

Breeding ruminants that emit less methane – development of consensus methods for measurement of methane

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A report from the Methane Phenotyping Working Group (MPWG) of the Animal Selection Genetics and Genomics Network of the Livestock Research Group of the Global Research Alliance for reducing greenhouse gases from agriculture.

Executive Summary

This report was prepared by a working group of the Animal Selection, Genetics and Genomics Network (ASGGN) of the Global Research Alliance for reducing greenhouse gases from agriculture.

It is a summary of published and yet to be published work on determining an appropriate measurement protocol for measurement of methane (CH₄) emissions from individual animals for the purpose of determining genetic options for breeding livestock that emit less CH₄. Its particular focus is to outline what is known about the factors that affect CH₄ production and its measurement in ruminants. Its purpose is to provide the background information required to evaluate methods that are potentially useful for measuring CH₄ emissions in individual animals to initially obtain genetic parameters and to subsequently screen animals for use in selective breeding programs.

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26 This report shows:

- 27 • Methane emissions are a heritable and repeatable trait at least in sheep.
- 28 • Repeated measurements of CH₄ emissions on individual animals add most
29 value when separated by at least 3-14 days.
- 30 • Methane emissions are strongly related to feed intake especially in the short
31 term (up to several hours) and less so in the longer term (days).
- 32 • When measured over days, in respiration chambers, CH₄ yield (MY:
33 gCH₄/kgDMI) i.e. CH₄ corrected (adjusted) for feed intake is a heritable and
34 repeatable trait albeit with less genetic variation than total CH₄ emission
35 (gCH₄/d). In sheep, heritability of MY is 0.13 and total CH₄ emissions 0.29
36 (Pinares-Patino et al, 2013)
- 37 • Methane emissions of individual animals are moderately repeatable across
38 diets and across feeding levels when measured in respiration chambers.
39 Repeatability estimates are lower when short term measurements are used,
40 possibly due to variation in time and amount of ingested feed prior to the
41 measurement. This needs to be investigated further.
- 42 • Given the above issues are resolved, short term (over minutes to hours)
43 measurements of CH₄ emissions show promise. However, we believe that for
44 short term measurements to be useful, for genetic evaluation, a number of (at
45 least 3) measurements will be required over an extended period of time
46 (weeks to months).
- 47 • Opportunities exist for “brief measurements” in standardised feeding
48 situations such as “sniffers” attached to milking parlours or total mixed ration

feeding bins, but we anticipate these are also subject to the caveats above about use of short term measurements.

- The measurement “protocol” (i.e. how the animal and its feeding behaviour are managed prior to measurement) is likely to be more important than the technology used to make the CH₄ measurement.
- While there is evidence that correlated and predictor traits exist for CH₄ emissions the current level of knowledge is insufficient to recommend the use of predictor traits in genetic selection to reduce MY.
- Genomic selection offers potential for use to reduce CH₄ emissions and MY, however, CH₄ measurements on thousands of individuals will still be required.
- In summary, we feel genetic and genomic selection offers a significant opportunity, but attention needs to be directed to a number of issues, if brief low cost measurements are to be implemented in industry.

As yet we have insufficient knowledge of the phenotypic and genetic correlations between CH₄ measurements made under different protocols (or methodologies) to be confident about how we combine such data. This will, at least in the short term, lead to different estimates of genetic parameters for CH₄ emission traits from different laboratories due to the measurement protocol/methodology employed. This is to be expected, because the cost of measurement of a trait will clearly affect the number of animals able to be measured, and low cost, accurate measurement procedures/protocols/methods will be sought. Different measurement protocols/methodologies may not impede genetic progress with selection for CH₄ traits in national or commercial programs (e.g. a breeding company). However, use

of different measurement protocols in different countries or species will almost certainly make pooling of data less efficient, and increase costs globally. The pooling of data would be especially beneficial to enable genomic selection for this trait. An additional consideration relates to how the IPCC process for accounting for genetic change in enteric emissions is implemented. The IPCC process utilizes peer reviewed publications to change to its accounting rules. We, the ASGGN, can help by providing leadership as to how best to include inherited differences in either feed intake or MY into the accounting framework for enteric emissions.

We recommend the following research be undertaken under the auspices of the ASGGN:-

- Wherever possible, measurement protocols used to obtain genetic parameters are compared with a standardised protocol. Ideally this should be to a level where heritabilities, repeatabilities and genetic correlations with key traits e.g. live weight and intake can be estimated from both techniques. At the minimum, a comparison of measurement repeatability across time, both within and between measurement protocols is essential. The assumption in this case is they both measure the same underlying trait just with different inherent error.
- Establish a process to enable at least meta-data of different measurement protocols to be shared across research groups in different countries. This could be extended across species.
- We encourage development of an international R & D project to analyse joint data sets and make recommendations that lead to improved lower cost protocols for measurement of methane emissions that can be employed in member countries. This would prepare the community for

development of preliminary genetic parameters and genomic estimated breeding values (GEBVs) to act as a catalyst for local/national development of breeding solutions for reduced emissions of methane from farmed ruminants.

- Continue to explore methods that use proxies of feed intake measured over the same time frame as CH₄, for example CO₂ output and O₂ uptake, to estimate MY. Establish relationships between proxy measures of MY and reference methods and the total CH₄ production/time measured on animals on pasture.

Introduction

Climate change is of growing international concern and it is well established that the release of greenhouse gases (GHG) are a contributing factor. Overall livestock activities, of which the largest single contribution is methane (CH₄) emissions by ruminants, contributes approximately 18% of total anthropogenic GHG emissions through the commodity chain (Steinfeld et al., 2006). Of the various GHG CH₄ is the most important contributor, with a global warming potential 25 times that of carbon dioxide (CO₂). Ruminant livestock occupy a significant niche in human activity through production of food, fibre and work. They normally consume fibrous low-quality diets (Hofmann, 1989), which are abundant and unable to be readily digested by man. Through domestication of ruminants man has increased his capacity to generate human food from the surrounding environment.

Globally GHG emissions from the agriculture sector accounted for 4.6 GtCO₂-eq/yr in 2010, of which enteric fermentation (emissions of CH₄ by ruminant animals)

contributed 2 GtCO₂ eq/yr (Tubiello et al., 2013), with an annual increase of 0.95% (1961 - 2010). Non-dairy cattle (beef and draft) were the single largest source of enteric CH₄, followed by dairy cattle, buffaloes, sheep and goats (Figure 1). Averaged over 2000 – 2010, the largest regional contributors to global enteric CH₄ production were Asia and the Americas (Figure 2). There was growth in annual enteric emissions in all regions except Europe and Oceania (FAOSTAT, 2013). After enteric emissions of CH₄, the next greatest contributor to agricultural emissions was deposition of manure onto pasture. Nitrous oxide emissions contributed 10% of total agricultural emissions, resulting from organic soils, crop residues and manure applied to soil. However, the effective area of land usable for domestic ruminant grazing is more than 30%, with 25% of the global land area as permanent pastures (Meadows & pasture, Figure 3; FAOSTAT, 2009).

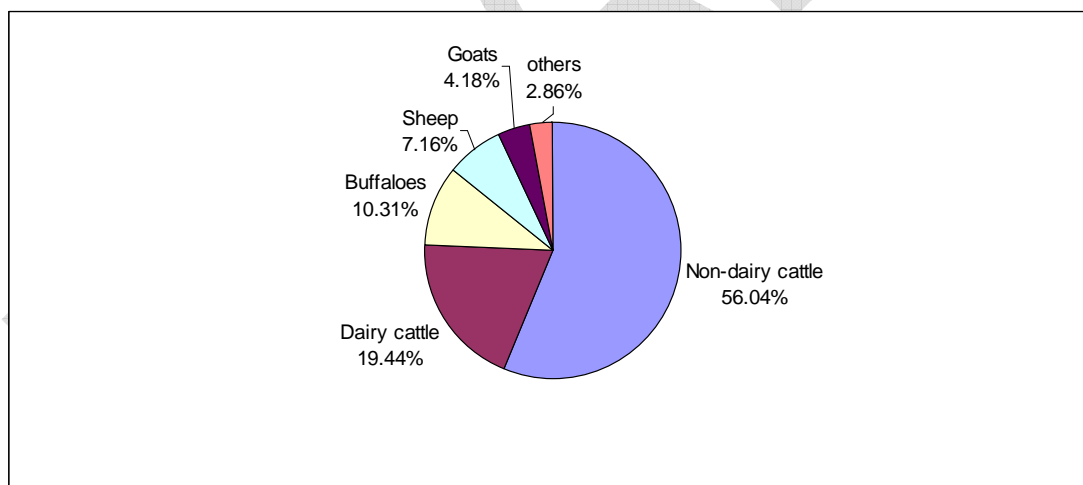


Figure 1. Contribution of different animal types and species to global livestock enteric methane production (source FAOSTAT, 2013).

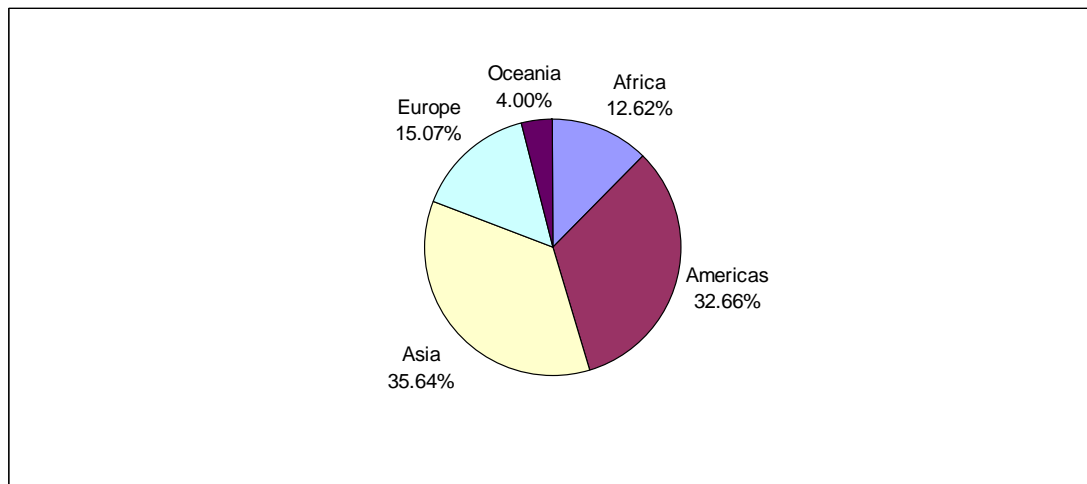


Figure 2. Contribution of region to global enteric methane production (source FAOSTAT, 2013).

