Many research centres have used the SF6 technique with variations in design of sampling and collection equipment, permeation tubes, and gas analysis (Berndt *et al.*, 2014). Reliable results depend on following standard protocols, with greatest variation coming from accuracy of determining SF6 release rate from permeation tubes and control of sampling rate. With capillary tubes, sampling rate decreases as pressure in the canister increases, whereas an orifice plate gives a steadier sampling rate over 24 hours (Deighton *et al.*, 2014). A source of error that has not been evaluated is that animals might interact and share CH4 emissions when the sampling tube of one animal is near the head of another animal. There is good agreement between CH4 emissions measured by the SF6 technique and respiration chambers, although results from the SF6 technique are more variable (Grainger *et al.*, 2007; Muñoz *et al.*, 2012).

6.4 Breath sampling during milking and feeding

Several research groups have developed methods to measure CH4 concentration in breath of cows during milking and/or feeding. These are often referred to as 'sniffer methods' because they use devices originally designed to detect dangerous gas leaks. Air is sampled near the animal's nostrils through a tube fixed in a feed bin and connected directly to a gas analyser. The feed bin might be in an automatic milking station (Garnsworthy *et al.*, 2012A, B; Lassen *et al.*, 2012; Pszczola *et al.*, 2017, 2018, 2019) or in a concentrate feeding station (Negussie *et al.*, 2017). Different research centres use different gas analysers (Nondispersive Infrared (NDIR), Fourier-transform infrared (FTIR) or photoacoustic infrared (PAIR)) and different sampling intervals (1, 5, 20 or 90-120 seconds). Methane concentration during a sampling visit of typically between 3 and 10 minutes may be specified as the overall mean, or the mean of eructation peaks. Some centres use CO2 as a tracer gas and calculate daily CH4 output according to ratio of CH4 to CO2 and daily CO2 output predicted from performance of the cow (Madsen *et al.*, 2010). Repeatability and rank correlations were higher for eructation peaks than for mean concentrations, and were higher for eructation peaks than for CH4 to CO2 ratio (Bell *et al.*, 2014). However, all methods show good repeatability.

6.5 GreenFeed

GreenFeed (C-Lock Inc., Rapid City, South Dakota, USA) is a sniffer system where breath samples are provided when animals visit a bait station (Huhtanen *et al.*, 2015). GreenFeed Emission Monitoring (GEM) systems are designed for measuring animal emissions in their production environment. As with other sniffer systems, GreenFeed samples breath from individual animals several times (in general 4 to 6 times) per day for short periods (3 to 7 minutes in which an under pressure is created to suck the whole breath of the animal to measure the flux). They record CH4 and carbon dioxide (CO2) fluxes during short-term periods of 3-10 minutes when cattle visit an automated feeder fitted with a semi-enclosed

head hood in which air is continuously drawn through an air-collection pipe (C-Lock, 2016; Huhtanen *et al.*, 2015; Hammond *et al.*, 2016A; Velazco *et al.*, 2016). Air samples are continually (every second) analyzed for CH4 and CO2 concentrations using non-dispersive infrared sensors. Gas fluxes are eventually calculated as the product of the air flow in the collection pipe and the concentration of gases corrected for the background concentrations and adjusted to standardized temperature, humidity and pressure. The position of the head in the feeder is detected by an infrared sensor. Gas fluxes are not calculated if the head is not correctly positioned in the feeder as not all the air in the feeder may be collected.

GreenFeed is a portable standalone system used in barn and pasture applications and incorporates an extractor fan to ensure active airflow and head position sensing for representative breath sampling (Hammond *et al.*, 2016B). Measurements are pre-processed by the manufacturer, and data are available in real-time through a web-based data management system (Hammond *et al.*, 2015). Because GreenFeed captures a high proportion of emitted air and measures airflow, which can be calibrated using a tracer gas, CH4 emission is estimated as a flux at each visit. Providing visits occur throughout the 24 hours, CH4 emission can be estimated directly as g/day (Hammond *et al.*, 2015; Huhtanen *et al.*, 2015). More importantly, repeatability of CH4 measurement must be high so the duration of the measurement period must be taken into account (Huhtanen *et al.*, 2013; Arbre *et al.*, 2016); (R=0.7 after 17 days duration of measurement period, or R=0.93 after 45 days, Arbre *et al.*, 2016).

6.6 Laser methane detector

The laser CH4 detector (LMD) is a highly responsive, hand-held device that is pointed at an animal's nostrils and measures CH4 column density along the length of the laser beam (ppm.m). In the first implementation of LMD on a farm, measurements for each cow were taken over periods of 15 to 25 seconds between eructation events and could detect CH4 emitted each time the animal breathed out (Chagunda *et al.*, 2009 Sorg *et al.*, 2016, 2017). In a later study with sheep and beef cattle, monitoring periods of 2 to 4 minutes allowed authors to separate breathing cycles from eructation events (Ricci *et al.*, 2014). Typically, animals are restrained either manually or in head yokes at a feed fence for the required length of time. The operator has to stand at the same distance (1 to 3 m) from each animal every time and must be careful to keep the laser pointed at the animal's nostrils throughout the measurement period.

7 Discussion of methods

7.1 SF6 vs. respiration chamber

For large-scale evaluation of CH4 emissions by individual animals, the SF6 technique is more useful than respiration chambers. Animal behaviour and intake might be affected by wearing the apparatus, and by daily handling to exchange canisters, but the technique is considerably less intrusive than respiration chambers because cows remain in the herd. Labour and monetary costs for changing canisters each day and for lab analysis are high. Throughput is limited by the number of sets of apparatus available, handling facilities, labour, and the capacity of the lab for gas analysis. Animals need to be measured for 5 to 7 days, and it is recommended that group size should be less than 15 animals (Berndt *et al.*, 2014), so maximum throughput would be about 750 animals per year. The method may be better suited for in housed conditions because of the labour and the potential movement restriction of the animals due to wearing the apparatus.

7.2 Breath sampling during milking and feeding (– vs respiration chamber)

For large-scale evaluation of CH4 emissions by individual animals, breath-sampling methods have significant advantages compared with other methods. Breath-sampling methods are non-invasive because, once installed, animals are unaware of the equipment and are in their normal environment. Animals follow their normal routine, which includes milking and feeding, so no training of animals, handling, or change of diet is required. Equipment is relatively cheap, although more expensive gas analysers are available, and running costs are negligible.

