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Animal Selection, Genetics & Genomics Network White Paper

Consensus methods for breeding low methane emitting animals

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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.

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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. The purpose is to evaluate methods that are potentially useful for measuring CH_4 emissions in individual animals so as to estimate genetic parameters and subsequently screen animals for use in selective breeding programs including its use in the development of genomic selection.

This report shows:

- Methane emissions are a heritable and repeatable trait in sheep and cattle.
- Repeated measurements of CH_4 emissions on individual animals add most value when separated by at least 3-14 days.
- Methane emissions are strongly related to feed intake especially in the short term (up to several hours) and less so in the medium term (days).
- When measured over days, in respiration chambers, CH_4 yield (MY: g CH_4/kg DMI) and CH_4 adjusted for feed intake are heritable and repeatable traits albeit with less genetic variation than total CH_4 emission (g CH_4/d).
- Repeatability estimates are lower 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 issues are resolved, short term (over minutes to hours) measurements of CH_4 emissions show promise. However, we believe that for short term measurements to be useful for genetic evaluation, multiple 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 likely to be more important than the technology used to make the CH_4 measurement.
- While there is evidence that correlated and predictor traits exist for CH_4 emissions, the current level of knowledge is insufficient to recommend the use of predictor traits in genetic selection to reduce MY or methane production (g CH_4/day).
- Currently, we have incomplete knowledge about the genetic relationships between CH_4 and production traits. This needs to be addressed before commercial implementation is contemplated. That said, to date there is no evidence of significant antagonistic relationships between production and CH_4 emitted per unit of production.
- Genomic selection offers potential to reduce CH_4 emissions and MY, however, CH_4 measurements on thousands of individuals will still be required to develop training sets with sufficient predictive accuracy.
- The “size of the prize” when combining lower MY with selection for low residual feed intake (RFI) may result in a reduction in methane emissions of 40-45% and may be possible through selection of individual animals on components that directly affect methane production.
- In summary, we feel genetic and genomic selection for CH_4 emission reduction offers a significant opportunity, but attention needs to be directed to a number of issues associated with brief, low cost measurements before it is to be implemented in industry.

A further caveat is we have insufficient knowledge of the phenotypic and genetic correlations between CH_4 measurements made under different protocols (or methodologies) to be confident about how to combine such data. This is to be expected, because the cost of measurement of a trait will clearly affect the number of animals able to be measured. Different measurement protocols/methodologies may not impede genetic progress for CH_4 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 its accounting rules. We, the ASGGN, can help by providing leadership as to how best to include inherited differences in either feed intake or CH_4 emission trait into the accounting framework for enteric emissions.

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

- Measurement protocols used to obtain genetic parameters are compared with a standardised protocol. This needs to be undertaken to a level where heritabilities, repeatabilities and genetic correlations with key traits e.g. live weight and feed intake, can be estimated from both (standardised protocol and other measurement protocol). At the minimum, a comparison of measurement repeatability across time, both within and between measurement protocols is essential. With the assumption the protocols 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 supporting development of genomic estimated breeding values (GEBVs) that can be employed in member countries.
- Exploration of the tripartite between CH_4, feed intake and animal production and between the three CH_4 traits; gross CH_4, MY and CH_4 emission intensity.
- Continue to explore methods that use proxies for feed intake measured over the same time frame as CH_4, for example CO_2 output and O_2 uptake, to estimate gross CH_4, MY and CH_4 intensity. Establish relationships between proxy measures of MY and reference methods and the total CH_4 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. Livestock activities contribute approximately 9-11% of total anthropogenic GHG emissions (Smith et al., 2007; Tubiello et al., 2013). Of the various GHG, CH_4 is the most important contributor, with a global warming potential 25 times that of carbon dioxide (CO_2).

Globally GHG emissions from the agriculture sector accounted for 4.6 $GtCO_2$-eq/yr in 2010, of which enteric fermentation (emissions of CH_4 by ruminant animals) contributed 2 $GtCO_2$ 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 CH_4, followed by dairy cattle, buffaloes, sheep and goats (Figure 1). Averaged over 2000 to 2010, the largest regional contributors to global enteric CH_4 production were Asia and the Americas (Figure 2). There was an increase in annual enteric emissions in all regions except Europe and Oceania (FAOSTAT, 2013).

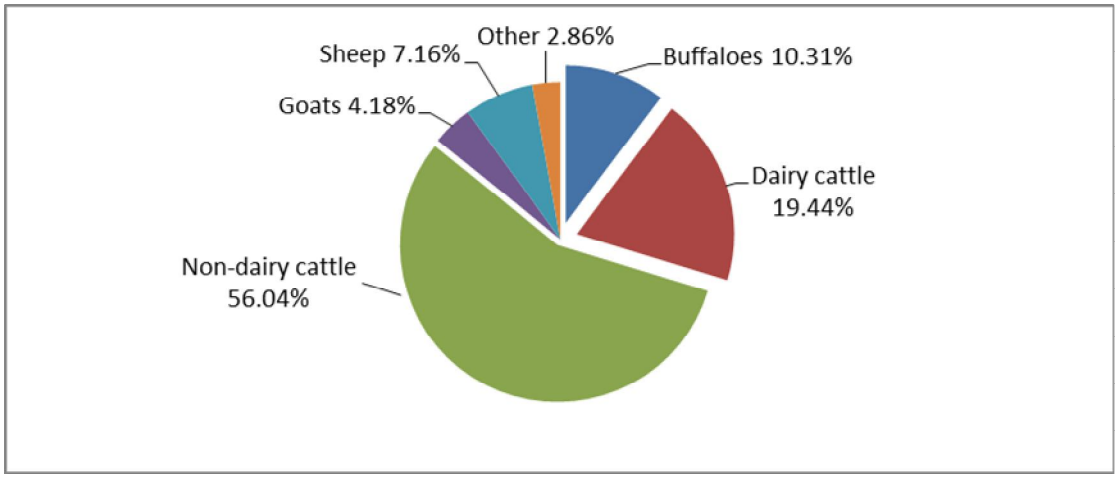

Figure 1. Contribution of different animal species and cattle types to global livestock enteric methane emission (source FAOSTAT, 2013).

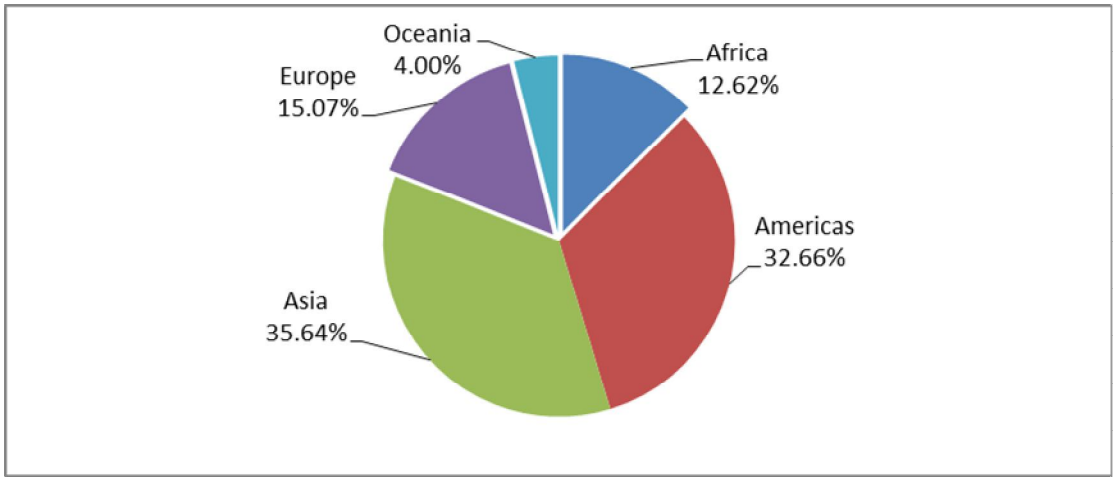

Figure 2. Contribution of region to global enteric methane production (source FAOSTAT, 2013).

Enteric CH_4 production by ruminant livestock (cattle, sheep and goats) accounts for 2 to12% of gross energy intake (Blaxter, 1962; Johnson and Johnson, 1995). Although CH_4 production is an energy loss to ruminants, it can also be considered a small price to pay for their adaptation to digest cellulose based feeds. Sources of systematic variation in CH_4 production by an individual animal include: total feed intake, the nutrient composition of the feed eaten, the proportion and rate of fermentation of that feed in the rumen (for recent reviews see Hristov et al., 2013a; 2013b), rumen volume and rate of passage of digesta from the rumen (Goopy et al., 2013), physiological state of the animal and variation between individual animals including that between sire families (Pinares-Patiño et al., 2013a).