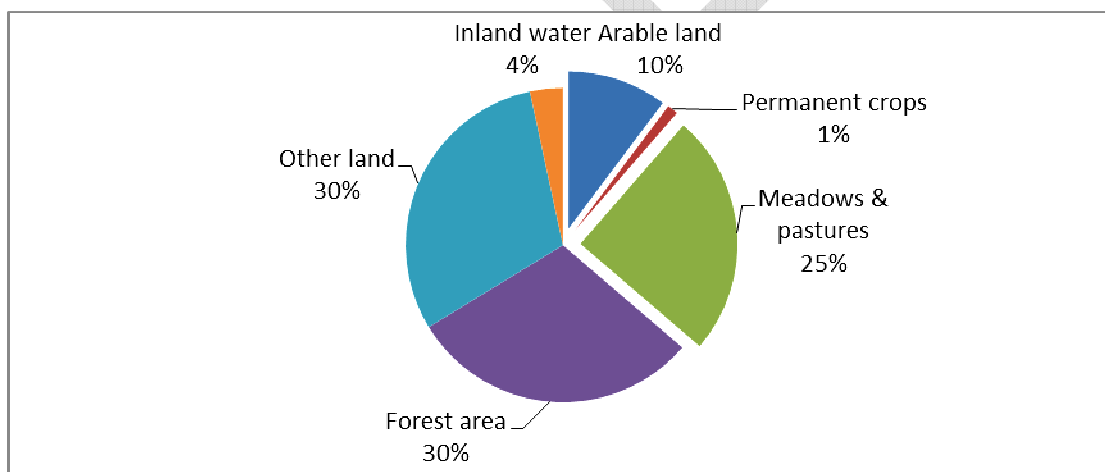


Figure 3. Global land use (source FAOSTAT, 2009).

The range of options to reduce enteric CH₄ emissions include: changing feed type (for example from pasture to concentrate feed, or to new pasture varieties), use of supplements that reduce CH₄ emissions (fats, oils, plant extracts and nitrate), immunisation against methanogens (Williams et al, 2009) and selective breeding of animals with low methane emissions, without compromising production characteristics (Eckard et al., 2010; Martin et al., 2010; Wall et al., 2010; Cottle et al., 2011).

Selective breeding for reduced emissions, with no loss of productivity, is a mitigation strategy which could deliver a permanent reduction in CH₄ emissions provided selection pressure is maintained. The technologies for implementation of selective breeding programs are well established and provide a low cost option for control. Nonetheless, within animal production, there is currently little or no concerted research effort on long-term breeding strategies to mitigate GHG in ruminants. Unlike many production traits, where the traits may be measured as part of the day to day management processes (e.g., weight, milk production, number of offspring and carcase quality), CH₄ emissions are not routinely measured in livestock.

To implement a breeding program requires the trait be measured. Alternatively, if there are strong genetic correlations between heritable indicator traits that can be readily measured in the industry and a CH₄ emissions trait, then that correlated trait may be used for indirect selection. However, in the first instance, the CH₄ trait itself must be measured on enough animals to confidently establish the genetic correlations with indicator trait(s). At present we are not confident that breeding low CH₄ emitting livestock is a practical option. However, studies are now underway to determine if it is possible to breed low CH₄ emitting livestock (e.g. Pinares-Patiño et al., 2013a).

If breeding for methane reduction is to be successful in industry a number of criteria must be met. Firstly, the trait should define and demonstrate, at least in simulation modelling, that it can achieve the intended outcomes if implemented by industry. Then the trait must be shown to be heritable, and readily measured in at least research situations. During early stages of development of a new trait such as methane emissions, these steps may well be repeated as new knowledge becomes available. Once established that a new trait such as methane emissions is feasible, it

may be implemented by direct selection using CH₄ measurements, or it may be possible to use correlated traits, or incorporate genomic information to estimate breeding values for methane emissions into breeding schemes (Meuwissen et al., 2013). For the latter to be implemented, a reference population of several thousand genotyped industry relevant animals, with the CH₄ phenotype measured, is required to provide initial estimates of the contribution of each genomic region to the expression of the phenotype under investigation (Calus et al., 2013). Similarly, genomic information could be used to increase the rate of progress for reduction in methane emissions through selection on GEBV for correlated indicator traits, if the CH₄ trait is impractical to measure on enough animals to establish a reference population. Secondly, to implement a CH₄ 'trait' into an existing production selection index, there is a need to identify and quantify any associations between CH₄ emissions and production traits. The expected genetic progress in reducing emissions while, at the same time, maintaining or improving other desirable traits can then be calculated. Finally, there must be an economic (and/or social) incentive to breed animals with the trait which is incorporated in the selection objective, so the CH₄ trait receives the appropriate weighting in any breeding program.

In the case of CH₄, there are a number of other considerations in defining a trait for genetic selection. The research question is "where should investment be made to further increase rate of genetic improvement for low CH₄ emissions, without reducing productivity?" It is known that there is already on-going improvement in intensity i.e. yield of CH₄ emissions per unit product arising from genetic selection for current production traits (Wall et al., 2010, Hayes et al, 2013). One could argue that further research investment into this area is not necessary. However, selection solely on productivity traits such as live weight gain and/or milk production, will increase feed

intake and CH₄ emissions per animal, and hence total CH₄ emissions unless a constraint is imposed on total emissions. In some markets, particularly for dairy products, there is a market constraint on total production which has resulted in an increase in productivity per cow and a decrease in number of animals. This may suit some industries but poses the question “is it possible to increase productivity and reduce CH₄ emissions per animal at the same time?” This could be achieved by reducing the yield of CH₄ per feed ingested (Methane Yield; MY), provided that there is no concomitant reduction in productivity. This provides options to either reduce emissions while holding net enterprise feed consumption constant, or alternatively, allowing intake to increase supporting a production boost per animal without raising total emissions. It is not yet clear if MY (defined as CH₄ production per unit feed eaten) is under genetic control, although early results from a number of studies around the world, suggest it is both a heritable and repeatable trait (e.g. Hegarty and McEwan, 2010; Pinares-Patiño et al., 2013a). However, the means by which the host influences fermentation in the gut to affect CH₄ production is largely unknown. The extent to which genetic selection can be used to reduce MY is also not known. It is possible to make genetic progress without detailed knowledge of the biological mechanism. Because CH₄ emissions are derived from the internal milieu of the rumen, and we currently know little of the means by which the host controls rumen function, nor are we likely in the short term to gather enough data to reliably estimate genetic correlations with production traits, we believe it would be prudent to obtain some information of the associated phenotypic changes in rumen function before large scale industry implementation.

The methods by which CH₄ emissions of individual animals can be measured are an important factor because the method used to measure the CH₄ trait will also

influence the resulting genetic parameters and is therefore an integral part of the selection program.

We anticipate that the CH₄ emission trait will be implemented as part of a selection index. Current selection indices use production traits and returns and allocate costs associated with production (costs are principally related to expected feed intake). These are weighted in proportion to the genetic contribution the trait makes to the economic breeding objective based on costs and returns from historic production system data. We anticipate implementation of a CH₄ emissions trait will need to account for an anticipated future carbon price. Given the lags and delays implicit in genetic improvement this should probably be the best estimate of the carbon price 20 years hence (to account for a number of intangibles: likely time of implementation of breeding solutions to reduce methane emissions from livestock, development of mature carbon markets and extension of current emissions trading schemes to include agriculture). There may also be a social cost placed on CH₄ emissions which operates over and above a rational market framework. In that context, finding breeding solutions for reducing livestock CH₄ is akin to establishing a method to ensure future freedom to operate for the ruminant livestock industries. The impact, pace and extent of these factors are unknown, but nonetheless important considerations.

In this manuscript we outline what is known about the animal factors which potentially affect production of CH₄ in individual livestock, ***with the explicit objective of informing methods that can be used to derive genetic parameters to underpin a process to selectively breed livestock for lower CH₄ emissions.*** The expectation is that genetic selection is possible and will require robust, low cost,

emission measurement methodologies to identify suitable candidates for breeding and that it will be integrated with genomic selection.

Evidence of genetic control of emissions

To justify investment of effort and money in developing protocols for measurement of emissions to support genetic improvement in a CH₄ trait, it is worth summarising evidence supportive of this breeding strategy. Genetic diversity in a range of digestive parameters likely to be associated with enteric CH₄ production was apparent when reviewed in 2002 (Hegarty, 2002). The prospect for selection for a CH₄ trait was initially investigated by multiple groups; some identified variation in CH₄ traits amenable to animal selection (Robinson et al., 2010) and some did not (Münger and Kreuzer, 2008). More recent research in beef (Arthur et al., 2012) and sheep (Pinares-Patiño et al., 2011a; 2013a) is increasingly supportive of CH₄ traits being heritable with improvement by direct selection achievable. Arguably the strongest data set is that from New Zealand sheep studies summarised in Table 1 (Pinares-Patiño et al., 2013a).

Based on records of 1,277 pedigreed sheep, estimated heritability and repeatability of CH₄ across days, rounds and years, using the total 24hr measurement are shown in Table 1. There are high repeatabilities across consecutive days. Across rounds and across years the repeatability estimates are lower, but, relatively stable. Estimation of genetic and phenotypic correlations with some of the main New Zealand production traits; weaning weight (WWT), live weight at 8 months (LW8), fleece weight at 12 months (FW12), eye muscle depth (EMD) and dag score (accumulation of faeces on the perineum region) at 3 or 8 months

(DAG3, DAG8) are shown in Table 2. Correlations with MY (gCH₄/kg dry matter intake (DMI)) are low or close to zero, only exception is FW12. The negative genetic and phenotypic correlations of FW12 with MY (-0.32 ± 0.11 and -0.08 ± 0.03 , respectively) imply that selecting for increased hogget fleece weight would in part result in lower CH₄ emissions expressed as gCH₄/kg DMI.

Table 1: Heritability (h^2), repeatability estimates (\pm standard errors; s.e.) for methane traits and live weight (LW) at measurement (Pinares-Patiño et al., 2013).