The compromise for non-invasiveness of breath-sampling is that concentrations of gases in the sampled air are influenced by cow head position relative to the sampling tube (Huhtanen *et al.*, 2015). The use of head position sensors and data filtering algorithms can remove the effects when the cow's head is completely out of the feed bin (Difford *et al.*, 2016), but not within the feed bin. Consequently, sniffer measurements are more variable than flux methods, with factors like variable air flow in the barn increasing measurement error (imprecision), and head position, a highly repeatable character, inflating between-cow variability.

Using CO2 as a tracer gas partly addresses the issue but, because CO2 arises from metabolism as well as rumen fermentation, variability of CO2 emissions has to be considered. A further consideration is diurnal variation in breath concentrations of CH4 and CO2 because animals are spot-sampled at different times of day and night. Diurnal variation can be accounted for either by fitting a model derived from the whole group of animals, or by including time of measurement in the statistical model (Lassen *et al.*, 2012).

The number of observations per analyser is limited only by number of cows assigned to one automatic milking station or concentrate feeding station and length of time equipment is installed. Typically, each analyser will record 40 to 70 animals 2 to 7 times per day for 7 to 10 days, although the number of sampling stations per analyser can be increased by using an automatic switching system (Pszczola *et al.*, 2017). Throughput per analyser is likely to be 2,000 to 3,000 animals per year.

7.3 NDIR vs LMD

Both methods are low invasive. LMD needs larger labor force, wheras NDIR can be used during milking and feeding. According to Rey at al. (2019), the repeatability of the CH4 concentration was greater for NDIR (0.42) than for LMD (0.23). Correlation between methods was moderately high and positive for CH4 concentration (0.73 and 0.74, respectively) and number of peaks (0.72 and 0.72, respectively), and the repeated measures correlation and the individual-level correlation were high (0.98 and 0.94, respectively). A high coefficient of individual agreement for the CH4 concentration (0.83) and the number of peaks (0.77) were observed between methods. The study suggests that methane concentration measurements obtained from NDIR and LMD cannot be used interchangeably. But the use of both methods could be considered for genetic selection purposes or for mitigation strategies only if sources of disagreement, which result in different between-subject and within-subject variabilities, are identified and corrected for.

7.4 GreenFeed

A limitation of the GreenFeed system is that animals require training to use the system, although animals which have been trained to use the system will readily use it again (Velazco *et al.*, 2014). However, some animals will not use the system or will use it infrequently, and frequency of visits is affected by diet (Hammond *et al.*, 2016B). This can be a challenge when

screening commercial herds for CH4 emission under genetic evaluation. On the other hand, animals seem to get used to the equipment rapidly, and the sound produced by the system is remembered by the animals easily (personal information Dr. Finocchiaro). Alternatively, as practised in Canada, the unit is moved to individual animals in a tie-stall setting multiple times a day (personal information Prof C.F. Baes). Thus, action of individual animals is not needed.

The manufacturer recommends 15 to 25 animals per GreenFeed unit, and recordings are made typically for 7 days. If all animals visit the unit adequately, throughput per unit is likely to be 750 to 1,250 animals per year. Sebek *et al.* (2019A, B) and Bannink *et al.* (2018) showed the usefulness of the GreenFeed method in an on farm setting.

7.5 Laser methane detector

The LMD can be used in the animal's normal environment, although for consistency restraint is required during measurement. Because the LMD measures CH4 in the plume originating from the animal's nostrils, results can be affected by factors such as: distance from the animal; pointing angle; animal's head orientation and head movement; air movement and temperature in the barn; adjacent animals; and operator variation (Sorg *et al.*, 2017). Operator variation is likely to be one of the biggest factors because the operator controls distance and pointing angle, and is responsible for ensuring the laser remains on target. The structure of the barn and the resulting ventilation conditions and wind speed at the location of the measurement are also considerable sources of variation in recorded CH4.

Assuming operator fatigue does not limit measurements, each LMD could record up to 10 animals per hour. If each animal is recorded 3 times (on 3 consecutive days, for example, as in Mühlbach *et al.* (2018)), throughput is likely to be up to 1000 animals per year.

8 Comparison of methods to measure methane

8.1 Correlations among methods

Table 5 shows correlations between the respiratory chamber method as the gold standard to measure CH4 emission from cows and other methods. Data were taken from Garnsworthy *et al.* (2019), Table 2.

Table 5. Correlations between Ch4 measuring methods. Data were taken from Garnsworthy et al. (2019).

Method	Correlation	(S.E.)
Respiratory chamber - SF6	0.87	-0.08
Respiratory chamber - GreenFeed	0.81	-0.1
Respiratory chamber – NDIR	-0.07	0.88
Respiratory chamber – NDIR peak	0.72	-0.11
Respiratory chamber – PAIR	-0.08	0.7
SF6 – GreenFeed	0.4	-0.18
LMD – GreenFeed	0.77	-0.23
NDIR – GreenFeed	0.64	-0.18
NDIR – LMD	0.6	-0.11

Method	Correlation	(S.E.)
FTIR – LMD	0.57	-0.25
NDIR - NDIR peaks	0.58	-0.15
FTIR – NDIR	0.97	-0.02
FTIR – NDIR	0.53	-0.17

In method comparison studies, simultaneous repeated measures per cow with two or more methods are required in order to assess systematic differences between methods (means) and random differences (precision) and correlation between methods free of residual error. Furthermore, adequately short time differences between repeated measures per subject are needed to ensure the underlying biology of the cow has not changed. Not all methods can be recorded simultaneously and CH4 emission of cows' changes both within day and over the lactation period. In such instances either cross-over designs or matched pair repeated measures designs are needed. Members of METHAGENE WG2 provided data from studies in which two or more methods had been used to measure CH4 output (g/day) by individual dairy cows. Methods were applied to each cow either concurrently or consecutively within a short timeframe.

Seven main methods were represented: respiration chambers; SF6; GreenFeed; LMD; and three breath-sampling systems based on different gas analysers. Gas analysers incorporated different technologies to measure CH4, which were NDIR (e.g. Guardian Plus, Edinburgh Instruments, Edinburgh, UK), FTIR (e.g. Gasmet 4030, Gasmet Technologies Oy, Helsinki, Finland), or PAIR (e.g. F10, Gasera Ltd, Turku, Finland). In the contributing studies, NDIR and FTIR were used in automatic milking stations, and PAIR was used in concentrate feeding stations. One NDIR study and all FTIR and PAIR studies used CO2 as a tracer gas, with daily CO2 output calculated either from milk yield, live weight and days pregnant or from metabolisable energy intake. Two NDIR studies were based on CH4 concentration in eructation peaks rather than mean CH4 concentration, so were treated as separate methods. By separating NDIR studies, a total of 8 distinct methods were available giving a matrix of 28 potential combinations for comparisons. Data were available for 13 method combinations (Garnsworthy *et al.*, 2019).