Production of CH_4 (and other GHGs) per unit of production has declined over the past 50 years in most ruminant livestock industries in developed countries due to ongoing improvements in productivity. For example, the carbon footprint, in terms of CO_2-eq/billion kg of milk produced, of the US dairy industry in 2007 was 37% of that in 1944 (Capper et al., 2009). In 1944 there were 25.6 million cows producing 53 billion kg milk per annum compared to 9.2 million cows producing 84.2 billion kg milk per annum in 2007. Productivity improvements included a change of breed type of the dairy cow (to Holstein), improved genetics within the Holstein breed and a shift from a forage based to total mixed ration feeding system (see Capper et al., 2009). Analysis of the carbon footprint of total US beef production indicates a reduction of CO_2-eq of 16% per

billion kg of beef produced in 2007 compared with 1977 (Capper, 2011), due to a reduction in total feedstuff used, changed industry structure, improved nutritional management and improved herd genetics.

The extent to which genetic improvement can contribute to improvement in individual animal milk production and consequent impacts on GHG emissions has been highlighted by Wall et al (2010). They describe how systematic improvement in environmental outcomes has resulted from productivity improvements and discuss how direct and indirect measures of emissions can be incorporated into breeding objectives to reduce emissions.

There are 3 levels in which a methane trait can be defined (Figure 3). 1. The farm system level which uses information on the number of animals present within a system boundary with a related estimate of CH_4 emissions per head, calculated for example from the IPCC (2006) Tier 2 calculations. These calculations have embedded within them a number of assumptions about the factors which affect CH_4 per head, i.e. feed intake, feed quality and CH_4 yield. 2. The animal production level which uses information about productivity per head i.e. milk yield or kg carcass weight, from individual animals to give us CH_4 intensity (g CH_4 /kg product). 3. At the animal level, individual CH_4 emissions and feed intake measurements to enable genetic progress on CH_4 yield (g/d) or RFI.

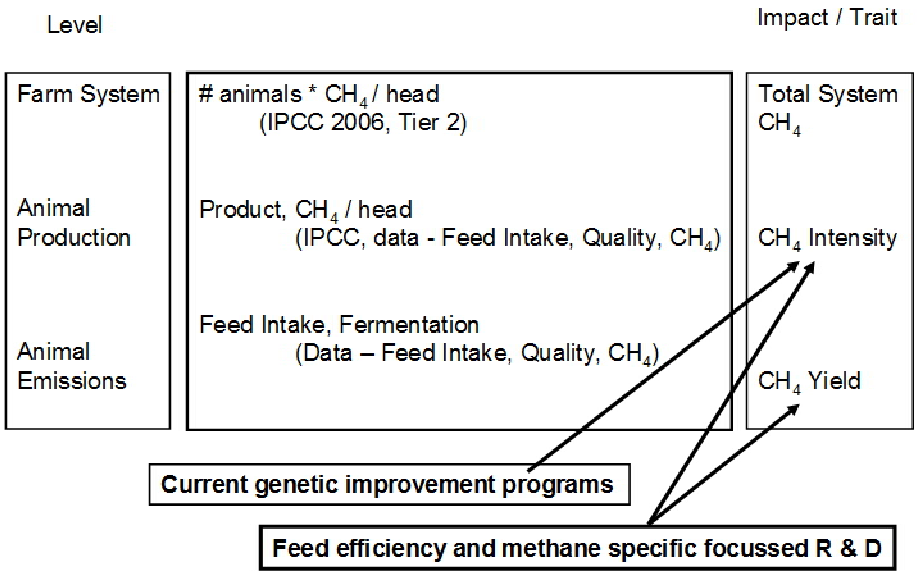

Figure 3. Levels at which a methane trait can be defined.

There are many potential methods to reduce enteric CH_4 emissions per head and thereby intensity of CH_4 production per unit product. These include: changing feed type (for example from pasture to concentrate feed e.g. Capper (2012) or to new pasture varieties), use of supplements that reduce CH_4 emissions (fats, oils, plant extracts and nitrate), improving productivity through management change including use of growth enhancers and improved genetics, immunisation against methanogens and selective breeding of animals with low methane emissions, through either reduced feed intake per product or reduced CH_4 production per feed consumed, without compromising production characteristics (Williams et al., 2009; Eckard et al., 2010; Martin et al., 2010; Wall et al., 2010; Cottle et al., 2011).

In addition to reduction in intensity of CH_4 production per unit animal product as a consequence of selection for improved productivity, selection for reduced methane per head either through reduced feed intake (e.g. selection for residual or net feed intake) or reduced CH_4 per kg feed eaten (selection for a low MY) are potential additional strategies. It has been shown that selection for residual feed intake (less feed per live weight and live weight gain) in beef cattle results in lower total emissions of CH_4 per head and a small non-significant increase in MY (Hegarty et al, 2007). Selection for low MY has been demonstrated in sheep (Pinares-Patiño et al., 2013a).

Although genetic selection is possible the potential magnitude of combined selection for reduced feed intake (through improved efficiency of feed use) and reduced MY is unknown. Differences in feed intake of 1.17 kg/d between beef cattle selected for and against RFI were observed after 2.4 generations of single character selection on RFI, this is equivalent to a difference of 18g CH_4/d around a mean 180g CH_4/d, or a 10% difference (Hegarty et al., 2007). Pinares-Patiño et al (2013a) report a difference of 8% in methane yield (g CH_4/kg DMI) between sheep after 1 generation of selection for and against methane yield. The extent to which variation in residual feed intake and MY can be exploited depends on the stability of the underpinning relationships. For example, selection for low RFI in beef cattle is associated with a small but inconsistent reduction in fatness (rg = 0.49 to -0.30; Arthur and Herd, 2012). This is favourable so long as there are no adverse consequences on either reproductive performance of the female

on market specifications for slaughter stock or on fitness. While index selection can be used to restrain these changes progress in the trait of interest will be reduced. The extent to which differences in RFI resulting from ongoing selection can be projected into the future is unknown, but is unlikely to exceed 25% (R.M. Herd, pers comm.). The lower limit of MY potentially attainable by selection is unknown. The mechanisms that contribute to genetic variation in MY of individual animals may include: reduced fermentation of organic matter in the rumen (due to shorter retention time of digesta; (Pinares-Patiño et al., 2011) and smaller rumen volume (Goopy et al., 2013)), instability of fermentation (natural occurring defaunation; Faichney and Graham, 1996), different microbial population in the rumen and potentially reductive acetogenesis (inferred from Faichney et al., 1999). The extent to which these combine to produce natural variation in MY is unknown, but data from measurements of MY in sheep using RCs suggest that the coefficient of variation is 10.3% (Pinares-Patiño et al., 2013a) and for cattle is 14% (Donoghue et al., 2013). It would not be unreasonable to anticipate a response to long term selection to exceed 2 standard deviations from the mean, suggesting that a reduction of up to 25% in MY may be feasible through selection of livestock for low MY. Combined with potential reduction in CH_4 emissions due to selection for low RFI, this suggests that a reduction in CH_4 emissions of 40-45% may be possible through selection of individual animals on components that directly affect CH_4 production. It remains to be seen if this is independent of animal productivity traits, although in practice, selection for reduced feed intake and CH_4 emissions will be conducted using an index that includes production traits.

The trade-off between the effect on CH_4 emissions as a consequence of selection for low RFI (reduced feed intake for the same level of production) and potential effect on MY was modelled as follows. Whole-farm greenhouse gas (GHG) emissions from four western Canadian beef production systems were determined using data outlined by Basarab et al (2012), and following IPCC Tier 2 methodology (IPCC, 2006) and modified for nitrogen excretion according to NRC (2000). Farm GHG emissions included enteric CH_4, manure CH_4 and nitrous oxide (N_2O), cropping N_2O and energy use CO_2. A baseline simulation resulted in carbon intensities of 21.09, 19.87, 22.52 and 21.21 kg CO_2e-/kg carcass weight for calf-fed

hormone free, calf-fed implanted, yearling-fed hormone free and yearling-fed implanted beef production systems, respectively (Basarab et al., 2012). A 10% reduction in DMI at equal productivity was simulated to reflect a 10% improvement in feed efficiency due to selection for low RFI. This scenario (1) resulted in carbon intensities of 19.22, 18.10, 20.54 and 19.34 kg CO_{2}-e/kg carcass weight for calf-fed hormone free, calf-fed implanted, yearling-fed hormone free and yearling-fed implanted beef production systems, respectively, or an average reduction in carbon intensity of 8.85% compared with the baseline scenario. A second scenario (2) was simulated to reflect a 10% decrease in DMI at equal productivity and where a 10% decrease in DMI leads to a 1.4% increase in the MY following the general equations of Blaxter and Clapperton (1965). In this case, increased MY resulted from slower rate of rumen passage and more hydrogen ions being available for methanogenesis. This scenario resulted in carbon intensities of 19.29, 18.16, 20.62 and 19.41 kg CO_{2}-e/kg carcass weight for calf-fed hormone free, calf-fed implanted, yearling-fed hormone free and yearling-fed implanted beef production systems, respectively, or an average reduction in carbon intensity of 8.55% compared with the baseline scenario. The difference between Scenario 1 and 2 was small (0.3 percentile points), indicating that the rise in MY associated with reduced feed intake will do little to offset the drop in CH_4 production (g CH_4/day) resulting simply from less substrate; so improving RFI is still a good strategy for reducing GHG emissions in beef cattle. In addition, the difference may be even smaller since DM and crude protein digestibility has been reported to be improved by 1-2% percentile units in low RFI beef cattle (Nkrumah et al., 2006; Gomes et al., 2013) and dairy cattle (Rius et al., 2012).