Trait	n records	mean	σ_p	$h^2 \pm \text{s.e.}$	Repeatability		
					consecutive days	across rounds	across years
gCH ₄ /day	5236	24.6	3.18	0.29 ± 0.05	0.94 ± 0.003	0.55 ± 0.02	0.53 ± 0.02
gCH ₄ /kgDMI	5235	15.7	1.62	0.13 ± 0.03	0.89 ± 0.005	0.26 ± 0.02	0.24 ± 0.02
LW (kg)	4869	48.5	5.12	0.46 ± 0.07	0.93 ± 0.004	0.88 ± 0.01	0.80 ± 0.01

CH₄: methane; DMI: dry matter intake

Table 2: Estimates of SIL production trait heritabilities (h^2) (\pm standard errors; s.e.) and genetic (r_g) and phenotypic (r_p) correlations with methane traits. (Pinares-Patiño et al., 2013)

Trait	single trait analysis					2-trait with gCH ₄ /day		2-trait with gCH ₄ /kgDMI	
	n records	mean	σ_p	direct $h^2 \pm \text{s.e.}$	dam $h^2 \pm \text{s.e.}$	r_g	r_p	r_g	r_p
WWT (kg)	48591	27	4.11	0.23 ± 0.01	0.23 ± 0.01	0.88 ± 0.04	0.31 ± 0.02	0.06 ± 0.12	0.01 ± 0.02
LW8 (kg)	34742	40	4.95	0.56 ± 0.01	-	0.89 ± 0.03	0.50 ± 0.02	0.10 ± 0.09	0.03 ± 0.03
FW12 (kg)	15186	3.1	0.48	0.53 ± 0.02	-	0.23 ± 0.07	0.09 ± 0.03	-0.31 ± 0.09	-0.08 ± 0.02
EMD (mm)	22141	26.7	2.86	0.50 ± 0.02	-	0.64 ± 0.06	0.28 ± 0.03	-0.03 ± 0.11	-0.01 ± 0.03
DAG3 score	22809	1.03	1.12	0.43 ± 0.02	-	-0.18 ± 0.07	-0.06 ± 0.03	-0.07 ± 0.10	-0.02 ± 0.02
DAG8 score	8072	1.14	1.25	0.51 ± 0.03	-	-0.04 ± 0.10	-0.01 ± 0.04	-0.13 ± 0.12	-0.03 ± 0.03

SIL: Sheep Improvement Limited (www.sil.co.nz); CH₄: methane; DMI: dry matter intake; WWT: weaning weight at 3 months; LW8: live weight at 8 months; FW12: fleece weight at 12 months; EMD: eye muscle depth; DAG3, DAG8: dag score at 3 and 8 months, respectively.

While data in tables 1 and 2 are based on 24hr respiration chamber (RC) measurement with known feed intake, the cost of this is thought to be prohibitive for a testing program using industry animals. Therefore, protocols for measuring or estimating CH₄ production and feed intake that require less time and cost need assessment. To inform development of these protocols, an overview of variation in CH₄ production and feed intake are described.

Understanding animal variation in methane production over time

Sources and transfer of methane within the ruminant

While CH₄ is produced in both the reticulo-rumen and the hindgut, some transfer within the animal occurs before the CH₄ is emitted. For example, in ewes eating lucerne, 97.5% of CH₄ emission was via the oesophagus and lungs and only 2.5% via flatus; 23% of CH₄ production occurred in the lower gut and most (89%) of this hindgut CH₄ was excreted via the lungs, presumably after absorption into the blood (Murray et al., 1976). The proportion of CH₄ derived from the hindgut increases with feeding level (Murray et al., 1978; Hofmeyr et al., 1984). A small difference has been observed in some, but not all, experiments in which sulphur hexafluoride (SF₆) has been used as a tracer in confinement studies, in which the whole animal is confined, and flatus is included in the emission measures (Johnson et al., 1994; Boadi et al., 2002; McGinn et al., 2006; Grainger et al., 2007; Pinares Patiño et al., 2011b).

Most of the CH₄ leaving the rumen in oesophageal eructation is thought to be subsequently drawn into the lungs and then emitted in exhaled breath. This has been confirmed by dosing and radiotracer studies (Dougherty et al., 1962; Heywood

and Wood 1985). Some rumen produced CH_4 , is also absorbed directly into the lungs without passing back through, up the oesophagus.

Cattle eructate on average every 1.5 mins and take between 25-40 breaths per min (Ulyatt et al., 1999; Mortola and Lanthier, 2005). The recently developed GreenFeed emission monitor (GEM; www.c-lockinc.com/greenfeedonline.php) has provided the opportunity to monitor the pattern of those emissions (Figure 4). Distinct emission peaks carrying both CO_2 and CH_4 , at 40-60 second intervals, are apparent when cattle are measured by a GEM. The frequency of eructation peaks is reduced when drinking (Hegarty et al., 2013).

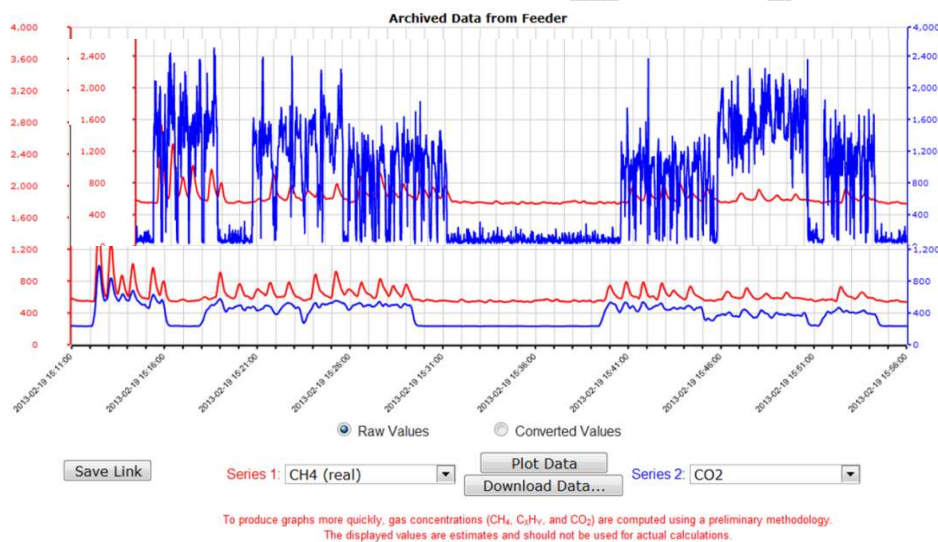


Figure 4. Methane (red) and Carbon dioxide (blue) concentration in breath of a cow measured using the Greenfeed Emission Monitoring system (Hegarty 2013)

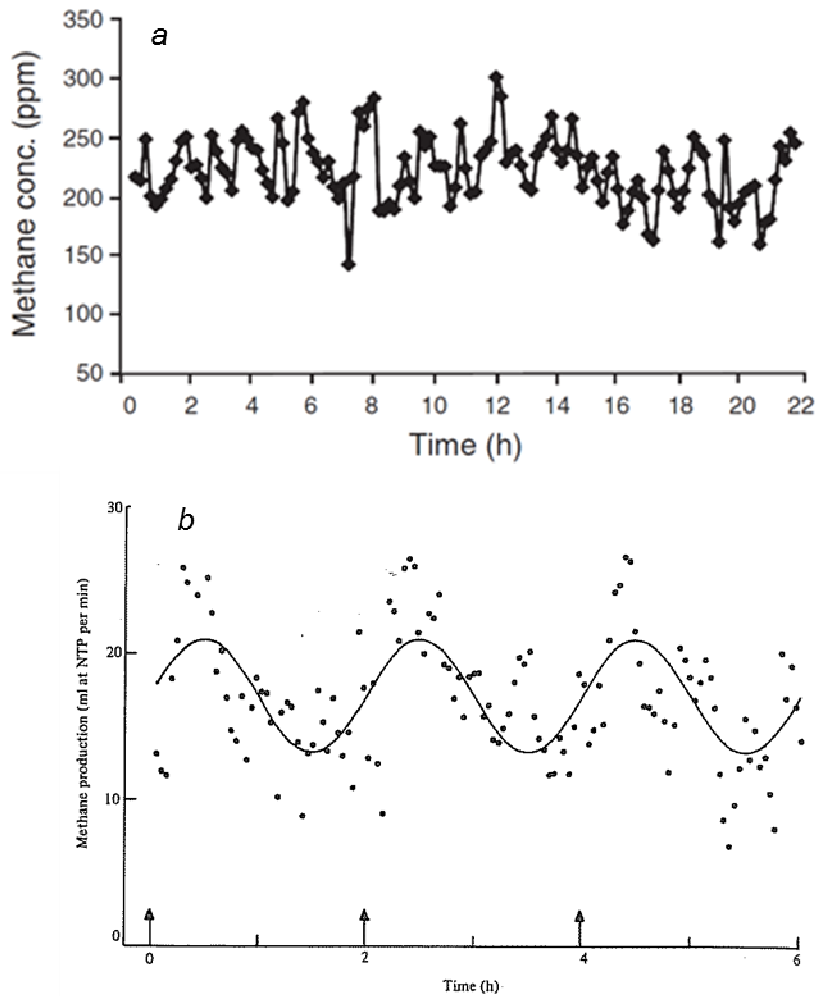
Studies with tracheostomised cattle (Dougherty and Cook, 1962; Hoernicke et al., 1965) have revealed that before feeding, 25–94% of the total CH_4 emission (flatus not included) was by exhalation, whereas after feeding exhalation accounted for 9–43% of total CH_4 emission. Furthermore, with small amounts of rumen gas, CH_4 was almost completely absorbed from the rumen into bloodstream and exhaled via the

lungs. The fraction of CH₄ absorbed into the bloodstream decreased with increasing volume of eructated gas (Hoernicke et al. 1965). The proportion of tracheal inhalation of eructated gases is also greater when an animal is not ruminating than when it is ruminating and is highly variable between individuals (Hoernicke et al., 1965).

From the above, it seems that in cattle, absorption of CH₄ from the rumen and subsequent exhalation is an important source of CH₄ excretion, but it is highly variable between animals. However, irregularities in emission occur, as evidenced by the large oscillations in CH₄ release rate (but not necessarily methanogenesis rate) observed during calorimetry. Animal position and activity is known to affect pooling of gas in the rumen (McCauley and Dziuk, 1965). Pooling of gas in the rumen may be part of the reason that variable short term CH₄ production rates are seen during RC studies even when animals are fed at 2 hr intervals (e.g. Figure 5a: Nolan et al., 2010; Figure 5b: Mathers and Walters, 1982). Enteric CH₄ production rate varies widely over 2 hr intervals (Figure 5b), potentially contributing to a highly variable estimate of emission rate if measurements are short term. Mathers and Walters (1982) acknowledged “violent short-term variations were evident in the plots of the observations”. Emission rates were averaged, over various periods, to generate smoother emission profiles. Even with slowly fermented high-fibre diets, such variations in emission (not necessarily production) are apparent (e.g. McCrabb and Hunter, 1999; Figure 6).

Breathing frequency in cattle not only oscillates within a day, but it also varies largely between animals (Piccione et al., 2004). Thus, differences in gas excretion mechanisms (eructation, tracheal inhalation, exhalation and expiration) might differ considerably among individual animals as well as with diets.