Method comparisons were conducted using bivariate models (repeatability animal models) to obtain correlations between 'true values', also known as repeated measures correlations or individual level correlations (Bakdash and Marusich, 2017). Variance components including between cow variation and within cow variation (precision) and means (accuracy) were used in the calculation of between cow coefficient of variation (CV, %) and total CV and repeatability. Where single measurements were available for each method Pearson's correlation was reported and where repeated measures per subject were available repeated measures correlation was reported.

Respiration chambers were the most precise method, as can be seen by the smaller between cow CV% and total CV compared to alternative methods, and respiration chambers are by definition the most accurate. All methods tested showed high correlations with respiration chambers but none of the correlations exceeded 0.90. This is in part due to the increased imprecision of alternative methods, as even the most accurate and precise method will compare poorly to a less precise method. These correlations are also likely to be underestimated because none of the methods could be recorded simultaneously with

respiration chambers and had to be recorded in cross over designs. Consequently, the true value for each cow may have changed due to changes in the underlying biology of the cow over time between measurements. **Comparisons among alternative methods generally had lower correlations than comparisons with respiration chambers, despite having relatively higher numbers of animals and in most cases simultaneous or near simultaneous repeated measures per cow per method due to the increased variability and imprecision of alternative methods** as is seen by the increased CVs or due to the possibility that different aspects of CH4 emission are captured using different methods.

For the methods with repeated measures per cow the two mass flux methods, **SF6** and **GreenFeed**, had the highest repeated measures correlations (0.87 ± 0.08 and 0.81 ± 0.10) which outperformed the concentration based NDIR method using CO2 tracer gas. Of the two concentration methods evaluated against respiration chambers using single measurements, NDIR Peaks had a higher correlation (0.89 ± 0.07) than the PAIR CO2 tracer gas (0.80 ± 0.10). The study of Hristov *et al.* (2016) comparing SF6 and GreenFeed reported a low Pearson correlation of 0.40, despite having a large number of animals with repeated measures per method, the authors appear not to have estimated a repeated measures correlation, which could be larger. Estimating a repeated measures correlation between these two mass flux methods is a priority as it would clarify the inexplicable disagreement between two methods which both correlate highly with the gold standard method. With the exception of the aforementioned study, the imprecision was low in the mass flux measure comparisons as compared to the concentration-based methods.

Two of the **sniffer methods** evaluated, **FTIR CO_2t1** and **NDIR CO_2t1**, correlated close to unity (0.97), most likely due to the shared prediction equation for CO2 tracer gas. Nevertheless, all correlations derived from actual data were positive. This suggests that combination of datasets obtained with different methods is a realistic proposition for genetic studies. **Calculation of adjustment or weighting factors for bias, accuracy and precision would improve the value of combined datasets.**

8.2 Pro's and con's of devices

8.2.1 Daily methane emission measures

Due to the large diurnal variation in enteric CH4 emission in relation with feeding pattern (Grainger *et al.*, 2007; Jonker *et al.* 2014), the highest accuracy of daily CH4 production rate (DMPR) will be obtained with methods that encompass the whole day emissions. Two methods are available: **Respiration Chambers** (RC) and **SF6** methods.

Alternative methods are based on short-term measures of CH4 production rate: **Portable Accumulation Chambers** (PAC) for sheep and **GreenFeed Emission Monitoring** (GEM) systems for cattle and sheep (Hegarty, 2013).

8.2.2 DMPR with Respiratory Chambers (RC)

It should be noted that CH4 emissions recorded in RC also include gases from flatulence in addition to eructed and expired CH4. Compared with mouth exhaled CH4, CH4 from flatulence is generally considered as limited.

Feed intake in the RC may not be representative of the normal animal feed intake (Bickell *et al.*, 2014; Llonch *et al.*, 2016; Troy *et al.*, 2016). As a consequence, the DMPR measured could be biased. Animals are usually not fed ad libitum when recorded in RC. It is therefore recommended to compare animal or diet effects on Methane Yield (MY) calculated as the

ratio of the observed DMPR/DMI during the RC recording in order to take into account possible differences among animals in DMI bias. Animal effects can also be compared on the Residual Methane Production Rate (RMPR) the difference between the observed DMPR and the expected DMPR obtained by regression of observed DMPR on DMI recorded during RC test. Residual traits, however, require a large number of recorded animals for valid adjustment.

Repeatability coefficients between measures taken on consecutive days are very high, rep=0.85 [0.75 to 0.94] for MeY and RMPR of cattle and sheep (Grainger *et al.*, 2007; Donoghue *et al.*, 2016; Pinares-Patino *et al.*, 2013). It has been concluded that 1-day measurement duration could be recommended as it will have a limited impact, less than 5%, on the efficiency of selection of MeY as compared to a selection on a 2-day measurement duration.

When repeated measures of CH4 emission of sheep are taken few days to two weeks apart the repeatability coefficients of MeY and RMPR drops to rep=0.36 [0.26 to 0.41] on average (Pinares-Patino *et al.*, 2013; Robinson *et al.*, 2014a). Interestingly, repeatability maintains at a moderate level, rep=0.27 [0.23 to 0.53], when animals were measured several months or even years apart. Similar results were found in Angus cattle, rep=0.20, between MeY and RMPR measures taken more than 60 days apart (Donoghue *et al.*, 2016).

8.2.3 Conclusions and recommendations

All these results show that animal effects exist on daily CH4 emissions and animal differences are partially under genetic determinism. This trait, as any other physiology trait, is subject to number of environmental effects and to evolution with time. Ranking animals on their CH4 emission requires standardization of the testing environment. **Although highly precise, a single measure recorded in RC is not sufficient for characterizing an animals emission aptitude**. In order to characterize a long term phenotype it is therefore **recommended to record several 1-day measures, each a few weeks apart, instead of one single 2-day measure, keeping the testing environment as constant as possible**.

8.2.4 DMPR with GEM

At each visit CH4 and CO2 fluxes are measured and animal emission rates are obtained by averaging the short-term flux measures recorded during the testing period. In a review of published results (Dorich *et al.*, 2015; Hammond *et al.*, 2015; Velazco *et al.*, 2016) Hammond *et al.* (2016A) concluded that the **GEM** system provides similar DMPR values as the RC or SF6 methods. Similar accuracy was found by Arbre *et al.* (2016) for CH4 yield measured with GEM as compared with **RC** and **SF6** measures.