Breeding to reduce methane emissions from livestock.

Selective breeding for reduced emissions, with no loss of productivity, may be a mitigation strategy which could deliver a permanent reduction in CH_4 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 emissions from 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), CH_4 emissions are not routinely measured in livestock.

To implement a breeding program the trait needs to be measured. The trait should demonstrate, at least in simulation modelling, that it can achieve the intended mitigation if implemented by industry. The trait must be shown to be heritable, have a reasonable amount of genetic variation and readily measured in at least research situations. Further, there is a need to identify and quantify any associations between CH_4 emissions and production traits. The expected genetic progress in reducing emissions while, at the same time, maintaining or improving other traits is desirable. Once established that a new trait such as methane emissions is feasible, it may be implemented by direct selection using CH_4 measurements. Alternatively, if there are strong genetic correlations between a CH_4 emissions trait and heritable indicator traits that can be readily measured in the industry, then the correlated trait may be used for indirect selection. In the first instance, the genetic correlation of CH_4 trait with indicator trait(s) must be confidently established on an appropriate population. Or it may be possible to incorporate genomic information to estimate genomic breeding values (GEBVs) for CH_4 emissions into breeding schemes (Meuwissen et al., 2013). For GEBVs to be implemented, a reference population of several thousand genotyped industry relevant animals, with the CH_4 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, selection on GEBV for correlated indicator traits can be used where it is impractical to directly measure CH_4 on enough animals to establish a reference population. 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 CH_4 trait receives the appropriate weighting in any breeding program.

Utilising all technologies and ignoring cost, genetic selection (either direct from recorded phenotype, or utilising genomic techniques) provides a reliable route towards permanent and cumulative reductions in enteric CH_4 emissions. There are a number of considerations in defining

a CH_4 trait for genetic selection. It is known that there is already on-going improvement in intensity i.e. yield of CH_4 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 (i.e. selection for reduced intensity of methane emissions) is not necessary. However, selection solely on productivity traits such as live weight gain and/or milk production will increase feed intake and CH_4 emissions per animal and hence total CH_4 emissions unless a constraint is imposed on total emissions. 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 CH_4 emissions per animal at the same time?" This could be achieved by reducing MY, provided that there is no concomitant reduction in productivity. Selection on MY 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. Early results from a number of studies around the world, suggest MY 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 CH_4 production is still largely unknown. The extent to which genetic selection can be used to reduce MY is also not known. The methods by which CH_4 emissions of individual animals can be measured are an important factor because the method used to measure the CH_4 trait will also influence the resulting genetic parameters and is therefore an integral part of the selection program. We anticipate that the CH_4 emission trait will be implemented as part of a selection index.

In this manuscript we outline what is known about the host animal factors which potentially affect production of CH_4, ***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 CH_4 emissions without detriment effect on other production traits***. 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 CH_4 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 CH_4 production was apparent when reviewed in 2004 (Hegarty, 2004)**.** The prospect for selection for a CH_4 trait was initially investigated by multiple groups; some identified variation in CH_4 traits amenable to animal selection (Robinson et al., 2010) and some did not (Münger and Kreuzer, 2008). More recent research in beef (Donoghue et al., 2013) and sheep (Pinares-Patiño et al., 2011a; 2013a) is increasingly supportive of CH_4 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 CH_4 across days, rounds and years, using the total 24hr measurement are shown in Table 1. There were high repeatabilities across consecutive days. Across rounds and across years the repeatability estimates were lower than for consecutive days, but, relatively stable. Estimation of genetic and phenotypic correlations with some of the main New Zealand production traits; weaning weight, live weight at 8 months, fleece weight at 12 months (FW12), eye muscle depth and dag score (accumulation of faeces on the perineum region) at 3 or 8 months of age are shown in Table 2. Correlations with MY (gCH_4/kg dry matter intake (DMI)) are low or close to zero, only exception was 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 CH_4 emissions expressed as gCH_4/kg DMI.

Table 1: Heritability (h^2) and repeatability estimates (± standard errors; s.e.) for methane emission traits in sheep (Pinares-Patiño et al., 2013a).

Trait	n records	mean	σ_p	h^2 ± 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

CH_4: methane; DMI: dry matter intake
#: rounds were measured 2 weeks apart

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

Trait	single trait analysis					2-trait with gCH4/day		2-trait with gCH4/kgDMI	
	n records	mean	σ_p	direct h^2 ± s.e.	dam h^2 ± 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); CH_4: 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.

Results from Donoghue et al (2013) on Australian Angus beef cattle showed very similar heritabilities. Based on 530 pedigreed cattle, fed at a proportion of maintenance (1.2x), heritability estimates for gross methane (L/d), and MY (L/kgDMI) were 0.40 ± 0.11 and 0.19 ± 0.10, respectively. Genetic and phenotypic correlations of gross methane with eye muscle area were 0.17 ± 0.29 and -0.01 ± 0.05, respectively. With MY, the genetic and phenotypic correlations were -0.02 ± 0.30 and -0.03 ± 0.05, respectively.

While data in Tables 1 and 2 and from Donoghue et al (2013) are based on 24hr respiration chamber (RC) measurement with known feed intake, the cost of routinely measuring methane emissions using respiration chambers is thought to be prohibitive for a testing program using industry animals. Therefore, protocols for measuring or estimating CH_4 production and feed

intake that require less time and cost need assessment. To inform development of these protocols, an overview of variation in CH_4 production and feed intake are described.

Methodologies for measurement of methane from Ruminants

Before considering short term breath-based measures, it is worth considering the constraints of the respiration chamber (RC) system that is often viewed as a 'gold standard' for emission measurement. There is little question RC measurements accurately quantify CH_4 output over the 1-3d measurement period typically used, and they achieve this by frequently monitoring emissions. The variability in emission rate resulting from eructation cycles, animal position and feed intake that occur in 24hr, are typically damped within the large chamber volume. However, even if emission rate was monitored every second, a 1-3d collection seems unlikely to describe the CH_4 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, 2004). However, RC rarely monitor CH_4 outflow on a second by second basis, the chambers used to estimate CH_4 parameters, for example those reported in Table 1, do so by measuring volume of air flow coupled with intermittent samples of CH_4 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, CH_4 is largely emitted intermittently via brief eructations or burps lasting only seconds, albeit with a basal level of emission. Using respiration chambers with intermittent sampling as described above has produced estimates of repeatability of 0.53 and 0.24 for gCH_4/d and MY across years, respectively (Table 1). Such results indicate that the high frequency emission monitoring in a RC over a single 1-2d period does not by itself adequately describe the long term emission rate of methane in an individual animal.

The SF_6 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 SF_6 from the permeation tube is no longer stable. While repeatability of daily CH_4 production is being improved as the methodology is refined (Deighton, et al., 2013), SF_6 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), monitoring eructations in feeding stations (Negussie et al., 2012) or voluntary milking systems for cattle (Garnsworthy et al., 2012; Lassen et al., 2012). 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 CH_4 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, SF_6, 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 CH_4 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 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 gross 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. Alternately, spot breath samples taken during 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 would allow

merging of data to generate 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 to perform selective breeding.

In summary, there are three biological factors affecting CH_4 measurement outside of the technology itself: variation within short periods within a day, variation across time and the influence of the animal containment, diet selection and feeding regime. Understanding these factors requires knowledge of the digestion process.