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360 **Figure 5.** Time course of **a)** methane concentrations (ppm) (reproduced Nolan et al.,
361 2010, figure 1a), and **b)** methane production (ml/min) (reproduced from Mathers and
362 Walters, 1982, figure 2a), of sheep fed using an automated feeder at 2-hourly
363 intervals

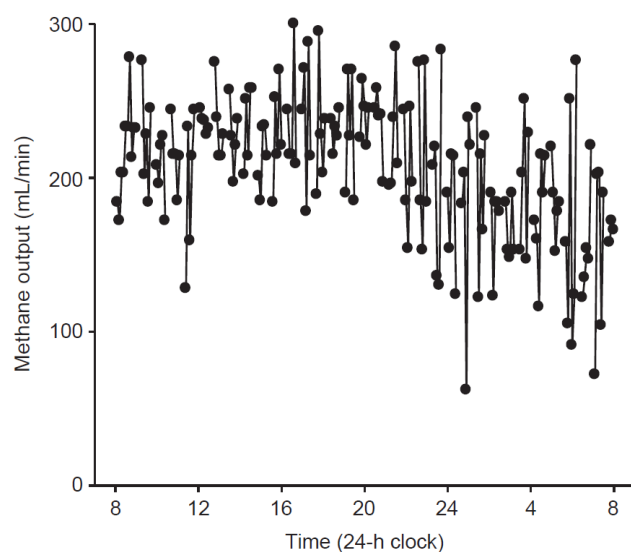


Figure 6. Pattern of methane emissions from a Brahman steer fed *ad-libitum* Rhodes grass diet once at 0800hrs (reproduced from McCrabb and Hunter, 1999, figure 1)

Diurnal and longer term emission cycles

In the grazing environment, ruminants are considered to ingest most of their feed intake in morning and late-afternoon feeding sessions (see Gregorini, 2012 for recent review). Emulation of this pattern in RCs (Robinson, 2009) shows a biphasic diurnal CH₄ emission pattern, consistent with timing of feed intake but there was no difference in either total daily emission or MY when feed was provided in a single meal or as 4 equal meals in the morning and 4 equal meals in the afternoon (Figure 7). Murray et al (2001) found a similar pattern of biphasic emissions in grazing sheep using a polytunnel (Figure 8).

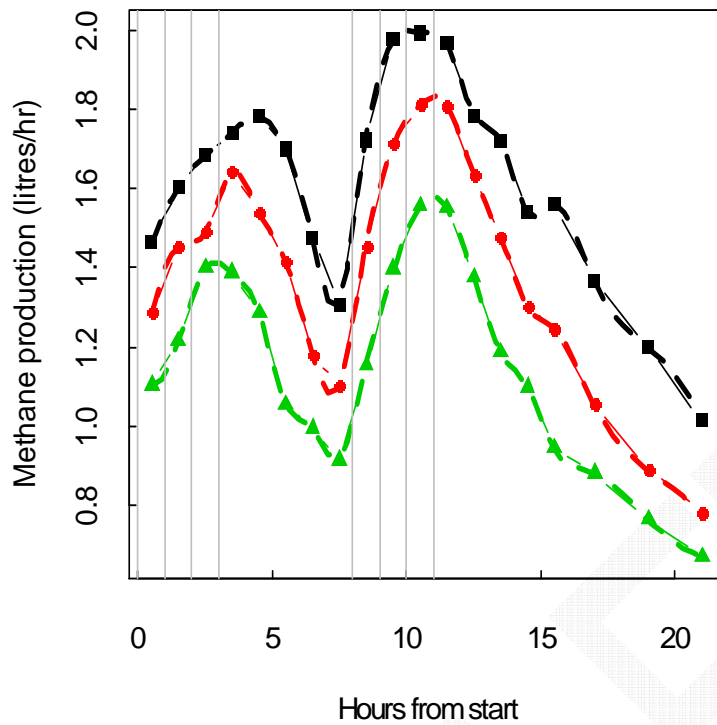


Figure 7. Production of methane from sheep fed three levels of chaffed lucerne hay (0.7, 1, 1.3 times maintenance requirements) in 2 sessions each of 4 hours in which feed was presented hourly for 4 hours /day. The feeding pattern was intended to represent the anticipated pattern of feed intake by sheep under pasture conditions. The x- axis is hours from start of feeding, the y- axis is methane production rate (l/hr). (After Robinson 2009; concentration data multiplied by flow rates to get methane production rate)

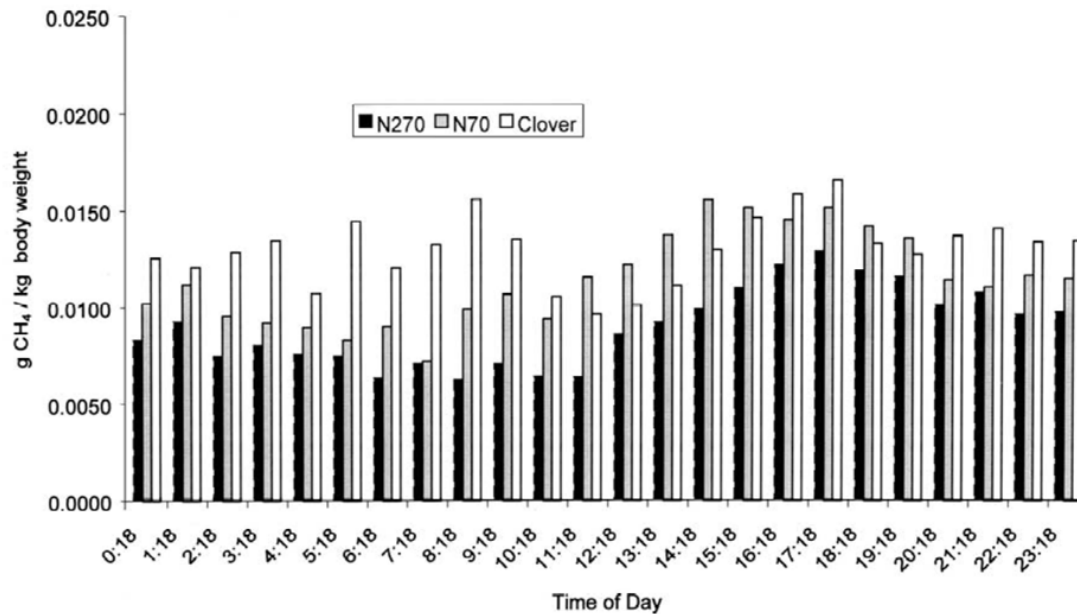


Figure 8. Biphasic emission profile in sheep grazing ryegrass pasture (N270 , N70) or clover (Murray et al., 2001).

A number of studies offer evidence of repeatability of emissions over prolonged periods, but the repeatability is confounded by the variations in pasture that occur with seasonal pasture change, (Knight et al., 2008; Munger and Kreuzer, 2008), so do not reflect innate repeatability of emission by the animal as would occur if the same diet was fed for a prolonged period.

Recent sheep genetics research provides evidence of repeatability over extended time intervals when a consistent diet is fed (Pinares-Patiño et al., 2013a) and confounding with changes in feed composition do not occur. Within year repeatabilities of daily CH₄ production and of MY were 0.55 and 0.26, respectively (Table 1) and repeatability declined as the period between measurement increases.

Implications for measurement

The highly variable dynamics of CH₄ excretion in relation to feed intake implies that methods, based on discrete and low frequency measurements of emissions from animals feeding intermittently and with asynchronous timing, may not accurately rank individuals.

Before considering short term breath-based measures, it is worth considering the constraints of the RC system that is often viewed as a 'gold standard' for emission measurement. There is little question RC measurements accurately quantify CH₄ output over the 1-3d typically used, and they achieve this by frequently monitoring emissions, with the variability in emission rate resulting from eructation cycles, animal position and feed intake that occur in 24hr, being typically damped within the large chamber volume. However, even if emission rate was monitored every second, a 1-2d collection seems unlikely to describe the CH₄ phenotype of an animal over a year or a lifetime. Feeding in RCs can also cause a reduction in feed intake (relative to pre-chamber intakes) and completely eliminates diet selection and feeding pattern which has strong genetic control and may well be a means by which animal genetics moderates emission in the grazing environment (Hegarty, 2002). However, RC rarely monitor CH₄ outflow on a second by second basis, the chambers used to estimate CH₄ parameters in table 1 do so by measuring volume of air flow coupled with intermittent samples of CH₄ concentrations every 5 to 6 minutes. This means that hourly measurements described here consist of averages of 9-13 measurements each taken over a few seconds (albeit averaged via dilution in a large volume that is the chamber). In reality, CH₄ is emitted intermittently via brief 5-30 second eructations or burps, albeit with a basal level of emission, so these results are not derived via integrating instantaneous emissions over time. This system has shown

repeatabilities of 0.53 and 0.24 for CH₄/d and MY across years in table 1, highlighting that the high frequency emission monitoring of a RC over 1-2d cannot describe the long term emission rate or variation.

The SF₆ technique is one tool that offers field measurement over a longer time, but requires insertion of rumen boluses, daily animal handling and laboratory measurement of gases (McGinn et al., 2006). Moreover, the sampling procedures provide an average methane output for periods of typically 24hrs, but can be repeated over periods of 5-10d, or until the rate of release of SF₆ from the permeation tube is no longer stable. While repeatability of daily CH₄ production is being improved as the methodology is refined, SF₆ remains a very demanding method to get accurate emission measures over multiple days in individual animals.

Other systems that measure (or estimate) emissions over multiple short periods per day with minimal operator input have been developed. These include measuring all emissions from animals in short term confinement (Portable Accumulation Chambers: PAC; Goopy et al., 2011), or monitoring eructations in feeding stations (Negussie et al., 2012) or voluntary milking systems for cattle (Garnsworthy et al., 2012a; Lassen et al., 2012; de Hass et al., 2013). Also laser gun methodology has been used to make short term measurements in dairy cattle (Chagunda et al., 2013). Tables 3 and 4 present the average CH₄ emissions in various units, heritability estimates, where known, and various repeatability estimates e.g. across days, across periods and across rounds. There are a wide variety of methods used including; system (RC, SF₆, laser, GEM or PACs), diet (composition and particle size), feeding level (ad libitum or at a proportion of maintenance) and experimental period. Despite this, gross CH₄ output and repeatability estimates are not so different. However, MY is variable with a noticeable difference between studies where animals

are fed at a proportion of maintenance versus those that are fed at ad libitum. Those fed at maintenance are theoretically estimating CH_4 per live weight as much as CH_4 per unit intake; MY increases with live weight, and thus the ratio measure could be similar across time points in maintenance fed studies.

When collecting records for selective breeding, it will often be a choice between accuracy of the phenotype and number of records. In the case of CH_4 emission the most accurate method would be the RC method but in order to generate enough data to do selective breeding and make recordings in practice this method has limitations. On the other hand, spot samples from e.g. milking in dairy cattle might be an inaccurate phenotype for selective breeding but can generate a huge number of individual animal records. A correlation structure between these methods together with 1hr RC methods, SF_6 and other methods seems obvious and merging data therefore seems to be an appropriate way to get enough data for use in selective breeding. The value of the recordings is enhanced by the family structure in the given population analysed. Often half-sibs will be recorded in different systems and that will help in order to perform selective breeding.

471

Table 3. Summary of methane measurement experiments in cattle, including average emissions (\pm sd), and repeatability (Rep) estimates.

