



The road to genetic selection for methane emission from ruminants: A global approach

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Abstract

Measuring and mitigating methane (CH₄) emissions from livestock is of increasing political and economic importance. Potentially, the most sustainable way of reducing CH₄ emission from ruminants is through the estimation of genomic breeding values to facilitate genetic selection. Enteric CH₄ emissions are difficult and expensive to measure, thus genomic prediction could provide significant, long term economic benefits. Implementation will require global collaboration to define a suitable measure and many thousands of records to ensure valid and accurate evaluations. A number of approaches for individual measures on a large scale have been recently proposed including fixed and portable respiratory chambers, SF₆ tracer gas, laser detector systems and sniffers or spot samples at milking. Some studies have also shown promising results in predicting individual animal CH₄ emission from mid infra-red milk spectra data. It is currently unclear, however, how well measures between these approaches correlate. Comparison and validation of these novel phenotypes presents a huge task over the coming years. A crucial first step is to define a trait phenotype and measuring protocols to create a robust resource for the global sharing and comparison of data, and further, to measure correlations with production traits. Proposed phenotypes could be measures of “total methane emissions”, measured in grams CH₄ per day, or “methane yield” measured in grams CH₄ per kg dry matter intake (DMI) when DMI is available as a phenotype, or “methane intensity” measured in grams CH₄ per kg of produced human edible protein. Here, we describe how two recently established entities; an ICAR Working Group and the EU COST-Action network METHAGENE are working in close collaboration to successfully address these major challenges together with the Animal Selection Genetics and Genomics Network (ASGGN) of the Livestock Research Group of the Global Research Alliance (GRA) on agricultural greenhouse gases.

Keywords: methane emission, international collaboration, phenotypes, genetic selection

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 (Tubiello *et al.*, 2013). Of the various GHG, methane (CH₄) is the most important agricultural contributor, with a global warming potential 25 times that of carbon dioxide (CO₂).

There are many potential methods to reduce enteric CH₄ emissions per head and thereby intensity of CH₄ production per unit product. These include: changing feed type (for example from

pasture to concentrate feed or to new pasture varieties); the use of supplements that reduce CH₄ emissions (fats, oils, plant extracts and nitrate); improving productivity through management change including the use of growth enhancers and improved genetics; immunisation against methanogens, and selective breeding of animals with low CH₄ emissions, through either reduced feed intake per product or reduced CH₄ production per feed consumed, without compromising production characteristics (Wall *et al.*, 2010).

Animal breeding that exploits natural animal variation in CH₄ emissions is an additional mitigation strategy that is cost-effective, permanent, and cumulative. Nonetheless, within animal production, there is little or no concerted world-wide effort on long-term breeding strategies to mitigate against GHG from ruminants. There have been several, largely nationally funded, studies that have taken place (or are underway), but are too small in size to draw definitive conclusions and also are of insufficient size to make any meaningful contribution to national, EU-wide, or international mitigation strategies through breeding. This is because successful animal breeding strategies require measurements on a large population of animals, which can only be achieved through international collaboration. In this paper we provide an overview of the crucial first steps that are taken to define a trait phenotype and measuring protocols to create a robust resource for the global sharing and comparison of data.

Enteric Methane Emissions

Trait definitions

There are 3 levels in which a CH₄ trait can be defined; firstly, the farm system level which uses information on the number of animals present within a system boundary with a related estimate of CH₄ emissions per head, calculated for example from the Intergovernmental Panel on Climate Change (IPCC 2006) Tier 2 calculations. These calculations have embedded within them a number of assumptions about the factors which affect CH₄ per head, i.e. feed intake, feed quality and CH₄ yield. Secondly, 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 CH₄ intensity (g CH₄/kg product). Finally, at the animal level, individual CH₄ emissions and feed intake measurements to enable genetic progress on CH₄ yield (**MY**; g CH₄/kg dry matter intake (**DMI**)).

Measuring methods

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 CH₄ output over the 1-3 day 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 24 hours, are typically damped within the large chamber volume. 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 are also under strong genetic control (Hegarty, 2004).