The spot measures are highly variable since they include, in addition to the animal and environment effects, an important within-animal and within-day variance. The latter is considered as an error term. Consequently, the precision of the animal estimates increase with the number of spot measures averaged per animal. From the results reported by Renand and Maupetit (2016) with 124 beef heifers controlled indoors, it can be shown that the coefficient of variation of that error term (CVe) decreases exponentially with the number of spot measures: 13.7%, 10.8%, 7.9% and 4.9% with 5, 10, 25 and 100 measures respectively. Results reported by Arbre *et al.* (2016) with 7 lactating dairy cows controlled indoors, also show that CVe decreases from 12.8% to 11.4%, 9.5% and 6.8% when the number of measures increases from 5 to 10, 25 and 100. With dairy cows at pasture, Waghorn *et al.* (2016) showed that the coefficient of variation among 36 dairy cows at pasture was half (6.6 and 7.5%) when

CH4 production rate was averaged over 16 days with approximately 18 to 26 measures per cow, as compared with 4 day averages with 4 to 6 measures per cow (13.0 and 17.2%). These authors concluded that at least 16 days are required to give confident estimates.

With 45 to 50 spot measures recorded during 2 weeks Arbre *et al.* (2016) and Renand and Maupetit (2016) obtained repeatabilityof 0.78 and 0.73 for DMPR estimates of 7 dairy cows and 124 beef heifers, respectively. A similar repeatability coefficient (0.74) was obtained by Huhtanen *et al.* (2015) with 25 dairy cows recorded during 3 weeks, with 20 to 30 samples per cow. Interestingly, these latter authors fitted gas concentration, airflow and head position measurement equipments into two automatic milking systems that were used to measure CH4 emission of 59 dairy cows during two periods of 10 days. After filtering data for acceptable head-position, the repeatability of DMPR was 0.75.

Considering the need to average enough spot measures and the advantage of measuring DMPR over long periods to take into account the emission variability with time, **the GEM system should be run over several weeks**. Averaging 40 to 50 spot measures per animal should provide a precise measure of the animal DMPR. The minimum duration of CH4 recording will depend on the number of spot measures actually recorded per day.

The GEM system relies on animals that voluntarily visit the GEM unit when attracted with pellets dispensed by a feeder at a controlled rate. The visitation frequency appears to be highly variable among different studies reported up to now. While some experiments report a very high frequency of cattle visiting the GEM units (up to 96%), the proportion of not visiting animals may be very high in other studies (up to 60%) (Dorich *et al.*, 2015; Hammond et al, 2015A, 2015B; Arbre *et al.*, 2016; Renand and Maupetit, 2016; Velazco *et al.*, 2016; Waghorn *et al.*, 2016). The reason why some animals may not visit the unit is not obvious. That problem of no or low visiting frequency may jeopardize the precise ranking of animals on their DMRP. Training them is an important requisite for the success of DMPR recording with the GEM system (see recommendations on the C-Lock website). Palatability of the pellets used to attract the cattle should be high compared with the diet they receive in the trough or the grass they are grazing.

In addition to the effect on precision, the low visiting frequency may have an impact on accuracy if associated in some animals with specific time of visiting. Enteric CH4 emissions have a diurnal variation with a minimum at the end of night, before the first feeding, and a steady increase after each feeding. A weak diurnal pattern in CH4 emission was detected by Velazco *et al.* (2016) using GEM systems. Renand *et al.* (2013) observed significant differences between visit hours (CV=10%). If some animals visit the GEM at specific hours of the day, the rough average of spot measures will be biased. In order to get rid of this time effect on the DMPR measure, Dorich *et al.* (2015) and Hristov *et al.* (2016) came up with a protocol where the GEM units were moved sequentially from one cow to the next one over several days, so that all the cows were equally measured during different hours of the day. That protocol is possible only with tie stall cattle and is obviously not applicable for measuring large number of animals. However, with animals controlled in their production environment, the bias generated by potential specific visiting patterns can actually be removed if the measuring hour is taken into account in the linear model when estimating the animal effect.

As voluntary visiting of the GEM system may be a limiting factor under some conditions, measures of DMPR can be designed when animals are drinking or eating, i.e. several times per day. Velazco *et al.* (2016) showed that a GEM water unit prototype designed and built by C-Lock Inc., displayed different eructation patterns as compared with a plain GEM unit. They

concluded that further development appears necessary before any application. Troy *et al.* (2016) tested a CH4 hood (MH) system placed above an automated feeding bin. That system includes an air extraction fan for each hood with continuously recorded airflow. Methane concentration was measured using 4 infrared analyzers, one for 8 hoods. In this system one CH4 concentration value was recorded every 6 min. With 9 to 12 feeding events per day on average and feeding visits averaging 8 min, there were between 12 to 16 CH4 concentration values recorded and CH4 production rates calculated per day. The measurements were recorded during 46 days and ranking of animals in relation with the test duration was studied. However no repeatability coefficient was given for comparison with other methods. That system was compared with respiratory chambers results in two experiments with 82 and 80 steers fed different diet-treatment combinations. **Over the whole experimental design, a good concordance was found between MH and RC** results as a consequence that both methods detected similar effects for the diet-treatment effects. However no correlation was given between both methods within diet-treatment samples that are the essential information needed to evaluate the ability of this new method to predict individual DMPR.

8.2.5 Conclusions and recommendations

With only a single gas analyzer for 8 feed bins, the time when useful CH4 concentration is recorded is certainly too short for including several eructation peaks. Fitting one gas analyzer per feed bin will combine advantages of the measurement time during visits of the GEM system with the visiting frequency allowed by the MH system.

8.2.6 MPR with PAC

The delay between the measurement and the last feeding has to be carefully monitored and taken into account when calculating animal emission values. As individual DMI is difficult to record, direct measurement of CH4 yield (MY=MPR/DMI) turns out to be impossible. Although not representative of a whole day production rate, that method can be used to characterize individual CH4 emission rates if standardized protocols are applied. It was first validated with 40 ewes measured 1 hour in PAC after three 22-hour measures in RC: a correlation of 0.71 was found between the two measures of CH4 production rate over 1 or 22 hours (Goopy *et al.*, 2011). The 1-hour CH4 production measure in PAC has a moderate repeatability of rep=0.50 [0.37 to 0.60] when taken few days to seven weeks apart (Robinson *et al.*, 2015; Goopy *et al.*, 2016). Heritability coefficient of this 1-hour CH4 production measure is estimated to h^2=0.12 in a population of 2,279 sheep (Robinson *et al.*, 2014b) with a repeatability coefficient rep=0.25.