Understanding animal variation in methane production over time

Sources and transfer of methane within the ruminant

While CH_4 is produced in both the reticulo-rumen and the hindgut, some transfer within the animal occurs before the CH_4 is emitted. For example, in ewes eating lucerne, 97.5% of CH_4 emission was via the oesophagus and lungs and only 2.5% via flatus; 23% of CH_4 production occurred in the lower gut and most (89%) of this hindgut CH_4 is absorbed and subsequently excreted via the lungs, presumably after absorption into the blood (Murray et al., 1976). The proportion of CH_4 derived from the hindgut increases with feeding level while the proportion in rumen decreases (Murray et al., 1978; Hofmeyr et al., 1984). Most of the CH_4 leaving the rumen in oesophageal eructation is subsequently drawn into the lungs and then emitted in exhaled breath. This has been confirmed by dosing and radiotracer studies (Dougherty et al., 1964; Heywood and Wood, 1985). Some rumen produced CH_4, is also absorbed directly into the lungs without passing back 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). Distinct emission peaks carrying both CO_2 and CH_4, at 40-60 second intervals, were apparent when cattle were measured by a GreenFeed emission monitor (GEM; www.c-lockinc.com). The frequency of eructation peaks was reduced when drinking (Hegarty, 2013).

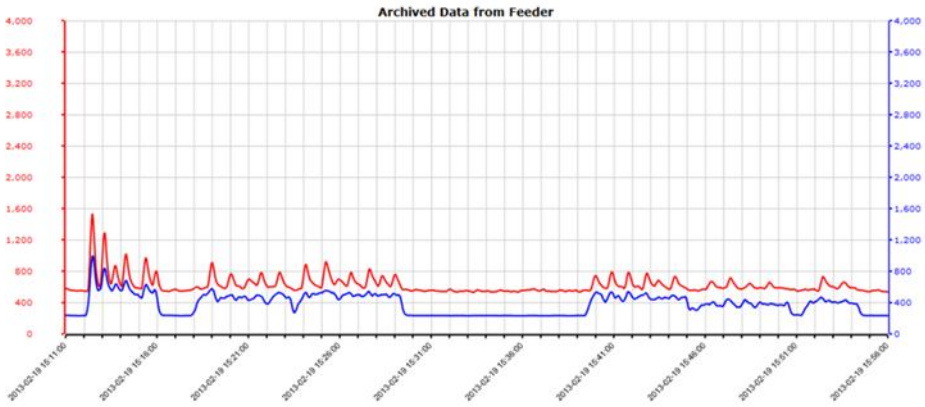

Figure 4. Example pattern of Methane (red) and Carbon dioxide (blue) concentrations in breath of a cow measured using the Greenfeed Emission Monitoring system (Courtsey of R. S. Hegarty, 2013).

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 CH_4 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, absorption of CH_4 from the rumen and subsequent exhalation is an important source of CH_4 excretion, but it is highly variable between animals. However, irregularities in emission occur, as evidenced by the large oscillations in CH_4 release rate (but not necessarily methanogenesis rate) observed during CH_4 measurements. 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 CH_4 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 CH_4 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”, so emission rates were averaged, over various periods, to generate smoother emission profiles. Even with slowly fermented high-fibre diets, such short term variations in emission (not necessarily production) are apparent (e.g. McCrabb and Hunter, 1999; Figure 6).

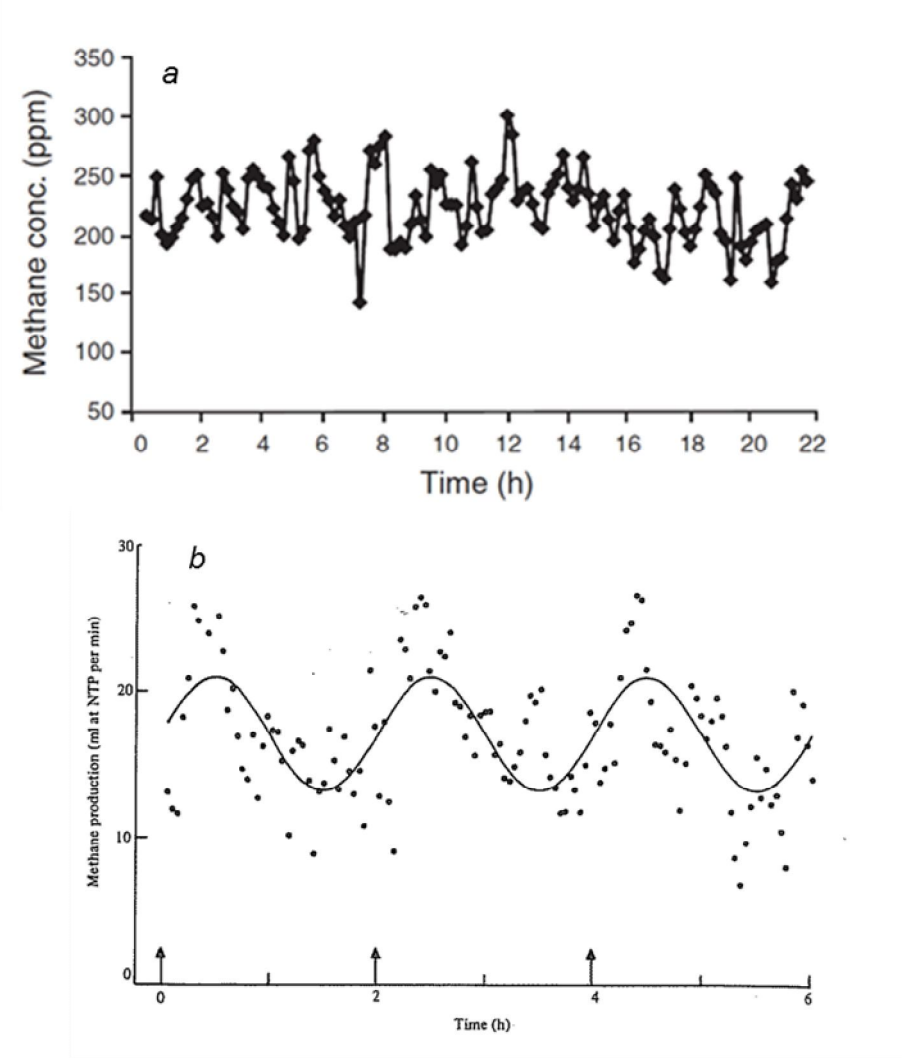

Figure 5. Time course of **a**) methane concentrations (ppm) in respiration chambers (reproduced Nolan et al., 2010, figure 1a), and **b**) methane production (ml/min) (reproduced from Mathers and Walters, 1982, figure 2a), of sheep fed using an automated feeder at 2-hourly intervals.

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.

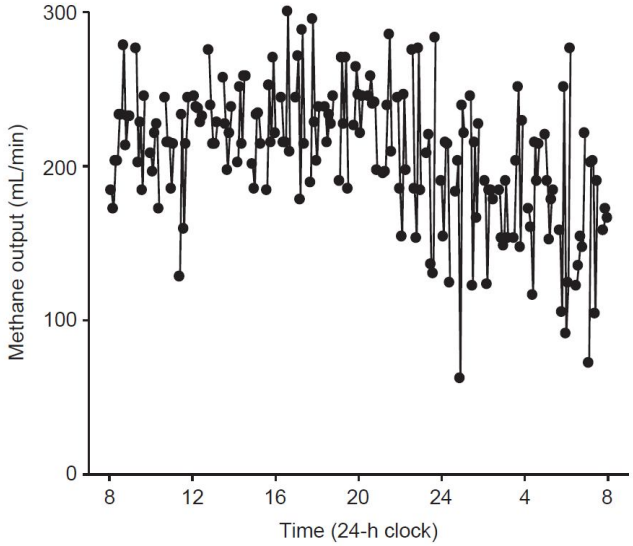

Figure 6. Pattern of methane emissions from a Brahman steer fed *ad-libitum* Rhodes grass diet once at 0800hrs (reproduced from McCrabb and Hunter, 1999, figure 1)

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 CH_4 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.