Animals	System ^a	Breed ^b	Diet ^c	Expt Period	Trait	Av Emissions		Rep	Country	Reference
4 Dairy cows	SF6	F x J	Forage based at M	23 days	gCH4/d	124.3 ± 11.1	across days	0.49	NZ	Vlaming et al., (2008)
					gCH4/kgDMI	22.8 ± 2.0		0.47		
			Cereal, lucerne and straw mix at M	30 days	gCH4/d	169.8 ± 11.0		0.73		
					gCH4/kgDMI	32.0 ± 2.0		0.73		
93 Dairy cows	FTIR – AMS	50 H	TMR <i>ad lib</i> , concentrate ^d	3 days	CH4		across visits to AMS	0.34 ± 0.01	Denmark	Lassen et al., (2012)
					CO ₂			0.46 ± 0.00		
					CH ₄ :CO ₂	0.065		0.37 ± 0.01		
		43 J		3 days	CH ₄			0.33 ± 0.01		
					CO ₂			0.40 ± 0.01		
					CH ₄ :CO ₂	0.05		0.33 ± 0.00		
30 heifers	RC	H, J and S	Forage <i>ad lib</i> plus concentrate ^e	6 periods of 3 days ^g	CH ₄ L/d		across stage of lactation	0.13	Swiss	Münger and Kreuzer (2008) data supplied
					CH ₄ L/kg DMI			0.039		
					DMI kg/d			0.12		
					CH ₄ L/kg DOMI			0.019		
					CO ₂ L/d			0.069		
					CH ₄ /CO ₂			0.25		
					LWT			0.65		
40 Yearling bulls	RC	Angus	Lucerne and cereal hay chaff 1.2x M	2periods of 24hrs ^h	DMI kg/d	7.61 ± 1.46		0.75	Aus	R.Herd pers. comm.
					Ch4 l/d	239 ± 24.04		0.69		
					ch4 l/kgDMI	31.75 ± 2.90		0.34		
10 steers	Greenfeed	Angus	Lucerne cereal mix chaff <i>ad lib</i> plus pellets ^f	6 periods of 2 days	LW	365.2 ± 49.97	across periods	0.95	Aus	J. Velazco pers. comm.
					DMI kg/d	8.93 ± 2.61		0.10		
					CH4/CO2	0.04 ± 0.00		0.26		
					CH4g/kgDMI	27.00 ± 13.50		0.02		

					CH ₄ g/d	216.54 ± 39.04		0.37		
					CO ₂ g/d	5675.7 ± 919.6		0.57		

^aSF₆: Sulphur hexafluoride; FITR: Fourier Transform InfraRed; AMS: Automatic Milking System; RC: Respiration Chambers; ^bF: Friesian; J: Jersey; H: Holsteins; S: Simmental; ^cM: maintenance; *ab lib*: *ab libitum*; ^dTMR: total mixed ration (corn silage, rapeseed meal and soybean meal). Concentrates were fed in the AMS as an attractant; ^eSpring: maize silage and fresh grass in Autumn: maize silage and hay. Concentrates 200g/kg daily DM fed in both seasons up to 22 week of lactation; ^f850g Horse pellets fed in Greenfeed as attractant; ^g6 periods: pre calving and weeks 8, 15, 23, 33 and 41 of lactation; ^h2 periods were 9 months apart;

Table 4. Summary of methane measurement experiments in sheep, including average emissions (± sd), and repeatability (Rep) estimates.

Animals ^a	System ^b	Breed ^c	Diet ^d	Expt days	Trait	Av Emissions	h ²		Rep	Country	Reference
684 sheep (10 mo)	RC	Rom, Coop, Peren and Comp	Lucerne pellet 2.1x M	24hr	gCH ₄ /d		0.38 ± 0.09	across days	0.89 ± 0.01	NZ	McEwan et al., (2012).
								across rounds	0.58 ± 0.02		
				1hr	gCH ₄ /d		0.20 ± 0.07	across days	0.62 ± 0.02		
								across rounds	0.37 ± 0.03		
				24hr	gCH ₄ /kgDMI		0.15 ± 0.06	across days	0.77 ± 0.01		
								across rounds	0.28 ± 0.03		
				1hr	gCH ₄ /kgDMI		0.08 ± 0.05	across days	0.51 ± 0.02		
								across rounds	0.21 ± 0.03		
1277 Sheep (10mo - 4yrs)	RC	Rom, Coop, Peren and Comp	Lucerne pellet 2.1x M	2x 48hr	gCH ₄ /d	24.89 ± 4.80	0.29 ± 0.05	across days	0.94 ± 0.00	NZ	Pinares Patino et al., (2013 ^a)
								across rounds	0.55 ± 0.02		
								across years	0.53 ± 0.02		
					gCH ₄ /kgDMI	15.74 ± 1.90	0.13 ± 0.03	across days	0.89 ± 0.01		
								across rounds	0.26 ± 0.02		
								across years	0.24 ± 0.02		
					LW	48.17 ± 13.31	0.46 ± 0.07	across days	0.93 ± 0.00		
X Sheep 12-15mo	RC and PACs		ab lib, M and pasture	9 measures ^e	LWT (kg)	51.50 ± 7.87		across days	0.93	Aus	H Oddy pers. comm.
					mICH ₄ /min	23.18 ± 4.53			0.47		
					mICH ₄ /gFI	24.39 ± 1.95			0.07		
					CH ₄ /CO ₂	0.06 ± 0.01			0.08		
					mICO ₂ /min	361.7 ± 52.17			0.42		
					mIO ₂ /min	-370.6 ± 46.38			0.20		

708 Sheep	PAC	Merino X	pasture ab lib	1hr	dLCH ₄ /h	5.5	0.30		0.30	Aus	Robinson,, (2009)
					adj for LW	5.5	0.13		0.32		

478 ^amo: month; ^b RC: Respiration chamber; PAC: Portable Accumulation chamber; ^c Rom: Romney; Coop: Coopworth; Peren: Perendale; Comp: Composites; X: cross; ^d M:
479 maintenance; *ab lib*: ab libitum; ^eAnimals were measured for 1hr in PACs for 2 consecutive days, done for 4 separate periods (period 1 *ab lib*, period 2 at maintenance, period
480 3 and 4 at pasture). Animals were also measured in RC for 1 day at *ab lib*, between period 1 and 2.

Three messages on repeatability emerge from tables 3 and 4. The repeatability of daily CH₄ emissions is highest between RC measures made on consecutive days, but diminishes as time between measures increases. Repeatability between CH₄ emissions measures is low for short term measurement systems (eg PACs) relative to RC measures. Consequently, more measures will be required from short-term sampling methods to capture variation within a day, but multiple samples across many days offers additional information about the robustness of emission phenotype that is not normally obtained by RC studies made only over 1-3d. This working group has not as yet been able to source sufficient structured data from these methods and protocols to develop a common procedure for measurement of rate of CH₄ emissions capable of being used for genetic selection

McEwan et al (2012) assessed the usefulness of multiple 1hr measures of emissions compared to 22hr RC measures using 684 sheep and found a high genetic correlation between 24hr total emission measure and a 1hr emission measure (0.89 for gCH₄/d and 0.76 for MY). From the data, they estimated there is little difference in measuring animals for 2 rounds of 2 days (by RC), 14d apart, or for measuring the animal 4 times for 1hr if intake is known. Such assessments indicate that using a range of measurement technologies is possible, but the intensity of sampling required and number of animals needing to be measured will be different for each system used.

It has been calculated that 3 x 1h PAC measurements will be as useful at describing CH₄ production rate as one RC measure for 1 day (Bickell et al., 2011)). Defining this comparability is a key requirement for developing measurement protocols of equivalent power to use in genetic selection.

Recent data from Oddy et al pers. comm. (Table 5) has started to build a framework for comparing the merit of emission measurement systems by estimating the correlation between them. For example, RC vs PACs (ml CH₄/min) measured on the same animal, same diet (*ad libitum*) have correlations of 0.58. The correlations between RC at *ad libitum*, and at maintenance was also 0.58, between PAC *ad libitum* and PAC maintenance was 0.60 (Table 6).

Where these short term emission measures for animal house or at pasture become constrained is that feed intake is not usually known and estimating the intake relevant to a CH₄ measure made over only a few minutes is challenging. For example, in the RC v PAC comparison in table 6 below, correlations between methods for estimating MY drop to 0.11 - 0.18 (RC+PAC *ad lib* fed), -0.12 - 0.01 (RC *ad lib*, PAC maintenance) and -0.14 - 0.16 (PAC *ad lib*, PAC maintenance), respectively. These results suggest we can't use PACs for estimating MY without a measure of feed intake temporally relevant to the measurement of CH₄. Because of the strong association between methane production and DMI, it is important to understand variation in feed intake if MY is to be considered as a trait. Variation in feed intake is assessed in the next section.

Table 5. Phenotypic correlation matrix (r) between methane production rate (ml CH₄/min) by sheep determined by RCs and repeated portable accumulation chambers (PAC) when fed at maintenance and *ad-libitum*.

	Ad-lib P1		M P2		Ad lib	pasture P3		pasture P4	
	PAC1	PAC2	PAC3	PAC4	RC	PAC5	PAC6	PAC7	PAC8
PAC1	1								
PAC2	0.751	1							
PAC3	0.518	0.582	1						
PAC4	0.691	0.719	0.815	1					
RC	0.583	0.579	0.563	0.597	1				
PAC5	0.394	0.515	0.454	0.439	0.556	1			
PAC6	0.539	0.468	0.415	0.473	0.44	0.634	1		
PAC7	0.508	0.55	0.492	0.484	0.531	0.635	0.552	1	
PAC8	0.545	0.619	0.489	0.556	0.463	0.539	0.505	0.58	1

Table 6. Phenotypic correlations between different measurement protocols, and different ways of expressing methane emissions from sheep.

CH ₄ ml/min	PAC ad-lib	PAC maint	
RC	0.58	0.58	
PAC maint	0.6	1	
CH ₄ ml/gDMI	PAC ad-lib	PAC maint	
RC	0.11-0.18	-0.12 - 0.01	
PAC maint	-0.14 - 0.16	1	
CH ₄ /CO ₂	PAC ad-lib	PAC maint	
RC	0.16 - 0.13	0.09 - 0.27	
PAC maint	0.36 - 0.40		
CH ₄ /Estimated FI	PAC ad-lib	RC	PAC pasture1
PAC pasture1	0.23-0.44	0.23-0.26	1
PAC pasture 2	0.28-0.36	0.10-0.12	0.16-0.47

CH₄: total methane emissions (ml/min, MY: CH₄/kgDMI; ratio of CH₄/CO₂ emissions: CH₄/CO₂). Measurement protocols: RC: respiration chamber 22hrs *ad-libitum* feed intake of chaffed hay (M/D = 9.5 MJ ME/kg DM); PAC *ad-lib*: 1hr measurement in portable accumulation chamber, *ad-libitum* intake of same chaffed hay; PAC maintenance: 1hr measurement in PAC maintenance intake of chaffed hay; PAC pasture 1 and 2: 1hr measurement in PAC of same sheep eating 2 different pastures.