Conclusions and recommendations

The authors recommend using the mean of 3 PAC measurements in order to get accurate phenotype estimates.

9 Proxies

9.1 Introduction

Large-scale measurements of enteric CH4 emissions from dairy cows are needed for effective monitoring of strategies to reduce the carbon footprint of milk production, as well as for incorporation of CH4 emissions into breeding programs. However, measurements on a sufficiently large scale are difficult and expensive. Proxies for CH4 emissions can provide an alternative, but each approach has limitations. Negussie *et al.* (2019) recently showed the

potential of proxies proxies that are easy to record in the farm. These proxies can be gathered in most farms and are a realistic threshold accuracy that can be obtained without more fancy proxies. Several techniques have been developed for the measurement of CH4 emissions from ruminants, with varying degrees of accuracy (see reviews by Cassandro *et al.*, 2013 and Hammond *et al.*, 2016A), but routine individual measurements on a large scale (a requisite for genetic selection) have proven to be difficult and expensive (Pickering *et al.*, 2015; Negussie *et al.*, 2016). Therefore, identifying proxies (i.e. indicators or indirect traits) that are correlated to CH4 emission, but which are easy and relatively low-cost to record on a large scale, is a much needed alternative. Proxies might be less accurate, but could be measured repeatedly to reduce random noise. The (potential) proxies range from simple and low-cost measurements such as body weight, to high-throughput milk MIR, to more demanding measures like rumen morphology, rumen metabolites or microbiome profiling.

Combining proxies that are easy to measure and cheap to record could provide predictions of CH4 emissions that are sufficiently accurate for selection and management of cows with low CH4 emissions.

9.2 Available Proxies

A large array of CH4 proxies differing widely in accuracy and applicability under different conditions have been reported. The ideal proxy would be highly phenotypically and genetically correlated with CH4 emissions and could easily, and potentially repeatedly, be measured on a large scale. A systematic summary and assessment of existing knowledge is needed for the identification of robust and accurate CH4 proxies for future use. Table 6 summarizes proxies for CH4 production, and Table 7 summarizes results from combining proxies to improve predictability of proxies for CH4 prediction.

Table 6. Available methane proxies include: (1) feed intake and feeding behaviour, (2) rumen function, metabolites and microbiome, (3) milk production and composition, (4) hind-gut and faeces, and (5) measurements at the level of the whole animal.

Proxy	Description / conclusion	Reference
(1) feed intake and feeding behavior		
Dry matter intake	DMI predict MeP with R^2= 0.06-0.64, and ME intake predict MeP with R^2= 0.53-0,55	Ellis *et al.* (2007); Mills *et al.* (2003); Negussie *et al.* (2019)
gross energy intake (GE)	predict MeP with RMSPE= 3.01.	Moreas *et al.* (2014)
Feeding behavior	magnitude and direction of relation to MeP varies across studies	Nkrumah *et al.* (2006); Jonker *et al.*, 2014
Rumination time	High rumination relates to more milk, consume more concentrate and produce more CH_4, lower RMP and MeI	Watt *et al.* (2015); López-Paredes *et al.* (2020)
Rumen microbiome	The metagenome can predict DMI, and classify high vs low intakes	Delgado *et al.* (2019)
(2) rumen function, metabolites and microbiome		

Proxy	Description / conclusion	Reference
Dietary anti-methanogenic compounds	Inhibitors of the enzyme methyl coenzyme-M reductase: bromochoromethane; chloroform; 3-nitrooxypropanol (not always)	Denman *et al.*, 2007; Knight *et al.*, 2011; Haisan *et al.*, 2014; Romero-Perez *et al.*, 2014, 2015
Dietary antimicrobial compounds	Induce reductions in both MeP and methanogens numbers: nitrates, anacardic acid (cashew nut shell liquid), monensin, isobutyrate	Iwamoto *et al.*, 2002; Kubo *et al.*, 1993; van Zijderveld *et al.*, 2010; Veneman *et al.*, 2015; Shinkai *et al.*, 2012; Wang *et al.*, 2015
Rumen microbiome profile	High Fibrobacteres, Quinella ovalis and Veillonellaceae and low Ruminococcaceae, Lachnospiraceae and Clostridiales associate with low-CH_4 phenotypes and high propionate Protozoa concentration	Kittelmann *et al.*, 2014; Wallace *et al.*, 2014; Sun *et al.*, 2015 Guyader *et al.*, 2014
	predict MeP with R^2 up to 0.55	Ross *et al.* 2013a; Ross *et al.* (2013b)
Microbial genes	20 (out of 3970 identified) related to CH_4 emissions	Roehe *et al.* (2016)
Rumen volume (X-ray Computed Tomography) and retention time	Low-MeY sheep had smaller rumens. Faster passage= less time to ferment substrate - explained 28% of variation in MeP	Pinares Patiño *et al.*, 2003; Goopy *et al.*, 2014; Okine *et al.* (1989)
blood triiodothyronine concentration	reduced MeY	Barnett *et al.* (2012)
Acetate to propionate ratio in ruminal fluid	positively associated with CH_4 emissions, but not confirmed in all studies, sometimes opposite relation	Mohammed *et al.*, 2011; Fievez *et al.*, 2012; Chung *et al.*, 2011; Van Zijderveld *et al.*, 2010
(3) milk Production and composition		
modelling approach	Doubling milk production only adds 5 kg to the MeP and so greatly reduces MeY	Kirchgessner *et al.* (1995); Hristov *et al.* (2014)
Milk fat content	key explanatory variable for predicting CH_4: A moderate negative genetic correlation with infrared predicted MeI: correlations MeP=0,08 and MeI=-0.13	Moreas *et al.* (2014); Kandel *et al.*, 2014A, B; Vanlierde *et al.* (2015)
	A positive relationship between VFA proportions and methanogenesis is expected as a consequence of the	Vlaeminck *et al.*, 2006; Van Lingen *et al.*, 2014