The sulfur hexafluoride (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). The sampling procedures provide an average CH₄ output for periods of typically 24 hours, but can be repeated over periods of 5-10 days, 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 (Deighton, *et al.*, 2013), 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 (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). A hand-held laser has been used to estimate CH₄ flux indirectly from dairy cattle (Chagunda *et al.*, 2013).

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 CH₄ emission the most accurate method would be the RC method, but for the generation of sufficient data to implement selective breeding this method has practical limitations. Alternately, spot breath samples taken during milking in dairy cattle might be an inaccurate phenotype for selective breeding, but can generate a large number of individual animal records. A correlation structure between all possible methods is needed and would allow merging of data to generate enough data for use in selective breeding.

Proxies

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 volatile fatty acids (VFA), or through selection on CH₄ predicted from feed intake and diet composition, or from milk composition.

Volatile fatty acids. The rumen microbial population converts the host ingested food in the rumen into CO₂, hydrogen (H₂), VFA and microbial cells. The host absorbs the VFA across the rumen for its own use and rumen methanogens utilise the H₂ to produce CH₄. High H₂ concentrations are thought to stimulate methanogenesis while suppressing production of acetate and VFA in general, while low H₂ concentrations will stimulate VFA production, especially acetogenesis, but suppress methanogenesis. VFA are thus a potential proxy for estimating CH₄ emissions. For sheep, Pinares-Patiño *et al.* (2013) measured 1,081 animals for VFA soon after exit from RCs. There were high genetic correlations (> 0.78) of MY with VFA concentrations.

For cattle, Herd *et al.* (2013) measured 532 young Angus bulls and heifers for VFA soon after exit from the RCs. Pearson correlation coefficients were estimated with CH₄ production (L/day), MY (L/kg DMI) and CH₄ intensity (L/kg live weight). There were correlations of 0.40 with MY and CH₄ intensity, but correlations with gross CH₄ production were almost zero.

Predicted methane from feed intake and diet composition. The objective of a Dutch study was to establish phenotypic and genetic variation in predicted CH₄ output (de Haas *et al.*, 2011). Records on daily feed intake, weekly live weights and weekly milk productions were available from 588 heifers. Along with residual feed intake (**RFI**), predicted methane emissions (**PME**, g/day) were estimated. PME is 6% of gross energy intake (method of IPCC) corrected for energy content of CH₄ (55.65 kJ/g). The estimated heritabilities for PME and RFI were 0.35 and 0.40, respectively. The positive phenotypic and genetic correlations between RFI and PME indicated that cows with lower RFI have lower PME as well (estimates ranging from 0.18 to 0.84 in different periods of the lactation). However, the association between these indicator traits and true CH₄ output is unknown.

Predicted methane from milk composition. Several studies have shown the link between milk composition and CH₄ output. However, these earlier studies focused on gas chromatography-measured milk composition traits mostly based on specific fatty acids (e.g. Chilliard *et al.*, 2009). Recent advances in the prediction of milk minor components from mid infra-red (**MIR**) spectral data allowed the development of fast and large scale fatty acid predictions (e.g., Soyeurt *et al.*, 2011). Currently, research is ongoing to validate initial results (e.g., Dehareng *et al.*, 2012) and to improve methods to predict individual animal CH₄ emission directly from MIR milk spectra data.

International networks

METHAGENE

METHAGENE is a European COST Action network that currently involves scientists from 19 different countries, that all want to collaborate for “large-scale methane measurements on individual ruminants for genetic evaluations” (www.methagene.eu). METHAGENE aims to discuss and agree on:

1. protocols to harmonise large-scale CH₄ measurements using different techniques;
2. easy to record and inexpensive proxies for CH₄ emissions to be used for genetic evaluations;
3. approaches for incorporating CH₄ emissions into national breeding strategies.

Successful breeding programs require large datasets of individual animal measurements that cannot be generated by any EU country working alone. However, smaller datasets of CH₄ measurements are being generated by individual countries across the EU, which could be combined if agreement could be reached on how best to harmonise the data.