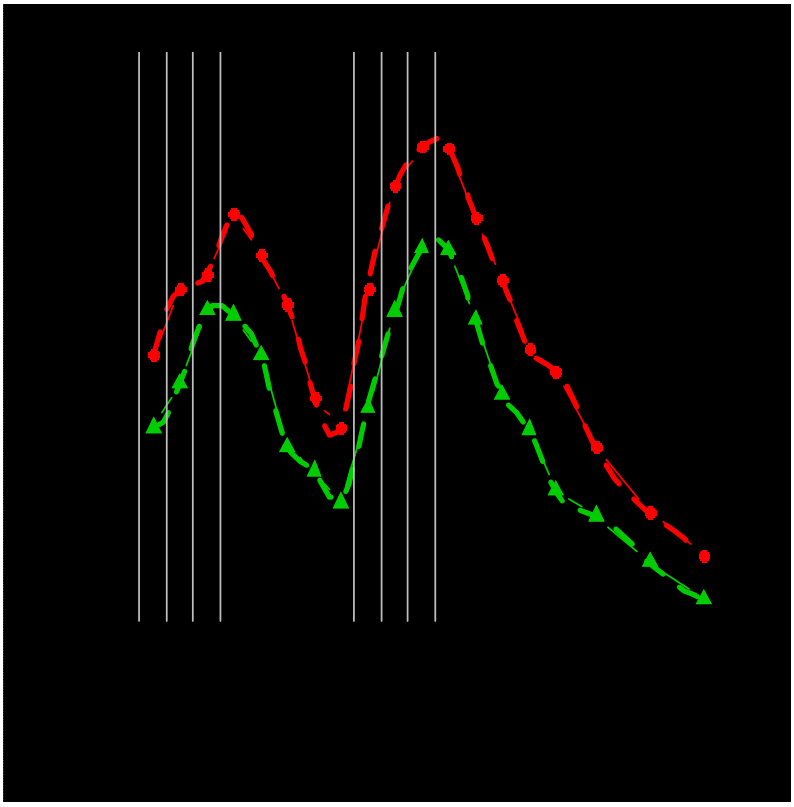

Figure 7. Production of methane from sheep fed three levels of chaffed lucerne hay (0.7, green; 1, red; and 1.3, black, times maintenance requirements) presented hourly for 4 hours starting at 0 and 8hrs from start of first feed (Generated from Robinson, 2009).

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; Münger 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 CH_4 production and of MY were 0.55 and 0.26, respectively (Table 1) and repeatability declined as the period between measurement increases.

Table 3. Summary of methane measurement experiments in cattle, including average emissions (± 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	gCH_4/d	124.3 ± 11.1		0.49	NZ	Vlaming et al., (2008)
					gCH_4/kgDMI	22.8 ± 2.0		0.47		
			Cereal, lucerne and straw mix at M	30 days	gCH_4/d	169.8 ± 11.0		0.73		
					gCH_4/kgDMI	32.0 ± 2.0		0.73		
93 Dairy cows	FTIR – AMS	50 H	TMR *ad lib*, concentrate[d]	3 days	CH_4 ppm/milking			0.34 ± 0.01	Denmark	Lassen et al., (2012)
					CO_2 ppm/milking			0.46 ± 0.00		
					CH_4:CO_2 ppm/milking	0.065	across visits to AMS	0.37 ± 0.01		
					CH_4 ppm/milking			0.33 ± 0.01		
		43 J		3 days	CO_2 ppm/milking			0.40 ± 0.01		
					CH_4:CO_2 ppm/milking	0.05		0.33 ± 0.00		
30 heifers	RC	H, J and S	Forage *ad lib* plus concentrate[e]	6 periods of 3 days[h]	LCH_4/d			0.13	Swiss	Münger and Kreuzer (2008) data supplied
					LCH_4/kg DMI			0.039		
					DMI kg/d		across stage of lactation	0.12		
					LCH_4/kg DOMI			0.019		
					LCO_2/d			0.069		
					CH_4/CO_2			0.25		
					LWT			0.65		
18 Yearling bulls (10 mo)	GEM	Charolais	Pellets[f]	42 days	gCH_4/d	233 ± 55		0.61	France	G. Renand pers. comm.
					gCO_2/d	5406 ± 738		0.75		
					kgDMI/d	6.06 ± 1.14	across days	0.22		
					gCH_4/kg DMI	39.8 ± 11.6		0.58		
					CH_4/CO_2	0.043 ± 0.009		0.43		
40 Yearling bulls	RC	Angus	Lucerne and cereal hay chaff 1.2x M	2periods of 24hrs[i]	kgDMI/d	7.61 ± 1.46		0.75	Aus	R. Herd pers. comm.
					gCH_4/ d	170.7 ± 17.2	across days	0.69		
					gCH_4/kgDMI	22.7 ± 2.10		0.34		
10 steers	GEM	Angus	Lucerne cereal mix chaff *ad lib* plus pellets[g]	6 periods of 2 days	LWT	365.2 ± 49.97		0.95	Aus	J. Velazco pers. comm.
					CH_4/CO_2	0.04 ± 0.00		0.1		
					gCH_4/kgDMI	27.00 ± 13.50	across periods	0.26		
					gCH_4/d	216.54 ± 39.04		0.02		
					gCO_2/d	5675.7 ± 919.6		0.37		
								0.57		

[a]SF_6: Sulphur hexafluoride: FITR: Fourier Transform InfraRed; AMS: Automatic Milking System; RC: Respiration Chambers; GEM: Greenfeed; [b]F: Friesian; J: Jersey; H: Holsteins; S: Simmental; [c]M: maintenance; *ad lib*: ad libitum; [d]TMR: total mixed ration (corn silage, rapeseed meal and soybean meal). Concentrates were fed in the AMS as an attractant; [e]Spring: 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; [f]30% dehydrated alfalfa, 39% wheat shorts and bran, 23% beet pulp; [g]850g Pellets (ME = 12.1), fed in GEM as attractant; [h]6 periods: pre calving and weeks 8, 15, 23, 33 and 41 of lactation; [i] 2 periods were 9 months apart; Where measures were made using GEM the values are given in gCH_4/d but the measurement is made over multiple periods of approximately 5 minutes.

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., (2013a)
								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		
					LWT	48.17 ± 13.31	0.46 ± 0.07	across days	0.93 ± 0.00		
96 Sheep 12-15mo	RC and PACs	Merino X	*ad lib*, M and pasture	9 measures[e]	LWT	51.50 ± 7.87		across days	0.93	Aus	H Oddy pers. comm.
					gCH$_4$/min	23.84 ± 4.66			0.47		
					gCH$_4$/gFI	17.42 ± 1.39			0.07		
					CH$_4$/CO$_2$	0.06 ± 0.01			0.08		
					mlCO$_2$/min	361.7 ± 52.17			0.42		
					mlO$_2$/min	-370.6 ± 46.38			0.20		
708 Sheep	PAC		pasture *ad lib*	1hr	gCH$_4$/h adj for LW	9.43	0.30		0.30	Aus	Robinson,, (2009)
						9.43	0.13		0.32		

[a]mo: month; [b] RC: Respiration chamber; PAC: Portable Accumulation chamber; [c] Rom: Romney; Coop: Coopworth; Peren: Perendale; Comp: Composites; X: cross; [d] M: maintenance; *ab lib*: ab libitum; [e]Animals were measured for 1hr in PACs for 2 consecutive days, done for 4 separate periods (period 1 *ab lib*, period 2 at maintenance, period 3 and 4 at pasture). Animals were also measured in RC for 1 day at *ab lib*, between period 1 and 2. Where measures were made using the PAC the values are given in g CH_4/d but the measurement is made over a period of 60 minutes.

Implications for measurement

The highly variable dynamics of CH_4 excretion in relation to time since eating 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.

Three messages on repeatability emerge from Tables 3 and 4. The repeatability of daily CH_4 emissions is highest between RC measures made on consecutive days, but diminishes as time between measures increases. Repeatability between CH_4 emissions measures is lower for short term measurement systems (e.g. 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 the emissions phenotype that is not normally obtianed 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 CH_4 emissions capable of being used for genetic selection.

McEwan et al (2012) assessed the usefullness 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 gCH_4/d and 0.76 for MY). They estimated there is little difference in estimates of total methane emissions and MY by measuring animals twice in RC, 14d apart, or by measuring an animal 4 times for 1hr, 14d apart (for MY if intake is known). Such assessments indicate that using a range of measurement technologies is possible, but the intenstity 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 CH_4 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, gross CH_4 production rate (ml CH_4/min), the correlation between RC and PACs measured on the same animal and same diet (*ad libitum*) is 0.58. The raw phenotypic correlations between RC (fed *ad libitum*) and PAC's (fed at maintenance) were 0.56 to 0.60 and between PAC (fed *ad libitum*) and PAC (fed at maintenance) the correlations ranged between 0.52 and 0.72 (Table 5). It is likely that the genetic correlations between measurements are higher.

Table 5. Phenotypic correlation matrix (r) between methane production rate (ml CH_4/min) by sheep determined by RCs and repeated portable accumulation chambers (PAC) when fed at maintenance and *ad-libitum*. (V. H. Oddy, pers. comm.)

		P1 Ad-lib		P2 M		Ad lib	P3 Pasture		P4 Pasture	
		PAC1	PAC2	PAC1	PAC2	RC	PAC1	PAC2	PAC1	PAC2
P1 Ad-lib	PAC1	1								
	PAC2	0.75	1							
P2 M	PAC1	0.52	0.58	1						
	PAC2	0.69	0.72	0.82	1					
Ad lib	RC	0.58	0.58	0.56	0.60	1				
P3 Pasture	PAC1	0.39	0.52	0.45	0.44	0.56	1			
	PAC2	0.54	0.47	0.42	0.47	0.44	0.63	1		
P4 Pasture	PAC1	0.51	0.55	0.49	0.48	0.53	0.64	0.55	1	
	PAC2	0.55	0.62	0.49	0.56	0.46	0.54	0.51	0.58	1

P1, P2, P3, P4: Period 1, 2, 3 and 4, respectively; Ad lib: *ad-libitum* feed intake of chaffed hay (M/D = 9.5 MJ ME/kg DM); M: fed at maintenance of chaffed hay; PAC1 and PAC2: 1 hr measurement in portable accumulation chamber on 2 different days within same period; RC: 22 hr measurement in respiration chamber; pasture 1 and 2: same sheep fed *ad-libitum* on 2 different pastures.