Proxy	Description / conclusion	Reference
	common biochemical pathways; Dietary unsaturated fatty acids are negatively associated with CH_4 emissions	
Milk protein yield	Correlation with MeI=-0.47 or -0.09, MeP=0.53	Kandel *et al.* (2014); Vanlierde *et al.* (2015)
Lactose	Variable correlations: MeP=0,33; MeI=-0.21; R = 0.19 for CH_4 emission	Miettinen and Huhtanen (1996); Dehareng *et al.* (2012)
Somatic cell score	Genetic correlation with infrared predicted MeI: R=0.07	Kandel *et al.* (2014A, B)
Prediction equations Milk FA and CH_4 emissions, including from MIR data	R^2 ranged between 47 and 95%; relationships between the individual milk FA and MeP differed considerably and the correlations between CH_4 and milk FA vary throughout the lactation	Chilliard *et al.* (2009); Delfosse *et al.* (2010); Castro-Montoya *et al.* (2011); Dijkstra *et al.* (2011); Kandel *et al.* (2013) Mohammed *et al.* (2011); Van Lingen *et al.* (2014); Williams *et al.* (2014); Dijkstra *et al.* (2016); Rico *et al.* (2016); Van Gastelen and Dijkstra (2016); Vanrobays *et al.* (2016); Bougoin *et al.*, (2019)
(4) hind-gut and feces		
Whole tract digestibility (potential as supporting factors in the prediction of enteric CH_4 emissions)	Main effects relate to rumen (see above), but energy digestibility as a supporting factor to GE intake improved the accuracy of CH_4 prediction, despite the fact that there was no direct linear relationship between energy digestibility and MeY and in % of GE intake	Yan *et al.*, 2009c
Ratio of acetic and butyric acid divided by propionic acid	Methane yield positive relation	Moss *et al.*, 2000
(5) Whole animal measurements		
Body weight and conformation	prediction models; primary predictor for enteric MeP	Moraes *et al.* (2014); Holter and Young, 1992; Yan *et al.*, 2009

Proxy	Description / conclusion	Reference
Body weight	Relationship with MeI: r = 0.44; relationship between body weight and rumen capacity	Antunes-Fernandes *et al.* (2016); Demment and Van Soest, 1985
Body weight	Key explanatory variable for enteric MeP	No reference available
Conformation traits: affects enteric MeP	indicators for rumen volume (via feed intake and rumen passage rates); BCS	Agnew and Yan, 2000
Lactation stage	Complementary proxy	Vanlierde *et al.* (2015)

It is evident that no single proxy offers a good solution in terms of all of these attributes, though the low cost and high throughput make milk MIR a good candidate for further work on refining methods, improving calibrations and exploring combinations with other proxies.

9.3 Combining proxies for methane

Although milk MIR shows promise as a single proxy for CH4 emissions, there may be advantages in using two or more proxies in combination. There are two potential reasons why a combination of proxies might be appropriate: (i) proxies may describe independent sources of variation in CH4 emissions, and (ii) one proxy allows correction for shortcomings in the way the other proxy describes CH4 emissions (e.g. taking into account lactation stage if CH4 emissions prediction coefficients change during the lactation). See also Negussie *et al.* (2019).

Table 7. Combinations of proxies for methane.

Proxy combinations	**Results**	**References**
Rumen microbiome + VFA	Combination of rumen VFA proportions and pH + modelling may be more informative	Brask *et al.* 2015
Methanogen abundance in rumen fluid + proxy, a chemical marker for methanogens (archaeol)		McCartney *et al.* (2013)
Fecal ether lipids (ratio of diether to tetraether lipids) + rumen pH	Combining measurements of rumen VFA, pH and the microbiome should be more informative for predicting CH_4 emissions	McCartney *et al.* (2014); Ann *et al.*, 1996
Feed intake (determined by body weight, production level, growth rate and feed quality)	Main driver for CH_4 emissions; should be all included in models for CH_4	Moraes *et al.* 2014,
DMI and diet composition	Combine database to predict CH_4	Niu *et al.*, 2018; Van Lingen *et al.*, 2019
Range of prediction equations for CH_4 production	Feed intake = primary predictor of total CH_4 production (accounted for 52 to 64%); Combining more factors did indeed improve the prediction equation by 15 to 35%	Ramin and Huhtanen (2013); Knapp *et al.*, 2015; Sauvant and Nozière (2016)
Rumen measurements (VFA, pH, protozoa counts) + feed intake (total DMI, forage DMI and FA intake) + production parameters (milk yield and composition) + milk FA	Suggest that milk FA predict CH_4 emission better (R^2= 0.74) compared to rumen variables, feed intake and production parameters (R^2 < 0.58). Total combination: R^2= 0.90	Mohammed *et al.* (2011)
Modelling	specific prediction equations may need to be developed, or diet composition may need to be included in the prediction equations	Mohammed *et al.* (2011)
Feed intake + diet composition + milk production + milk FA	CH_4 prediction equations: best fit = combining milk FA, feed intake, diet composition, and milk production (R^2 = 0.84)	Rico *et al.* (2016); Bougoin *et al.* (2019)
MIR + lactation stage	MIR spectroscopy (coefficient of determination = 0.68 and	Dehareng *et al.* (2012); Vanlierde *et al.* (2015)

Proxy combinations	Results	References
	0.79), predictions at different stages of lactation were not biologically meaningful + lactation stage refined the model: showing a biologically meaningful behavior throughout lactation: an increase in CH_4 production after calving up to approximately 100 DIM, followed by gradual decline towards the end of lactation	
Milk yield, fat percentage + type traits	Combine database to predict CH_4 using official milk recording system and type evaluation.	Cassandro *et al.*, (2010; Cassandro, 2013)

9.4 Building an index for methane

For some of the proxies, the heritability and correlations with CH4 output are known: e.g. Vanrobays *et al.* (2016) estimated heritability of 0.25 for CH4 production (g/d) and in the range 0.17 - 0.42 for different classes of milk FA; phenotypic and genetic correlations between MeP and milk FA varied between -0.03 and 0.16, and between -0.02 and 0.32 (C18:0), respectively. The genetic correlation between MeI and milk yield was estimated by Dehareng *et al.* (2012) at -0.45; that between milk yield and protein percentage at -0.54 (Miglior *et al.* 2007). This would give a genetic correlation between MeI and protein percentage in the range [-0.5, 0.9], with likelier values for positive correlations. The most probable value in the given range could then be estimated (from the prior distribution of the missing correlation and the joint likelihood of the two known correlations given the values in the range). Such data could in the future be used to develop an index for breeding on CH4 emission.

10 Proxies discussion

The greatest limitation of proxies today is the lack of robustness in their general applicability. Future efforts should therefore be directed towards developing combinations of proxies that are robust and applicable across diverse production systems and environments. Here we present the present status of the knowledge of proxies and their predictive value for CH4 emission. Proxies related to body weight or milk yield and composition are relatively simple, low-cost, high throughput, and are easy to implement in practice. In particular, DMI and milk MIR, along with covariates such as lactation stage, are a promising option for prediction of CH4 emission in dairy cows. No single proxy was found to accurately predict CH4, whilst combinations of two or more proxies are likely to be a better solution. Combining proxies can increase the accuracy of predictions by up to 15 - 35%, mainly because different proxies describe independent sources of variation in CH4 and one proxy can correct for shortcomings in the other(s). One plausible strategy could be to increase animal productive efficiency whilst reducing CH4 emissions per animal. This could be achieved by reducing

MeY and/or decreasing DMI provided that there is no concomitant reduction in productivity or increase in feed consumption (Pickering *et al.*, 2015).