ASGGN

The Animal Selection, Genetics and Genomics Network (ASGGN) is focused on bringing together scientists working in the area of reducing greenhouse gas emissions from ruminant livestock using animal selection, genetics and genomics techniques (www.asggn.org). The network was initiated at a May 2011 workshop in Auckland, New Zealand, and endorsed by the Livestock Research Group of the Global Research Alliance on agricultural greenhouse gases. It provides a forum to debate and reach agreement on a variety of topics including:

- common protocols for measurement of CH₄ emissions (and associated traits)
- calibrations of measurement procedures between countries
- co-measurement of appropriate correlated and productive traits
- formalised protocols for collection and storage of DNA from all animals measured and also protocols for collection and storage of rumen samples from all animals measured
- criteria for data sharing and analysis (including meta-analysis) among all contributing parties

International database

Global agreement on what to measure will create synergies ensuring that the value of expensive phenotypic measures is fully captured. Crucially, putting a framework in place will facilitate development of new high throughput technologies and biological proxies by enabling them to be evaluated quickly and efficiently using agreed protocols. Harnessing the power of these combined measures, however, is by no means straightforward. The pooling of data will lead to spurious results if levels of stratification are not identified and handled appropriately. A technical specification for the recording of a trait, therefore, needs to record not only the trait, but also the associated meta-data to make informed decisions about the combining of data sets.

A current well-evaluated set of guidelines, practised on a global level, is key to the successful sharing and transfer of information. ICAR is the world-wide organization for the standardization of identification, performance recording and evaluation of farm animals. Its aim is to promote improvement of farm animal recording and evaluation through the formulation of definitions and standards for the measurement of traits of economic importance.

Reasons for holding the information within a database are manifold. Data held in flat files, or on spreadsheets, are extremely difficult to interrogate efficiently. There can be associated data integrity problems, including inaccurate, inconsistent and out of date identifiers, data and formulas.

It can also be difficult to validate data e.g. where an incorrect formula has been used. A database consistently and systematically updates records and can organise information based on user defined parameters.

The information required divides into 5 categories; identification, information around the measurement and description of the trait itself, meta-data i.e. relates to all individual records, systematic effects considered of greatest importance when analysing the particular trait being described, and additional optional systematic effects that may improve analysis.

Example: Trait specification-file for methane record, divided in the 5 categories

Record	Code	Format	Example
International ID	ANIM	12 digit integer	554123456789
Dam ID	DAM	12 digit integer	554234567891
Sire ID	SIRE	12 digit integer	554345678910
Date of birth	DOB	dd/mm/yyyy	05/11/2012
Species	SPECIES	Character	Sheep
Breed	BREED	Character	COOPWORTH
Flock/Herd	GROUPM	Character	2638
Sex	SEX	M / F	M
Birth rearing rank	BRR	Integer	2
Contemporary group	CTG	Character	101
Trait description	DEPVARIABLE	Character	CO2
Units	UNIT	Character	g/day
Trait	Y	Number	27.4
Method of measurement	APPARATUS	Character	PAC
Date Measure start	DATEM	dd/mm/yyyy	01/04/2014
Date measure end	DATEE	dd/mm/yyyy	01/04/2014
Start of measure	TIMEMS	hh:mm:ss	09:00:00
End of measure	TIMEME	hh:mm:ss	10:00:00
Calibration/recovery	CAL	%	97
Scaling	SCALEFAC	Character	CTG
Feeding level	FEEDLEVEL	Character	ADLIB
Live weight at measure (kg)	LWTKGM	Number	35.2
Time off feed	TIMEOFF	hh:mm:ss	02:00:00

Conclusions

There is potential for adopting genetic selection and in the future genomic selection, for reduced CH₄ emissions in ruminants. From this review it has been observed, CH₄ emissions are a heritable and repeatable trait. Methane emissions are strongly related to feed intake both in the short term (minutes to several hours) and over the medium term (days). Repeated measurements add value; it is preferable the measures be separated by at least 3-14 days.

There are opportunities for using short term measurements in standardised feeding situations such as breath “sniffers” attached to milking parlours or total mixed ration feeding bins, to measure CH₄. We anticipate these are also subject to the caveats above about the 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.

Genomic selection has the potential to reduce CH₄ emissions and MY, however, measurements on thousands of individuals will be required. This includes the need to combine resources across countries in an international effort, emphasising the need for acknowledging the impact of the animal and production system on measurement of the CH₄ trait during design of experiments.

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