Where these CH_4 measures become constrained, is when feed intake is unknown, for example PAC measurements at pasture. This makes estimating the intake relevant to a CH_4 measure made over, at a maximum, one hour challenging. For example, where feed intake prior to PAC measurement was known for the hour prior to measurement (Table 6, PAC *ad libitum*, PAC fed at maintenance), phenotypic correlation of MY with RC (feed intake known) was 0.11 to 0.18 for PAC (fed *ad libitum*) and -0.12 to 0.01 for PAC (fed at

maintenance). Where feed intake prior to measurement was unknown and estimated (PAC measurement at pasture), phenotypic correlations of MY with PAC and RC (feed intake known) ranged between 0.23 and 0.44, and 0.10 and 0.26, respectively. The discrepancy between animals fed ad libitum and fed maintenance and between known feed intake and estimated intake suggests more work is required in this area. These results suggest more work is required when using PACs for estimating MY without a measure of feed intake temporally relevant to the measurement of CH_4. Because of the strong association between CH_4 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 6. Phenotypic correlations between different measurement protocols and methane yield feed intake known (CH_4 ml/gDMI) and unknown (CH4/Estimated FI) from sheep. (V. H. Oddy, pers. comm.)

CH_4 ml/gDMI			
	PAC *ad-lib*	PAC M	
RC	0.11-0.18	-0.12 - 0.01	
PAC M	-0.14 - 0.16	1	
CH_4/Estimated FI			
	PAC *ad-lib*	RC	PAC pasture1
PAC pasture 1	0.23-0.44	0.23-0.26	1
PAC pasture 2	0.28-0.36	0.10-0.12	0.16-0.47

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 M: 1hr measurement in PAC, fed at maintenance on chaffed hay; PAC pasture 1 and 2: 1hr measurement in PAC of same sheep fed *ad-libitum* of 2 different pastures.

Pinares-Patiño et al (2011) showed that groups of animals selected to be high or low methane yield (MY) when consuming 2.2X maintenance lucerne pellets retained their ranking when fed lucerne and concentrate pellets. Subsequently they (C.S. Pinares-Patiño 2012, pers. comm.) demonstrated that with 5 different diets the groups remained different in MY although individuals in the groups sometimes re-ranked (Table 7). This suggests that using a standard diet to assess rank of animals for MY is useful, and the

rankings are likely to hold across diets. The data also suggest that the differences in MY between animals in high and low MY groups (and therefore individuals) are greater when they are eating a more digestible diet. This suggests that the discriminatory power of a phenotype test could be expanded by feeding a mixed ration of forage and concentrate, although this requires testing with more animals.

Table 7. Consistency of response of sheep selected on basis of Methane yield (g CH4/kgDMI) across time and a range of diets (C.S. Pinares-Patiño pers. comm.).

Time of measurement	Diet (fed at 1.3-1.6M)	CH4 yield (g/kg DMI)		
		Low Group (n = 10)	High Group (n= 10)	% difference between High and Low Group
Aug 2008	Grass Silage	17.8	19.2	7.8
May 2009	Fresh Grass	22.5	24.4	8.4
June 2009	60% Forage, 40% Concentrate P	18.6	23.6	27.4
Jan 2010	Fresh Grass	22.2	25.3	13.8
March 2010	40% Forage 60% Concentrate P	8.9	12.8	43.8

Understanding variability of feed intake over time

Of the factors that influence CH_4 emissions, feed intake (quantity and extent of and rate of fermentation in the rumen) accounts for most of the variation in daily CH_4 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 CH_4 emissions within 15 minutes and elevated CH_4 emissions continue for several hours (e.g. Figure 7 above). Gas production and consequently the rate of eructation is higher 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).

Because variation in CH_4 production is predominantly related to variation in timing, extent and composition of nutrients ingested, a systematic assessment of sources of variation in feed intake is required. This should be done principally using experience from previous studies 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 CH_4 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 CH_4 as part of the process for measuring animals to improve efficiency of feed utilisation.

Repeatability of feed intake in confined systems

Knowledge of variation in feed intake is useful for deciding the best strategy for measurement of CH_4 emissions because of the dominant effect of intake on CH_4 emissions. When combined with a clear breeding objective, trait definition and knowledge of variation in rate of CH_4 emission in response to feed ingestion, it should then be possible to work out an optimal protocol for measuring CH_4 emissions. For example, if the trait under selection is total CH_4 emissions, some knowledge of pattern of intake is useful, but not essential to measurement of CH_4. However, if the trait is MY, we need to know enough about the characteristics of feed intake as well as CH_4 emissions to derive an estimate of MY. In practice we need to know that the correlations between intake and CH_4 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.

144 **Table 8.** Summary of feed intake experiments in cattle, including average daily dry matter intake (av kgDMI/d ±

145 sd), coefficient of variation (CV% ± sd) and repeatability (Rep) estimates.

Animals	System	Breed[i]	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		0.33	Canada	
128 Feeder steers	GrowSafe[a]	Beefbooster TX	1 - 84	9.7 ± 0.7	19.3 ± 3.0		0.34	Canada	
61 Beef heifers	GrowSafe[b]	AA x H & C x RA	1 - 108	7.0 ± 0.8	15.1 ± 2.5	Between animal	0.29	Canada	J. Basarab pers. comm.
99 Young bulls	GrowSafe[c]	AA x H x G	1 - 77	9.1 ± 0.9	16.4 ± 1.9		0.39	Canada	
40 Beef cows (3-5y)	GrowSafe[d]	AA x H & C x RA	1 - 79	14.4 ± 1.3	20.9 ± 4.6		0.49	Canada	
50 Feeder heifers	Insentec[e]	Limousin X Friesian	1 – 84	10.8 ± 1.0		Within finishing period	0.34	Canada	Kelly et al., (2010)
						Between growing and finishing period DMI	0.61		
						Between growing and finishing period RFI	0.62		
64 Steers	Tullimba feeder[f]	Mixed Bos Taurus	10 - 100	11.8 ± 3.1	26	Daily	0.26	Australia	Robinson & Oddy (2004)
			40 - 100	11.7 ± 3.0	26		0.25		
93 Steers	GrowSafe[g]		8 - 46	14.1 ± 2.4	17		0.15	Australia	J. Cook pers. comm.
Dairy cattle: Feedlot									
554 Dairy cows	Insentec [h]	Holstein	8 - 305			Weekly across lactation	0.31	Denmark	J. Lassen pers. comm.
						Weekly within lactation	0.64		
						Daily across lactation	0.24		
						Daily within lactation	0.65		
Dairy cattle: tracer									
755 Dairy cows	C32 n-alkane[j]	Holstein-Friesian		13.9 - 17.8[k]		Within stage lactation	0.18 - 0.57	Ireland	Berry et al., (2007)

146

147 *Diets:* [a]Finishing diet (56.6% barley grain, 20% corn-DDGs, 20% barley silage and 3.4% protein supplement/minerals); [b]90% barley
148 silage and 10% barley grain diet; [c]Growing diet (72.1% barley silage, 24.6% barley grain and 3.3% protein/mineral supplement;
149 [d]Hay-straw cube (25% straw, 75% grass hay-alfalfa ix); [e]70:30 concentrate and corn silage; [f]75% cracked barley, 15% chopped
150 ceral hay, 8% molafos and 2% minerals; [g]70.8% cracked Barley, 6% whole fuzzy cottenseed, 4.6% cottenseed hulls, 5% mill run,
151 4.6% chopped hay, 5% liquid supplement, 4% H_20; [h] TMR ad lib based on corn and grass silage together with soybean meal and
152 concentrate in VMS;; [i]Pasture or pasture plus concentrate; [j] TX: Terminal cross; AA: Aberdeen Angus; H: Hereford; G: Gelbvieh; C:
153 Charolais; RA: Red Angus; Mixed Bos Taurus: Angus, Hereford and Shorthorn; [k]Within stage of lactation.

154 **Table 9.** Summary of feed intake experiments in sheep, including average daily dry matter intake (av kgDMI/d ±

155 sd), coefficient of variation (CV%) and repeatability (Rep) estimates.