10.1 Combining diet-based measurements with other proxies for methane emissions.

Feed intake appears a reasonably adequate predictor of MeP: generally, heavier animals have higher maintenance requirements, so eat more and produce more CH4. However, a substantial level of variation is left unaccounted for. This suggests that more detailed information on dietary composition is needed. This is also important when one wants to account for MeP on diets of similar DMI but of different nutrient profiles.

The prediction accuracy of MeP strongly depends on the accuracy of quantifying the VFA produced in the rumen (Alemu *et al.*, 2011). The type of VFA formed during rumen fermentation depends on the type of substrate fermented (Bannink *et al.*, 2011), such as the dietary content of neutral detergent fiber and starch. The type of substrate fermented thus appears a useful factor for predicting MeP (Ellis *et al.*, 2007), indicating that including a description of variation in dietary quality caused by nutritional factors results in improved prediction accuracy of CH4 emission (Ellis *et al.*, 2010; Moraes *et al.*, 2014).

10.2 Rumen

When feed intake is kept constant, a higher rumen capacity results in a lower passage rate (Demment and Van Soest, 1985), resulting in a higher MeP (Moraes *et al.*, 2014). Proxies based on rumen samples are generally poor to moderately accurate predictors of CH4, and are costly and difficult to measure routinely on-farm. VFA are a proxy for rumen CH4 emissions. Using rumen fermentation data obtained from in vitro gas production, Moss *et al.* (2000) reported a negative linear relationship between CH4 production and the ratio of (acetic + butyric acid)/propionic acid. However, by combining different information sources, either related to feed intake or to the impact of feed intake on the VFA composition, a better proxy with an improved accuracy can be achieved. This way, the prediction equation for CH4 production can be optimized (higher accuracy).

The relationship between rumen methanogen abundance and methanogenesis is less clear when changes in enteric CH4 emissions are modulated by diet or are a consequence of selecting phenotypes related to feed efficiency or MeY. Whereas in some reports there was a significant positive relationship (Aguinaga Casanas *et al.*, 2015; Arndt *et al.*, 2015; Sun *et al.*, 2015; Wallace *et al.*, 2015), in many others the concentration of methanogens was unrelated to methanogenesis (Morgavi *et al.*, 2012; Kittelmann *et al.*, 2014; Shi *et al.*, 2014; Bouchard *et al.*, 2015). Bouchard *et al.* (2015) even reported a reduction in methanogens without significant decrease in MeP for steers fed sainfoin silage. Sheep selected for high or low MeY showed no differences in methanogen abundance, though there was a strong correlation with expression of archaeal genes involved in methanogenesis (Shi *et al.*, 2014).

Hindgut and Feces: whole tract digestibility variables cannot serve as primary predictors for enteric MeP in cattle or sheep, but might be used as supporting factors to improve the accuracy of prediction of CH4 output.

10.3 Protozoa and other rumen microbes

Protozoa are net producers of H2 and their absence from the rumen is associated with an average reduction in enteric MeP of approximately 11% (Hegarty, 1999; Morgavi *et al.*, 2010; Newbold *et al.*, 2015). Using a database of 28 experiments and 91 dietary treatments, Guyader *et al.* (2014) showed a significant decrease of 8.14 g CH4/kg DMI for each log unit reduction in rumen protozoal abundance. About 21% of experiments within this dataset

reported CH4 changes unrelated to protozoal abundance, highlighting the multifactorial nature of methanogenesis.

Roehe *et al.* (2016) observed that the ranking of sire groups for CH4 emissions measured with respiration chambers was the same as that for ranking on archaea/bacteria ratio, providing further evidence that host control of archaeal abundance contributes to genetic variation in CH4 emissions - at least in some circumstances. Across a wide geographical range, the methanogenic archaea were shown to be highly conserved across the world (Henderson *et al.*, 2015). This universality and limited diversity could make it possible to mitigate CH4 emissions by developing strategies that target the few dominant methanogens. However, one clear limitation of metagenomic predictions compared to genomic predictions was that the microbiome of the host is variable - that is, it may change in response to diet or other environmental factors over time, whereas the hosts DNA remains constant.

10.4 Rumen microbial genes

These included genes involved in the first and last steps of methanogenesis: formylmethanofuran dehydrogenase subunit B (fmdB) and methyl-coenzyme M reductase alpha subunit (mcrA), which were 170 times more abundant in high CH4 emitting cattle. Whilst gene-centric metagenomics is not low-cost or high-throughput, these results point to potential future proxy approaches using low-cost gene chips.

The difference in gene expression activity as opposed to abundance was also reported by others (Popova *et al.*, 2011). However, there are also studies in which there was no relationship with gene expression (Aguinaga Casanas *et al.*, 2015). There are some methodological and experimental differences that might explain some of the apparent contradictions, such as the type of gene target and primers used for nucleic acid amplification. Effects are seen most clearly when the difference in MeP between groups of animals is large (e.g. Wallace *et al.* (2015) used treatments that generated a 1.9-fold difference CH4 emissions).

10.5 Proxies based on measurements in milk

Milk yield alone does not provide a good prediction of MeP by dairy cows. Yan *et al.* (2010) indicated that CH4 as a proportion of GE intake or milk energy output was negatively related to milk production. It is less clear if MeY can be predicted from milk yield when making comparisons across studies.