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545 **Proxies for methane production**

546 Soon after exit from RCs (fasted or pre-feeding stage), sheep used in the
547 studies of Pinares-Patiño et al., (2013a) were sampled for rumen contents
548 (20–50 mL) by stomach-tubing for volatile fatty acid (VFA) analysis from 1,081
549 animals (Pinares-Patiño et al., 2013b). Animals were fed at 8.30-9am and
550 4pm, rumen samples were taken at 8am after released from RC, therefore,
551 well into the fasting period. There were also 96 animals measured before
552 going into the chamber, ~ 3hrs after the last feeding. Individual and classes of
553 VFA were analysed as log of concentrations (mM) or alternatively as molar
554 percentages (% molar). There is no agreement in the literature on the validity
555 and representativeness of sample of rumen contents collected via stomach
556 tube, but representativeness of stomach tube sample seems be related to
557 feeding time and depth of insertion (Shen *et al.*, 2012). In the present study,
558 sampling took place at fasting and the operation was completed within one
559 minute. Results are shown in Table 7. There were high genetic correlations of
560 MY with \log_e mM VFA concentrations. Genetic correlations are lower,
561 however, still moderate when VFAs were expressed as molar %. However,
562 other studies (Robinson et al., 2010, McPhee and Hegarty, 2008), suggest
563 that information on VFA has limited utility in predicting CH₄ emissions.

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Table 7. Rumen VFA (log_e mM or molar %), heritability (h²), repeatability (rep) and genetic correlation (r_g) with gCH₄/kgDMI (Pinares-Patiño et al., 2013b)

	mM			molar %		
	h ²	rep	r _g	h ²	rep	r _g
VFA	0.10±0.04	0.33±0.03	0.92±0.10			
Ace	0.09±0.04	0.34±0.03	0.95±0.10	0.04±0.03	0.08±0.03	-0.01±0.28
Pro	0.10±0.04	0.31±0.03	0.78±0.15	0.09±0.04	0.15±0.03	-0.18±0.17
But	0.09±0.04	0.28±0.03	0.86±0.13	0.04±0.04	0.18±0.03	0.36±0.34
Ace/Pro				0.10±0.04	0.11±0.03	0.08±0.18

CH₄: methane; DMI: dry matter intake; VFA: volatile fatty acid; Ace: acetate; Pro: propionate; But: butyrate; Ace/Pro: acetate/propionate ratio

Understanding variability of feed intake over time

Of the factors that influence CH₄ emissions, feed intake (quantity and extent of and rate of fermentation in the rumen) accounts for most of the variation in daily CH₄ emissions. Methane production and excretion from the rumen is synchronised with and consequent to feeding pattern (Johnson et al., 1998). Ingestion of a meal and subsequent fermentation increases CH₄ emissions within 15 minutes and elevated CH₄ emissions continue for several hours (e.g. Figure 7 above). Gas production and consequently the rate of eructation is highest soon after feeding than when an animal is ruminating or resting (Colvin et al., 1958; Colvin et al., 1978; Dougherty and Cook, 1962; McCauley and Dziuk, 1965; Waghorn and Reid, 1983).

Feed intake, variation and repeatability

Because variation in CH₄ production is predominantly related to variation in timing, extent and composition of nutrients ingested, a systematic assessment of sources of variation in feed intake, principally using experience from previous studies, is required where feed intake has been measured to assess

variation in production efficiency. This is an important consideration, because it has been suggested (Alcock et al., 2011), that selection of animals for improved efficiency will also reduce CH₄ emissions (Nukumah et al., 2006; Hegarty et al., 2007). In practice, if selection for reduced MY is to be implemented, it would be reasonable to couple measurement of both feed intake and CH₄ as part of the process for measuring animals to improve efficiency of feed utilisation.

Repeatability of feed intake

Knowledge of variation in feed intake is useful for deciding the best strategy for measurement of CH₄ emissions because of the dominant effect of intake on CH₄ emissions. When combined with a clear breeding objective, trait definition and knowledge of variation in rate of CH₄ emission in response to feed ingestion, it should then be possible to work out an optimal protocol for measuring CH₄ emissions. For example, if the trait under selection is total CH₄ emissions, some knowledge of pattern of intake is useful, but not essential to measurement of CH₄. However, if the trait is MY, we need to know enough about the characteristics of feed intake as well as CH₄ emissions to derive an estimate of MY. In practice we need to know that the correlations between intake and CH₄ measured across time are sufficiently high as to be useful for genetic evaluation.

Many factors affect the DMI of cattle and include factors such as body size, growth, body composition, gender, age, season, ambient temperature, physiological status, previous nutrition and diet (NRC, 2000). Most of these factors are either standardised between animals during a feed intake test (e.g.,

gender, season, ambient temperature and physiological status) or adjusted for factors such as age, body size, body composition, and growth. However, considerable within- and between- animal variation exists for DMI and measures of feed efficiency. Table 8 and 9 below present the average daily feed intakes, coefficients of variation and various repeatability estimates e.g. between animal, across days, within periods and across lactations from known feed efficiency trials, for cattle and sheep respectively. As can be seen there is variation between each system, diet and experimental time periods. Within an experiment, with repeatability conducted in 10 day or 30 day intervals (e.g. 1-10, 1-20, 1-100 days) estimates decreased as the time interval increased. For example for feeder steers (Figure 9) between-animal repeatability decreased from 0.407 (1-10 days) to 0.341 (1-84 days) and for beef heifers decreased from 0.380 (1-10 days) to 0.286 (1-108 days) (J. Basarab, pers. comm.). These levels of repeatability are weak to moderate and would mean that an animal does not have consistent feed intake over time as reflected by the decreasing repeatability estimates for the same group of cattle as the feeding interval increased. Similar trends were found for repeatability of daily FI in sheep (K. Cammack, pers. comm.; Oddy and Sainz, 2002).

Wang et al. (2006) reported that the phenotypic variances for DMI decreased rapidly from 7 to 35 days of feed intake data collection and then stabilized after 35 days, indicating that extending the duration of data collection beyond 35 days resulted in only small improvement in accuracy. The same trend for ADG was not as clear and a test period of at least 63 days was recommended. The feed intake measures should be taken for at least 35

days for a given diet and animal type (e.g., feeder steers on a finishing diet, replacement heifers on a growing diet). This is consistent with the reductionist approach of Archer et al., (1997)

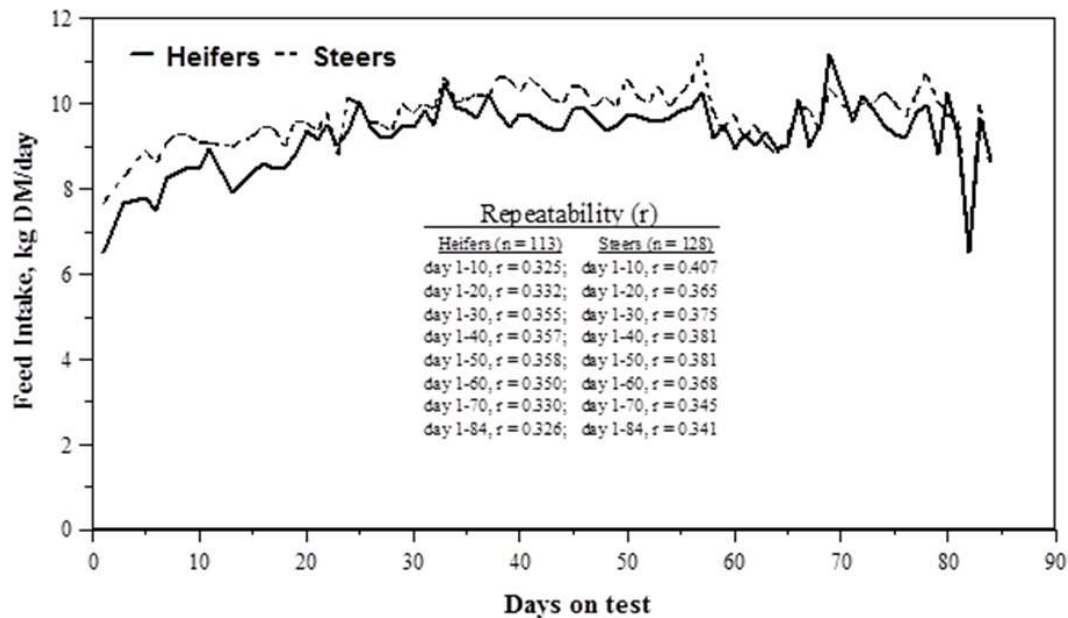


Figure 9. Daily feed intake for heifers (solid line) and steers (dashed line) fed a finishing diet (56.6% barley grain, 20% corn-DDGs, 20% barley silage and 3.4% protein supplement/minerals, dry matter basis) over 84 days.

647 **Table 8.** Summary of feed intake experiments in cattle, including average daily dry matter intake (av kgDMI/d \pm sd), coefficient of
648 variation (CV% \pm sd) and repeatability (Rep) estimates.

Animals	System	Breed ^j	Expt days	Av kg DMI/d	CV %	Rep	Country	Reference
Beef Cattle: Feedlot								
113 Feeder heifers	GrowSafe ^a	Beefbooster TX	1 - 84	9.3 ± 0.8	19.2 ± 2.3	0.326 ^k	Canada	J. Basarab pers. comm.
128 Feeder steers	GrowSafe ^a	Beefbooster TX	1 - 84	9.7 ± 0.7	19.3 ± 3.0	0.341 ^k	Canada	
61 Beef heifers	GrowSafe ^b	AA x H and C x RA	1 - 108	7.0 ± 0.8	15.1 ± 2.5	0.286 ^k	Canada	
99 Young bulls	GrowSafe ^c	AA x H x G	1 - 77	9.1 ± 0.9	16.4 ± 1.9	0.386 ^k	Canada	
40 Beef cows (3-5y)	GrowSafe ^d	AA x H and C x RA	1 - 79	14.4 ± 1.3	20.9 ± 4.6	0.491 ^k	Canada	
50 Feeder heifers	Insentec ^e	Limousin X Friesian	1 - 84	10.8 ± 1.0		0.34 ^l	Canada	Kelly et al., (2010)
						0.61 ^m		
						0.62 ⁿ		
64 Steers	Tullimba feeder ^f	Mixed Bos Taurus ^j	10 - 100	11.8 ± 3.1	26	0.257 ^o	Australia	Robinson & Oddy (2004)
			40 - 100	11.7 ± 3.0	26	0.245 ^o		
93 Steers	GrowSafe ^g		8 - 46	14.1 ± 2.4	17	0.15 ^o	Australia	J. Cook pers. comm.
Dairy cattle: Feedlot								
554 Dairy cows	Insentec ^h	Holstein	8 - 305			0.31 ^p	Denmark	J. Lassen pers. comm.
						0.64 ^q		
						0.24 ^r		
						0.65 ^s		
Dairy cattle: tracer								
755 Dairy cows	C32 n-alkane ⁱ	Holstein-Friesian		13.9 - 17.8 ^t		0.18 - 0.57 ^t	Ireland	Berry et al., (2007)