Animals	System	Breed	Expt period	Av kgDMI/d	CV%	Rep trait[f]	Rep	Country	Reference
Autofeeders									
61 ewes (7 mo)	GrowSafe[a]	Targhee X Rambouillet	68	1.5 ± 0.2	14.1	Daily FI	0.26	USA	K. Cammack pers. comm.
610 progeny (5mo - 2 Yr olds)	Auto feeder[b]	Merino X Awassi	1 – 90	1.1 ± 0.5		Weekly FI	0.20	Australia	Jonas et al., (2009)
						Weekly DMI	0.14		
						Weekly RFI	0.02		
Individual penned and refusals weighed									
96 Ewes (12 mo)[b]	Chaff[c]	Merino X	1 – 30	1.2 ± 0.2	13.7	Daily FI	0.711	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	Daily FI	0.24	Australia	Oddy & Sainz (2002); Hegarty et al., (1999)
	Pellet[e]		3 – 83	1.6 ± 0.2	12.6	Daily FI	0.44		
	Pellet[f]		3 – 83	1.7 ± 0.2	13.3	Daily FI	0.40		
Tracer on pasture									
300 Ewes	Cr marker	Merino		0.6 - 1.1		Within period DOMI	0.32-0.47	Australia	Lee et al., (1995)
						Across period DOMI	0.09-0.27		
350 Wethers	C32 marker	Merino				Across ages/sires DOMI	0.78	Australia	Lee et al., (2002)

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157 Diets: [a]Forage diet (15.2% Crude Protein; 50% DM); [b]Pellet (composition unknown); [c]50% chaffed lucerne hay 50% chaffed oated
158 hay; [d]Pellet energy density of diet MEMJ/kgDM (M/D) 7.7; [e]Pellet M/D 9.2; [f]Pellet M/D 10.9; [g] Border Leister X Merino ewes X Poll
159 Dorset sires; Repeatability estimates: [f]feed intake (FI); residual FI (RFI); Digestible organic matter intake (DOMI).

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 8) 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 deceasing 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).

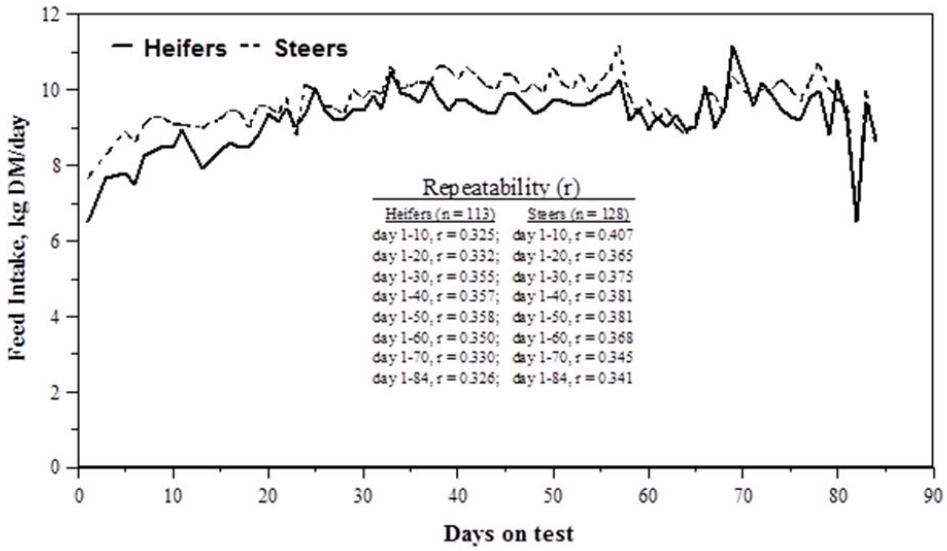

Figure 8. Daily feed intake for heifers (solid line) and steers (dashed line) fed a finishing diet (56.6% barley grain, 20% corn-DDGs, 20% barley silage and 3.4% protein supplement/minerals, dry matter basis) over 84 days. J. Basarab pers. comm.)

Wang et al (2006) reported that the phenotypic variances for DMI (cattle) 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 average daily gain 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).

Implications for measurement of methane phenotypes

The above data suggests that the system of measuring feed intake, and the system under which animals are fed, affect the repeatability of feed intake. However, we do not yet have sufficient data to estimate relationships between individual animal feed intake (and CH_4 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 CH_4 trait in different production systems. In the case of beef cattle, sheep and goats because females produce most CH_4 (on a system basis) and predominantly graze pasture, it puts emphasis on measurement of intake and CH_4 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; Jones et al., 2011). This gives hope that selection for CH_4 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 (Arthur 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 (cm^2) and marbling score at the start (optional) and end of the test period.

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 CH_4 emissions even when intake is not measured, is CO_2 production, and possibly O_2 uptake.

From the study of emissions by sheep fed at three levels of intake (Robinson, 2009), CH_4 and CO_2 production rates were (for 2 hours) proportional to substrate supply i,e, feed intake. This observation deserves further exploration.

Indirect selection to reduce emissions

Measuring CH_4 emission rates directly from animals is difficult and thereby hinders direct selection on reduced CH_4 emission. However, improvements can be made through selection on associated traits (e.g. RFI), volatile fatty acids (VFAs) or through selection on CH_4 predicted from feed intake and diet composition.

Volatile fatty acids

The rumen microbial population converts the host ingested food in the rumen into CO_2, H_2 and volatile fatty acids (VFAs). The methanogens act on the H_2 to product CH_4 and the host absorbs the VFAs across the rumen for its own use. A low concentration of H_2 in the rumen, due to increased activity of the H_2 consuming methanogens, promotes rapid fermentation of the feed. This in turn increases the other by-product and VFAs (Wolin, 1979), while High concentration of H_2 is thought to reduce the activity of the microbes that ferment the feed, thus reducing accumulation of VFAs (McAllister and Newbold, 2008). The VFAs are a potential proxy for estimating CH_4 emissions. The protocol of rumen sampling for profiling microbial populations has been standardise and also allows the measurement of VFAs.

For sheep, Pinares-Patiño et al (2013b) measured 1,081 animals for VFAs soon after exit from RCs (fasted or pre-feeding stage; Table 10). There were high genetic correlations of MY with $\log_e$ mM VFA concentrations. Genetic correlations are lower, however, still moderate when VFAs were expressed as molar %.

Table 10. Rumen Volatile Fatty Acid (VFA: $\log_e$ mM or molar %), heritability (h^2), repeatability (rep) and genetic correlation (r_g) with methane yield (gCH_4 /kgDMI). (Reproduced from 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

CH_4: methane; DMI: dry matter intake; VFA: volatile fatty acid; Ace: acetate; Pro: propionate; But: butanate; Ace/Pro: acetate/propionate ratio

For cattle, Herd et al (2013) measured 532 young Angus bulls and heifers soon after exit from the RCs (at least 12hrs post feed consumption). Pearson correlation coefficients with CH_4 (L/day), MY (L/kg DMI) and CH_4 intensity (L/kg LWT) were estimated (Table 11). There were strong correlations with MY and CH_4 intensity, but not with gross methane

production. Other studies (Robinson et al., 2010; McPhee and Hegarty, 2008), suggest that information on VFA has limited utility in predicting CH_4 emissions.

Table 11. Pearson correlations for methane production (MP), methane intensity (MI) and methane yield (MY) with volatile fatty acids. (Reproduced from Herd et al., 2013).

	MP (L/day)	MI (L/kg LWT)	MY (L/kg DMI)
Acetate (mM/L)	-0.07	0.33***	0.29***
Propionate (mM/L)	-0.05	0.16***	0.13**
Butyrate (mM/L)	-0.09*	0.40***	0.35***
Total VFA (mM/L)	-0.07	0.32***	0.28***
Acetate %	0.00	0.06	0.07
Propionate %	0.05	-0.41***	-0.39***
Butyrate %	-0.14**	0.44***	0.38***

*P<0.05; **P<0.01; ***P<0.001

Prediction from feed intake and diet composition

The objective of a Dutch study was to establish phenotypic and genetic variation in predicted CH_4 output, and to determine the potential that genetic selection has in reducing CH_4 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. Along with RFI, predicted CH_4 emissions (PME, g/d) and fat and protein corrected milk production (FPCM, kg/d) were estimated. The estimated heritabilities for PME and RFI were 0.35, and 0.40, respectively. The positive phenotypic (Table 12) and genetic (Table 13) correlations 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 CH_4 output is unknown. It is still 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 exist, for example the lack of true methane measurements to assess the estimate of PME, the key assumption that methane produced per unit feed is not affected by RFI level, and the limitations of recording and prediction of the biological

consequences of selection. To overcome these limitations an international effort is required to bring together data on milk production, feed intake and methane emissions of dairy cows.