Milk MIR spectroscopy is relatively inexpensive, rapid and already routinely used technology in milk recording systems to predict fat, protein, lactose and urea contents in dairy milk to assist farm management decisions and breeding. It can be used as a promising strategy to exploit the link between enteric CH4 emission from ruminants and microbial digestion in the rumen by assessing the signature of digestion in milk composition. Milk MIR data can be obtained through regular milk recording schemes, as well as, on a herd level, through analysis used for milk payment systems. Diverse milk phenotypes can be obtained by MIR spectrometry – including detailed milk composition (e.g. FA as reported by Soyeurt *et al.*, 2011), technological properties of milk, and cow physiological status (De Marchi *et al.*, 2014; Gengler *et al.*, 2016). Several of these novel traits (i.e. FA composition) have been identified as potential indicators of CH4 emission. Therefore, using MIR to predict MeP (Dehareng *et al.* 2012; Vanlierde *et al.* 2013, 2015; Van Gastelen and Dijkstra, 2016) is also a logical extension of its use to quantify the major milk components (i.e. fat, protein, casein, lactose, and urea) and minor components (e.g. FA). Dehareng *et al.* (2012) assessed the feasibility to predict individual MeP from dairy cows using milk MIR spectra. Their initial results suggest

that this approach could be useful to predict MeP at the farm or regional scale, as well as to identify low-CH4 emitting cows. According to Van Gastelen and Dijkstra (2016), MIR spectroscopy has the disadvantage that it has a moderate predictive power for CH4 emission, both direct and indirect (i.e. via milk FA), and that it lacks the ability to predict important milk FA for CH4 prediction. They concluded that it may not be sufficient to predict MeP based on MIR alone. It is, however, possible to improve the accuracy of prediction through the combination of MIR with some animal characteristics such as lactation stage (Vanlierde *et al.*, 2015). The advantage of this latter development is that this type of prediction can be done on a very large scale inside a routine milk recording system (Vanlierde *et al.*, 2015).

10.6 Proxies: future developments and perspectives

There is currently limited consensus on which phenotype to use to lower the carbon footprint of milk production through genetic selection. This could be MeP, MeI or MeY. The direct goal would be CH4 production; the relationship with milk production and/or feed intake could be accounted for by including these in the final selection index or scheme. However, one might argue that it would be more effective/accurate to directly use milk production- or feed intake-corrected CH4 (e.g. CH4 intensity or yield) as breeding goal.

The analysis of proxies in terms of their attributes shows that proxies that are based on samples from the rumen or related to rumen sources are poor to moderately accurate predictors of CH4. In addition, these proxies are too costly and difficult for routine on-farm implementation. On the other hand, proxies related to BW, milk yield and composition (e.g. milk FA) are moderately to highly accurate predictors of CH4 and relatively simple, low-cost and easier to implement in practice (Cassandro *et al.*,2010; Cassandro, 2013). Particularly, milk MIR and the prediction of CH4 based on milk MIR along with other covariates such as lactation stage is a promising alternative: that is accurate, cheaper and easy to be implemented in routine milk analysis at no extra cost.

Therefore, in the future advances in infrared, photoacoustic and related technologies will push the boundaries, particularly in focusing on developments of fast and portable technologies. Such developments will lead to better and promising proxies for CH4 that will enable a sizable throughput of CH4 phenotypes in dairy cows.

Antunes-Fernandes *et al.* (2016) already presented the use of metabolomics on milk to better understand the biological pathways involved in CH4 production in dairy cattle. The techniques used in that study are not suitable for large scale measurements, but rapid developments in omics may offer tests and assay methodologies on blood, urine or milk samples that will provide an additional tool for developing new / additional proxies for CH4 emissions in dairy cattle.

11 Conclusions

Measuring CH4 emission on large numbers of cows is a challenge. The high costs and low throughput of RC restrict their use to research studies measuring CH4 emissions on small numbers of individual animals. Respiration chambers remain the gold standard method, but benchmarking alternative methods against RC is challenging because simultaneous replicate measures per cow are not feasible. Methods like SF6 and GreenFeed require lower capital investment and running costs than RC, and have higher throughput and potential for use in extensive and grazing situations, but costs are still prohibitive for recording large numbers of animals. Methods based on concentration are less precise and accurate than flux methods, but they are viable for large scale measurement, which is a prerequisite of genetic

evaluations. Further development is needed to increase accuracy and precision of concentration methods. Several reviews of methods for measuring CH4 have made qualitative judgements based on individual comparison studies without expanding scope to genetic evaluations and considering repeated measure correlations between methods as proxies for genetic correlations. Results confirm that there is sufficient correlation between methods for all to be combined for international genetic studies and provide a much needed framework for comparing genetic correlations between methods should these be made available. Proxies have the potential to be used as predictors of CH4 production and emission. Although proxies are less accurate than direct CH4 measurements they can be easier, cheaper, and at high throughput, and may be therefore the best method in practical situations, especially proxies related to milk measurements. Therefore, these proxies at the population level, can provide useful information at genetic improvement that can be used to reduce emissions following 3 ways: (1) intensification of animal production; (2) improving of system efficiency and (3) the direct reduction of GHG emissions by breeding for reduced predicting animals that are high or low GHG emitters.

12 Merging and sharing data in genetic evaluations

Genetic parameters for CH4 using a multi-country dataset

Early 2016 an attempt to make cross country evaluations of CH4 emissions from Holstein dairy cattle was initiated. The work was based on data from NL, DK, AUS, UK and IR. In total, 12,820 weekly CH4 emission records from 2,857 cows were available. Although different equipment was used across countries to measure CH4 emissions, the research aimed to define similar CH4 output phenotypes in each country. The analysed CH4 traits, that are available in each country, are (1) CH4 production in g/d, and (2) CH4 intensity in g/d per kg fat protein corrected milk (FPCM). In addition to these CH4 traits, CH4 concentration (in ppm) was available in Denmark, the Netherlands and UK, and the ratio between CH4 and CO2 concentration was available in Denmark and the Netherlands.

Bivariate analyses were carried out to estimate genetic correlations between countries, using an animal linear mixed model for all traits. Both univariate and bivariate analyses were repeated with the GRM as well. With all weekly records, standardizing the trait in the full dataset increased the heritability for CH4 production from 0.03 to 0.06. The heritability for CH4 intensity was slightly higher. The highest heritability with the full dataset is estimated for the standardized CH4 concentration (0.19). Correlations estimated among CH4 traits estimated with either the pedigree or the GRM were in same direction and of similar magnitude. The genetic correlations show that when CH4 production increased, the CH4 concentration and the ratio between CH4 and CO2 increased as well.

The approach is novel, and no other attempt has been performed to make genetic analysis of CH4 traits across countries. The analysis can be repeated in future studies where more data hopefully will be available, and more effort can be made into improving both the fixed and random part of the model.

13 Recommendations

The most important question: what method to use if you need to measure CH4? The answer may be: it depends on what you like to do. In the Table 8 we summarize some experimental conditions and designs, and make recommendations.

Table 8. Recommendations for measuring methane in diverse experimental conditions and designs.

Experimental condition and design	**Methane mesurement method recommendation**
Need to measure absolute methane values – animal numbers and location not important	Respiration chamber; SF6; GreenFeed
Need to rank animals from low to high methane emission	Sniffer method
Need to measure methane on farm	Sniffer method; GreenFeed; PAC
Low budget measurements needed	Proxy / Proxies measurement
High animal numbers required	Sniffer method; Proxies measurement; LMD

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