649 *Diets:* ^aFinishing diet (56.6% barley grain, 20% corn-DDGs, 20% barley silage and 3.4% protein supplement/minerals); ^b90% barley silage and 10% barley
650 grain diet; ^cGrowing diet (72.1% barley silage, 24.6% barley grain and 3.3% protein/mineral supplement; ^dHay-straw cube (25% straw, 75% grass hay-alfalfa
651 ix); ^e70:30 concentrate and corn silage; ^f75% cracked barley, 15% chopped cereal hay, 8% molasses and 2% minerals; ^g70.8% cracked Barley, 6% whole fuzzy
652 cottenseed, 4.6% cottenseed hulls, 5% mill run, 4.6% chopped hay, 5% liquid supplement, 4% H₂O; ^hTMR ad lib based on corn and grass silage together with
653 soybean meal and concentrate in VMS;; ⁱPasture or pasture plus concentrate;

^j TX: Terminal cross; AA: Aberdeen Angus; H: Hereford; G: Gelbvieh; C: Charolais; RA: Red Angus; Mixed Bos Taurus: Angus, Hereford and Shorthorn;
^k Repeatability estimates: ^kBetween animal; ^lWithin finishing period for DMI; ^mBetween growing and finishing phases for DMI and ⁿresidual feed intake (RFI);
^oDaily feed intake; ^pWeekly DMI repeatability across lactation and ^qwithin lactation; ^rDaily DMI repeatability across lactation and ^swithin lactation; ^tWithin stage
of lactation.

Table 9. Summary of feed intake experiments in sheep, including average daily dry matter intake (av kgDMI/d \pm sd), coefficient of
variation (CV% \pm sd) and repeatability (Rep) estimates.

Animals	System	Breed	Expt period	Av kgDMI/d	CV%	Rep	Country	Reference
Autofeeders								
61 ewes (7 mo)	GrowSafe ^a	Targhee X Rambouillet	68	1.5 ± 0.2	14.1	0.26 ^h	USA	K. Cammack pers. comm.
610 progeny (5mo - 2 Yr olds)	Auto feeder ^b	Merino X Awassi	1 – 90	1.1 ± 0.5		0.20 ⁱ	Australia	Jonas et al., (2009)
						0.14 ^j		
						0.02 ^k		
Individual penned and refusals weighed								
96 Ewes (12 mo) ^b	Chaff ^c	Merino X	1 – 30	1.2 ± 0.2	13.7	0.711 ^h	Australia	H. Oddy pers. comm.
36/group Weathers (6-8 mo)	Pellet ^d	BL X M X PD ^g	3 – 83	1.4 ± 0.2	11.1	0.24 ^h	Australia	Oddy & Sainz (2002); Hegarty et al., (1999)
	Pellet ^e		3 – 83	1.6 ± 0.2	12.6	0.44 ^h		
	Pellet ^f		3 – 83	1.7 ± 0.2	13.3	0.40 ^h		
Tracer on pasture								
300 Ewes	Cr marker	Merino		0.6 - 1.1		0.32-0.47 ^k	Australia	Lee et al., (1995)
						0.09-0.27 ^l		
350 Wethers	C32 marker	Merino				0.78 ^l	Australia	Lee et al., (2002)

Diets: ^aForage diet (15.2% Crude Protein; 50% DM); ^bPellet (composition unknown); ^c50% chaffed lucerne hay 50% chaffed oated hay; ^dPellet energy density
of diet MEMJ/kgDM (M/D) 7.7; ^ePellet M/D 9.2; ^fPellet M/D 10.9; ^g Border Leicester X Merino ewes X Poll Dorset sires; Repeatability estimates:
^oDaily feed intake (FI); ^qWeekly FI; ^hWeekly DMI; ⁱWeekly residual FI (RFI); ^jDigestible organic matter intake (DOMI) within measurement period; and ^kacross
periods/seasons; ^lDOMI across 2 ages/sites.

The above data suggests that the system of measuring intake, and the system under which animals are fed, affects the repeatability of feed intake. However, we do not yet have sufficient data to estimate relationships between individual animal feed intake (and CH₄ emissions) across different measurement protocols and/or production systems. This is required to establish the extent to which measurement systems and feed types (for example) affect the ranking of individual animals.

Further work needs to be done to measure feed intake and the CH₄ trait in different production systems. In the case of beef cattle, sheep and goats because females produce most CH₄ (on a system basis), and predominantly graze pasture, it puts emphasis on measurement of intake, and CH₄ emissions, at pasture. An association between RFI measured in a feedlot and when grazing has been shown, supporting that selection for RFI measured in the feedlot will deliver changed RFI of the grazing maternal herd (Herd et al., 2002). This gives hope that selection for CH₄ or MY based on modest periods of measurement may also be adequate to deliver genetic improvement in these traits in the grazing herd. In the case of dairy cows measurement during milking seems to provide an appropriate period when emission measures can be made.

All direct measures of feed efficiency require an accurate measurement of feed intake and energy sinks such as body weight, growth and body composition in young cattle (Archer et al., 2001a and b; Basarab et al., 2003; 2007; 2011), and body weight, fat mobilization and milk fat, protein and yield in lactating dairy cattle (Rius et al., 2012). Typically, young cattle (7-10 months of age; maximum age difference = 60 days) are placed into a feedlot

pen fitted with feeding stations for the automatic monitoring of individual animal feed intake and feeding behaviours (e.g., GrowSafe Systems Ltd., Airdrie, Alberta, Canada; Bindon 2001) and adjusted to their final test diet over 21-28 days which reduces the effect of non-genetic effects such as previous nutrition, age of dam and age of calf (Basarab et al., 2003, 2011; BIF, 2010). The adjustment period is followed by a 70 to 112 day test period, which has been recommended as being adequate for the determination of feed intake and growth (Wang et al., 2006). Cattle are weighed on two consecutive days at the start and end of the test period and at approximately 14-28 day intervals. They are also measured for ultrasound backfat thickness (mm), longissimus thoracis area (cm²) and marbling score at the start (optional) and end of the test period.

Indirect selection on feed efficiency to reduce emissions

Measuring CH₄ emission rates directly from animals is difficult and thereby hinders direct selection on reduced CH₄ emission. However, improvements can be made through selection on associated traits (e.g. residual feed intake), or through selection on CH₄ predicted from feed intake and diet composition. The objective of a Dutch study was to establish phenotypic and genetic variation in predicted CH₄ output, and to determine the potential that genetic has in reducing CH₄ emissions in dairy cattle (de Haas et al., 2011). Experimental data was used, and records on daily feed intake, weekly live weights and weekly milk productions were available from 588 heifers. Residual feed intake (MJ/d) is the difference between net energy intake and calculated net energy requirements for maintenance as a function of live

weight and for fat and protein corrected milk production. Predicted CH₄ emission in grams per day (PME) is 6% of gross energy intake (IPCC - method) corrected for energy content of methane (55.65 kJ/g). Along with RFI and predicted CH₄ emission (PME, g/d), milk production was expressed as kg/d corrected for fat and protein content (FPCM). The estimated heritabilities for PME and RFI were 0.35, and 0.40, respectively. The positive phenotypic (Table 10) and genetic (Table 11) correlation between RFI and PME indicated that cows with lower RFI have lower PME as well (estimates ranging from 0.18 to 0.84). However, the association between these indicator traits and true CH₄ output is unknown. Still, it seems possible to decrease methane production of a cow by selecting more efficient cows, and the genetic variation suggests that reductions in the order of 11 to 26% in 10 years are theoretically possible, and in a genomic selection program even higher. However, several uncertainties were discussed, for example related to the lack of true methane measurements (and the key assumption that methane produced per unit feed is not affected by RFI level), as well the limitations of recording and to predict the biological consequences of selection. To overcome these limitations an international effort is required to bring together data on feed intake and methane emissions of dairy cows.

Table 10. Phenotypic correlations between predicted methane emission (PME), fat and protein corrected milk production (FPCM), DMI and residual feed intake (RFI) in full lactation. Reproduced from de Haas *et al.*, (2011).

Item	PME (g/d)	FPCM (kg/d)	DMI (kg/d)
FPCM (kg/d)	0.26		
DMI (kg/d)	0.99	0.31	
RFI (MJ/d)	0.72	-0.45	0.72

Table 11. Estimated genetic correlations between predicted methane emission (PME g/d) and fat and protein corrected milk production (FPCM kg/d), between PME and residual feed intake (RFI MJ/d), between FPCM and RFI, between PME per FPCM (g/d per kg) and FPCM, and between PME per FPCM and RFI within the whole lactation (0-42 wk) and in different periods of the lactation. Reproduced from de Haas *et al.*, (2011).

Period (wk)	PME - FPCM	PME - RFI	FPCM - RFI	PME/FPCM - FPCM	PME/FPCM - RFI
0-42	0.31	0.32	-0.84	-0.87	0.98
1-5	-0.66	0.84	-0.98	-0.95	1.00
6-10	-0.18	0.50	-0.94	-0.91	0.99
11-15	0.42	0.18	-0.78	-0.86	0.94
16-20	0.67	0.21	-0.55	-0.84	0.83
21-25	0.70	0.34	-0.43	-0.85	0.76
26-30	0.60	0.43	-0.49	-0.85	0.82

The rate of change of CH₄ following feeding is clearly shown in a Dutch study as well. Data were collected from ten trials in the two RCs of Wageningen UR, each trial involving a pair of cows, reported by Van Zijderveld *et al.* (2011). Each trial reported data over a 72 hour period

spanning four calendar days. The recording equipment alternated between the two RCs and a reset period such that each observation within a trial represented a three minute yield with six minute intervals between them. There were a small number of both random and systematic (associated with feeding/milking events) missing observations in the data.

Lactating Holstein-Friesian cows producing 27.9 ± 7.0 kg of milk/d and 167 ± 99 days in milk (DIM) at the start of the experiment animals remained in tie-stalls for 12d to become accustomed to the diet and restriction in movement. After this period, animals were housed in one of two identical RCs to determine gaseous exchange, energy balance, and diet digestibility. The experimental unit for data measured in the RCs (e.g., CH₄ production, diet digestibility parameters) therefore consisted of a pair of cows. Animals were fed one out of 4 different diets at equal portions twice during milking. Feed intake was restricted per block to 95% of the *ad libitum* feed intake of the animal consuming the lowest amount of feed during d5 to d8 (i.e. still in the tie-stall) within a block (Van Zijderveld et al., 2011). This is not completely comparable with Dutch circumstances, where cows are usually fed roughage *ad lib*. The diet consisted of 40% grass silage, 26% corn silage, and 34% concentrates on a dry matter basis, which is comparable to Dutch circumstances.