Table 12. Phenotypic correlations between predicted methane emission (PME), fat and protein corrected milk production (FPCM), dry matter intake (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 13. 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 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

Information on methane production required to enable a genomic selection program

Methane emissions (as g CH_4/d or MY) certainly fit the description of hard to measure traits. Methods currently available are expensive and time consuming (RCs, SF_6) and

subject animals to artificial environments. Those that measure animals in production situations (pasture, feedlot or dairy feeding station) sample CH_4 for only a part of a day and require repeat measurements (PACs, Sniffers or GEM) and in some cases calculation back to known standard procedures. Those methods of estimating CH_4 emissions that rely on computation of differences between feeding standards and production account for only part of the potential variation in CH_4 emissions between animals.

Genomic selection opens the possibility to efficiently select for hard to measure traits. It is increasing being used to increase rate of genetic progress for production traits that are measured late in life (e.g. meat yield and quality), expensive to measure (e.g. RFI) and are sex linked (e.g. milk production and quality). In the dairy and increasingly in the beef and sheep industries leading sires are routinely genotyped and genomic breeding values (GEBVs) are used in making selection decisions. It is doubtful that adding the cost of genotyping onto a population in which CH_4 is measured would be cost effective, but by using industry animals which have measured production traits and have been genotyped it would be possible to estimate GEBVs for CH_4 emissions. This is predicated on having a large reference population, where CH_4 emission levels are measured and genome wide DNA marker effects have been estimated (e.g. to establish the prediction equation for marker effects).

The key question is how large does this reference population have to be, that is how many animals need to be measured for CH_4 and genotyped for the genome wide marker panels? Daetwyler et al (2008), Goddard (2008) and Hayes et al (2009) have all derived deterministic formula to estimate the accuracy of GEBV that could be achieved given the size of the reference population, the heritability of the trait and the effective population size. The accuracy of genomic selection for selection candidates (i.e. animals with a genotype, but no measured phenotype) with increasing size of reference population is shown in Figure 9. This was derived from the heritability of MY of 0.13 (reported in Table 1) and an effective population size of 150 using the procedure described by Hayes et al

(2009). This graph assumes perfect linkage disequilibrium between the SNP and QTL, which is unlikely for the current available chips and thus the graph will asymptote to the proportion of variance explained, for example, for dairy cattle using the Bovine 50K SNP chip this would be 90%. The estimates also assume unrelated individuals, if individuals were related, particularly the selection candidates to the reference population, the accuracy would be greater, as this is effectively reducing the effective population size (Ne). Finally, if the individuals in the reference population were progeny tested, this would make the "heritability" of the trait much higher and thus would require fewer animals genotyped to achieve the same accuracy, however the total number of animals measured for CH_4 to achieve the same accuracy would stay the same.

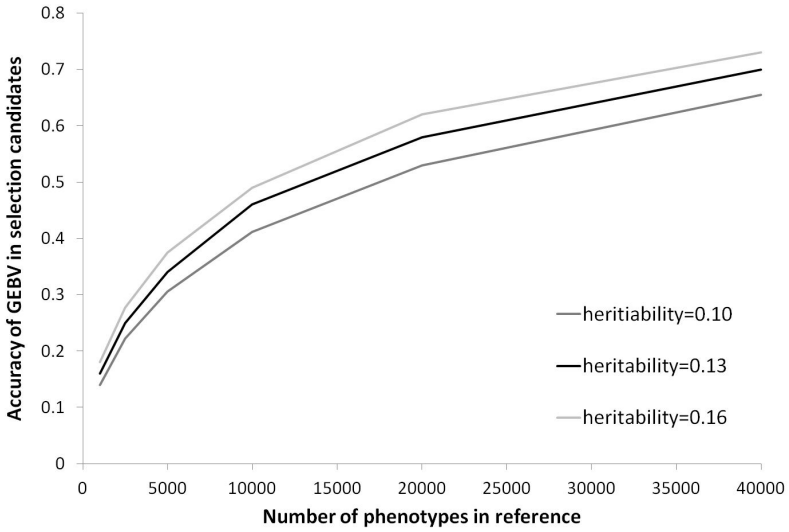

Figure 9. Accuracy of genomic estimated breeding values (GEBV) for methane yield in selection candidates as a function of heritability of the trait and number of animals with phenotypes in the reference population. Estimates of heritability of MY in sheep were obtained from Pinares-Patiño et al (2013a).

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.

The number of animals with phenotypes in the reference population required to obtain GEBVs of high accuracy for MY are large and almost certainly exceed the resources available in any one country. However, the research community has considerable experience with combining data from different countries to enable initial estimates of GEBVs for traits such as milk production, residual food intake and carcass traits. The challenge for the community now working on CH_4 related traits is to establish measurement procedures for phenotyping animals that can be combined to facilitate estimation of genetic parameters and GEBVs in particular. The ASGGN provides a forum to encourage such collaboration.

Summary: Expectations of methods for measuring methane

The key requirements of a methodology for measurement of CH_4 production and MY of individual animals for genetic selection are, firstly, the methodology must provide a reliable measure of the true CH_4 emission by the individual for the period of measurement and suitable for the production system under target. This requires that the recovery of CH_4 emissions by the measurement procedure be consistent and preferably 100%. The RC, PACs, GEMs and SF_6 all potentially meet this criteria (Table 14). Methods where recovery is less than 100% might be useful if they show consistent recovery, these include sniffers which permit losses of CH_4 between animal and sensor.

Secondly, the period of measurement (of CH_4 and for MY, feed intake) and number of measurement periods should be sufficient to reliably rank sires for estimation of BVs. In practice, this means multiple measures per animal. The optimal period and number of measurements will be determined by the pedigree structure of the data and the purpose of research. The repeatability of CH_4 measurements in PACs is only slightly less than in respiration chambers (Table 4; Pinares Patiño et al., 2013a; Oddy et al., 2013). There is limited data to reliably estimate repeatability of CH_4 emissions using the SF_6 and GEMs

(Table 3; Grainger et al., 2007), but it is anticipated that it would be less than in RCs. Having more progeny per sire will increase the accuracy of the estimate of sire eBVs and having more sires will improve the accuracy of the initial estimates of heritability. Finally, the measurement must be robust over time, as low cost as possible, not unduly influence animal behavior and permit a high rate of data capture with low labour requirements. Ideally it should replicate the normal production system as far as possible.

Table 14. Summary of the main methodologies for individual methane measurements.

Method	Robust	Intrusive	Cost	Throughput
Respiration Chamber	Yes	Yes	High	Low
Short term accumulation Chamber	Yes	Yes, but easily managed with grazing animals.	Low	High
Greenfeed	?	Moderately, requires modified grazing pattern	High	Moderate
SF_6	?	Yes for sampling, less so for grazing	High	Moderate

Conclusions

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

- CH_4 emissions are a heritable and repeatable trait.
- Repeated measurements add value, preferably separated by at least 3-14 days.
- CH_4 emissions are strongly related to feed intake both in the short term (minutes to several hours) and over the medium term (days).
- When measured over the medium term, MY (g CH_4/kg DMI, CH_4 corrected for feed intake) is a heritable and repeatable trait albeit with less genetic variation than total CH_4 emission (g CH4/d).
- CH_4 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 CH_4 emissions show promise. However we believe that for short term measurements to be useful for genetic evaluation, a number (between 3 and 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 breath "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 CH_4 measurement.
- While there is evidence that correlated and predictor traits exist for CH_4 emissions the current level of knowledge is insufficient to recommend there use in genetic selection to reduce CH_4 emissions.
- Genomic selection offers potential for use to reduce CH_4 emissions and MY, however, measurements on thousands of individuals will be required.
- The "size of the prize" when combining lower MY with selection for low residual feed intake (RFI) may result in a reduction in methane emissions of 40-45% and may be possible through selection of individual animals on components that directly affect methane production.
- In summary we consider genetic and genomic selection offers a significant opportunity to reduce CH_4 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 CH_4 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 CH_4 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 CH_4 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 CH_4 yield into the accounting framework for enteric emissions.

The above leads to the following recommendations for further work,

- 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.
- 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.

- 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 CH_4 emissions that can be employed in member countries. This would prepare the community for development of preliminary genetic parameters and GEBVs to act as a catalyst for local/national development of breeding solutions for reduced emissions of CH_4 from farmed ruminants.
- Exploration of the tripartite between CH_4, feed intake and animal production and between the three CH_4 traits; gross CH_4, MY and CH_4 emission intensity.
- Continue to explore methods that use proxies of feed intake measured over the same time frame as CH_4, for example CO_2 output and O2 uptake, to estimate MY. Establish relationships between proxy measures of MY and reference methods and the total CH_4 production/time measured on animals on pasture.

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