Methane data were provided as estimates of daily CH₄ production for the pair of cows. These data were converted back to three minute CH₄ yields in liters per cow by division by 960, there being 480 three minute periods in a day.

Proxies for intake measurement

Since intake of individual ruminants in a grazing environment remains a major challenge, the question of whether a proxy for feed intake (even relative level of intake) may exist and could be used in estimating MY of individuals is important. If not, it may be that a MY trait can only be determined under controlled feeding circumstances. A potential intake related parameter that is easily collected while measuring CH₄ emissions even when intake is not measured, is CO₂ production, and possibly O₂ uptake.

From the study of emissions by sheep fed at three levels of intake (Robinson 2009), CH₄ and CO₂ production rates were (for a hours) proportional to substrate supply i.e, feed intake. This observation deserves further exploration.

Alternate methods of selection

Methane emissions (as g CH₄/d or MY) certainly fit the description of hard to measure traits. Methods currently available are expensive and time consuming (RCs, SF₆) and subject animals to artificial environments. Those that measure animals in production situations (pasture, feedlot or dairy feeding station) sample CH₄ for only a part of a day and require repeat measurements (PACs, Sniffers, GEM) and in some cases calculation back to known standard procedures. Those methods of estimating CH₄ emissions that rely on computation of differences between feeding standards and production account for only part of the potential variation in CH₄ emissions between animals.

799 Genomic selection opens the possibility to efficiently select for hard to
800 measure traits. It is increasing being used to increase rate of genetic progress
801 for production traits that are measured late in life (e.g. meat yield and quality),
802 expensive to measure (e.g. RFI) and are sex linked (e.g. milk production and
803 quality). In the dairy and increasingly in the beef and sheep industries leading
804 sires are routinely genotyped and genomic breeding values (GEBVs) are used
805 in making selection decisions. It is doubtful that adding the cost of genotyping
806 onto a population in which CH₄ is measured would be cost effective, but by
807 using industry animals which have measured production traits and have been
808 genotyped it would be possible to estimate genomic breeding values for CH₄
809 emissions. This is predicated on having a large reference population, where
810 CH₄ emission levels are measured and genome wide DNA marker effects
811 have been estimated (e.g. to establish the prediction equation for marker
812 effects).

813 The key question is how large does this reference population have to be,
814 that is how many animals need to be measured for CH₄ and genotyped for the
815 genome wide marker panels? Both Daetwyler et al. (2009), Goddard (2008)
816 and Hayes et al. (2009) derived deterministic formula to estimate the accuracy
817 of GEBV that could be achieved given the size of the reference population,
818 the heritability of the trait and the effective population size. The accuracy of
819 genomic selection for selection candidates (i.e. animals with a genotype, but
820 no measured phenotype) with increasing size of reference population is
821 shown in Figure 10. This was derived from the heritability of MY of 0.13
822 (reported in Table 1) and an effective population size of 150 using the
823 procedure described by Hayes et al (2009). Figure 14 shows the accuracy of

prediction as a function of the number of animals in the reference population. Because MY is a new trait, it would be anticipated that even low initial accuracy will be useful to industry. As further animals are phenotyped the GEBVs would become increasingly useful. It remains to be determined if MY is independent of other (production) traits. If it is then adding information from the GEBVs for MY into a selection index is relatively straightforward.

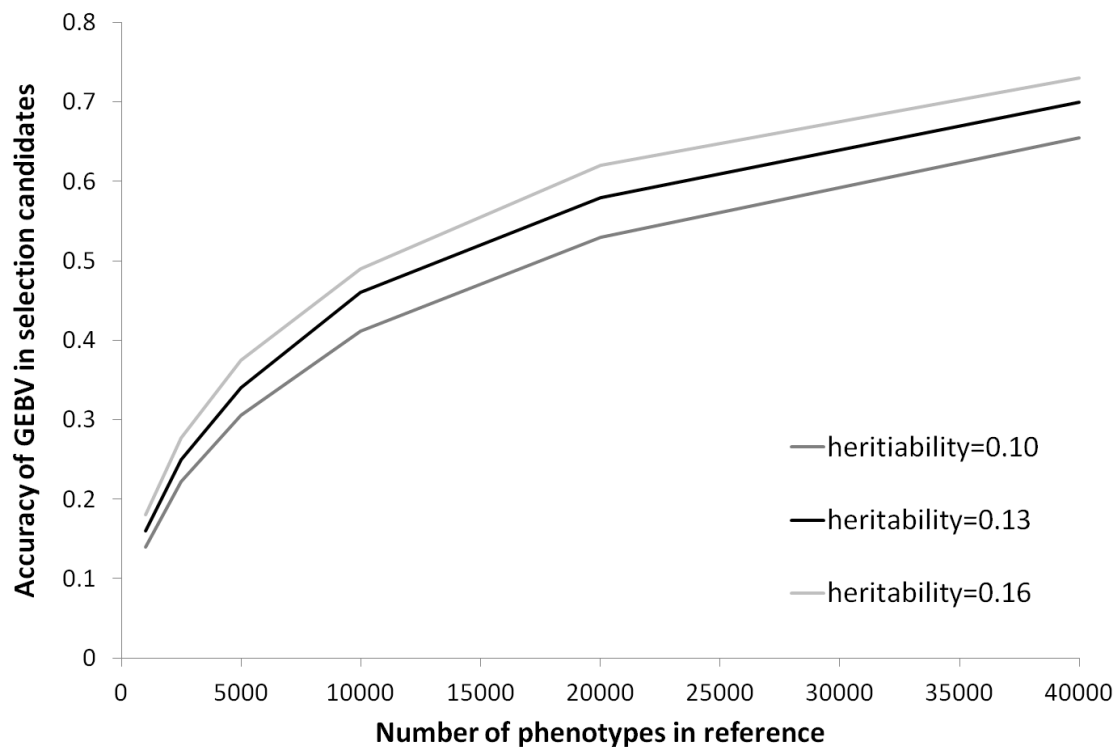


Figure 10. Accuracy of Genomic Breeding Values (GEBV) for methane yield in selection candidates as a function of heritability of the trait and number of animals with phenotypes in the reference population. Estimates of heritability of methane yield in sheep were obtained from Pinares-Patiño et al, (2013a).

838

839 The numbers of animals with phenotypes in the reference population
840 required to obtain GEBVs of high accuracy for MY are large and almost
841 certainly exceeds the resources available to any one country. However, the
842 research community has considerable experience with combining data from
843 different countries to enable initial estimates of GEBVs for traits such as milk
844 production, residual food intake and carcass traits. The challenge for the
845 community now working on CH₄ related traits is to establish measurement
846 procedures for phenotyping animals that can be combined to facilitate
847 estimation of genetic parameters and GEBVs in particular. The ASGGN
848 provides a forum to encourage such collaboration.

849

850 **Conclusions**

851 From this review of published and unpublished material the following
852 observations are made:

- 853 • Methane emissions are a heritable and repeatable trait.
- 854 • Repeated measurements add value, preferably separated by at least 3-
855 14 days.
- 856 • Methane emissions are strongly related to feed intake both in the short
857 term (minutes to several hours) and over the longer term (days).
- 858 • When measured over the long term methane yield (g CH₄/KgDMI) i.e.
859 CH₄ corrected (adjusted) for feed intake is a heritable and repeatable
860 trait albeit with less genetic variation than total CH₄ emission (g CH₄/d).

- Methane emissions of individual animals are moderately repeatable across diets, and across feeding levels, when measured in RCs. Repeatability is less when short term measurements are used, possibly due to variation in time and amount of ingested feed prior to the measurement. This needs to be investigated further.
- Given the above issue is resolved, short term (over minutes to hours) measurements of CH₄ emissions show promise. However we believe that for short term measurements to be useful, for genetic evaluation, a number (between 3 – 20) of measurements will be required over an extended period of time (weeks to months).
- Opportunities exist for “brief measurements” in standardised feeding situations such as “sniffers” attached to milking parlours or total mixed ration feeding bins, but we anticipate these are also subject to the caveats above about use of short term measurements.
- The measurement “protocol” (i.e. how the animal and its feeding behaviour are managed prior to measurement) is more important than the technology used to make the CH₄ measurement.
- While there is evidence that correlated and predictor traits exist for CH₄ emissions the current level of knowledge is insufficient to recommend their use in genetic selection to reduce CH₄ emissions.
- Genomic selection offers potential for use to reduce CH₄ emissions and methane yield, however, measurements on thousands of individuals will be required.

- In summary we consider genetic and genomic selection offers a significant opportunity to reduce CH₄ emissions from ruminants. However attention needs to be directed to a number of issues if brief low cost measurements are to be implemented in industry.

Recommendations for further work

As yet we have insufficient knowledge of the phenotypic and genetic correlations between CH₄ measurements made under different protocols (or methodologies), to be confident about how we combine such data. This will, at least in the short term, lead to different estimates of genetic parameters for CH₄ emission traits from different laboratories due to the measurement protocol/methodology employed. This is to be expected, because the cost of measurement of a trait will clearly affect the number of animals able to be measured and low cost, accurate measurement procedures/protocols/methods will be sought. Different measurement protocols/methodologies may not impede genetic progress with selection for CH₄ traits in national or commercial programs (e.g. a breeding company). However, use of different measurement protocols in different countries or species will almost certainly make pooling of data less efficient, and increase costs globally. An additional consideration relates to how the IPCC process for accounting for genetic change in enteric emissions is implemented. The IPCC process utilizes peer reviewed publications to change to its accounting rules. We, the ASGGN, can help by providing leadership as to how best to include inherited differences in either feed intake or CH₄ yield into the accounting framework for enteric emissions.

909 The above leads to the following recommendations for further work,

- 910 • Wherever possible measurement protocols used to obtain genetic
911 parameters are compared with a standardised protocol. Ideally this
912 should be to a level where heritabilities, repeatabilities and genetic
913 correlations with key traits e.g. live weight and intake can be
914 estimated from both techniques. At the minimum a comparison of
915 measurement repeatability across time, both within and between
916 measurement protocols is essential.
- 917 • Establish a process to enable at least meta-data of different
918 measurement protocols to be shared across research groups in
919 different countries. This could be extended across species.
- 920 • Encourage development of an international R & D project to analyse
921 joint data sets and make recommendations that lead to improved
922 lower cost protocols for measurement of CH₄ emissions that can be
923 employed in member countries. This would prepare the community
924 for development of preliminary genetic parameters and GEBVs to
925 act as a catalyst for local/national development of breeding
926 solutions for reduced emissions of CH₄ from farmed ruminants.
- 927 • Continue to explore methods that use proxies of feed intake
928 measured over the same time frame as CH₄, for example CO₂
929 output and O₂ uptake, to estimate MY. Establish relationships
930 between proxy measures of MY and reference methods and the
931 total CH₄ production/time measured on animals on pasture.

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934

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