Chapter 5

A Comparison of Methodologies for Measuring Methane Emissions from Ruminants

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Abstract

Accurate measurement techniques are needed for determining greenhouse gas (GHG) emissions in order to improve GHG accounting estimates to IPCC Tiers 2 and 3 and enable the generation of carbon credits. Methane emissions from agriculture must be well defined, especially for ruminant production systems where national livestock inventories are generated. This review compares measurement techniques for determining methane production at different scales, ranging from in vitro studies to individual animal or herd measurements. Feed intake is a key driver of enteric methane production (EMP) and measurement of EMP in smallholder production systems faces many challenges, including marked heterogeneity in systems and feed base, as well as strong seasonality in feed supply and quality in many areas of sub-Saharan Africa.

In vitro gas production studies provide a starting point for research into mitigation strategies, which can be further examined in respiration chambers or ventilated hood systems. For making measurements under natural grazing conditions, methods include the polytunnel, sulphur hexafluoride (SF_6) and open-path laser. Developing methodologies are briefly described: these include blood methane concentration, infrared thermography, pH and redox balance measurements, methanogen population estimations and indwelling rumen sensors.

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5.1 Introduction

Fermentation processes by rumen microbes result in the formation of reduced cofactors, which are regenerated by the synthesis of hydrogen (H_2) (Hungate 1966). Accumulation of excessive amounts of H_2 in the rumen negatively affects the fermentation rate and growth of some microbial consortia. Methanogens therefore reduce carbon dioxide (CO_2) to methane (CH_4) and water (H_20) thereby capturing available hydrogen (McAllister et al. 1996). It is predicted that total CH_4 emissions from livestock in Africa will increase to 11.1 mt $year^{-1}$ by 2030, an increase of 42% over three decades (Herrero et al. 2008). Production increases and efficiencies in the livestock sector are seen as complementary outcomes if enteric methanogenesis can be reduced. While mitigation strategies are focused on manipulation of nutritional factors and rumen function, animal breeding programmes for selecting highly efficient animals that produce less enteric CH_4 might also be useful. Regardless of the mitigation strategy imposed, any reduction in enteric methane production (EMP) must be quantified and for this to be achieved, accurate baseline emissions data are essential.

This chapter reviews existing and developing methodologies for gathering accurate data on ruminant methane production under a wide range of production systems. The principles of using predictive algorithms based on dietary, animal and management variables are considered here for modelling smallholder livestock emissions, but not in detail. Predictive models have been considered in detail elsewhere (Blaxter and Clapperton 1965; Kurihara et al. 1999; Ellis et al. 2007, 2008; Charmley et al. 2008; Yan et al. 2009). Major techniques are highlighted at

different levels -- in vitro, animal, herd and farm scale -- and their advantages and disadvantages, including implementation in practice, are discussed. These methodologies can be used to support mitigation strategies or quantify total national livestock emissions.

5.2 Indirect Estimation

5.2.1 In Vitro Incubation

The amount of gas released from the fermentation process and the buffering of volatile fatty acids is related to the kinetics of fermentation of a known amount of feedstuff (Dijkstra et al. 2005). Several systems have been developed for measuring in vitro gas production, varying considerably in complexity and sophistication. Menke et al. (1979) describes a manual method using gas tight syringes, which involves constant registering of the gas volume produced. More recently others have described a system using pressure transducers (Pell and Schofield 1993; Theodorou et al. 1994; Cone et al. 1996). Variants of this system are now available as proprietary systems (RF, ANKOM Technology®) using radio frequency pressure sensor modules, which communicate with a computer interface and dedicated software to record gas pressure values.

The basic principle of the in vitro technique relies on the incubation of rumen inoculum with a feed substrate under an anaerobic environment in gas tight culture bottles. Gas accumulates throughout the fermentation process and a cumulative volume is recorded. Gas volume curves can be generated over time. To estimate kinetic parameters of total gas production, gas production values are corrected for the amount of gas produced in a blank incubation and these values can be fitted with time using a non-linear curve fitting procedure in GenStat (Payne et al. 2011) or other suitable software. Headspace gas samples are taken to analyse the

gas compositions and determine actual CH_4 concentrations, typically by gas chromatography. A 'quick and dirty' alternative is to introduce a strongly basic solution, such as NaOH into the vessel, which will cause the CO_2 to enter solution. The remaining gas is assumed to be CH_4.

Gas is only one of the outputs of microbial fermentation, and the quality of the information derived can be improved by also considering substrate disappearance and production of volatile fatty acids (VFAs) (Blümmel et al. 2005).

5.2.2 Estimation from Diet

EMP can be estimated from intake and diet quality (digestibility). A number of algorithms can be used to do this, although estimates of emissions can vary by 35% or more for a particular diet (Tomkins et al. 2011). Diet quality can be inferred from analysis of representative samples of the rations or pasture consumed, but where intake is not measured, estimation of EMP faces considerable challenges. Models which estimate intake based on diet quality or particular feed fractions assume *ad libitum* access, and in situations where animals are corralled without access to feed overnight, the validity of this assumption is likely violated (Jamieson and Hodgson 1979; Hendricksen and Minson 1980). In such a case, intake can be inferred from energy requirement (Live Weight (LW) + Energy for: LW flux; maintenance + lactation and pregnancy + locomotion) using published estimates (such as National Research Council) to convert physical values to energy values and so infer intake of the estimated diet. If this method is chosen, multiple measurements are required to capture changes in these parameters, as well as seasonal influences on feed availability and quality. Where possible, estimates made using this methodology should be validated by measurements in respiratory chambers.

5.3 Direct Measurement

5.3.1 Open-circuit Respiration Chambers

Models to estimate national and global CH_4 emissions from sheep and cattle at farm level are mostly based on data of indirect calorimetric measurements (Johnson and Johnson 1995). Respiration chambers are used to measure CH_4 at an individual animal level. Their use is technically demanding, and only a few animals can be monitored at any one time (McGinn et al. 2008). However, these systems are capable of providing continuous and accurate data on air composition over an extended period of time.

Although the design of chambers varies, the basic principle remains the same. Sealed and environmentally controlled chambers are constructed to house test animals. All open-circuit chambers are characterized by an air inlet and exhaust, so animals breathe in a one-way stream of air passing through the chamber space. Air can be pulled through each chamber and, by running intake and exhaust fans at different speeds, negative pressure can be generated within the chamber. This is to ensure that air is not lost from the chamber (Turner and Thornton 1966). However, CH_4 can still be lost from chambers that are imperfectly sealed (down the concentration gradient), so gas recovery is an essential routine maintenance task. Thresholds for chamber temperature (<27°C), relative humidity (<90%), CO_2 concentration (<0.5%) and ventilation rate (250–260 L min^{-1}) have been described (Pinares-Patiño et al. 2011), but may vary in practice. It is very important, however, to ensure that test animals remain in their thermo-neutral zone while being measured, or intake is likely to be compromised. Some chambers may be fitted with air conditioning units, which provide a degree of dehumidification, and a ventilation system. This ensures that chambers can be maintained at constant temperature (Klein and Wright 2006) or at near-ambient temperature to capture normal

diurnal variance (Tomkins et al. 2011). Choices about temperature are governed by technical resources and experimental objectives. Feed bins and automatic water systems may also be fitted with electronic scales and meters to monitor feed and water intake.

Change in O_2, CO_2 and CH_4 concentrations is measured by sampling incoming and outgoing air, using gas analysers, infrared photoacoustic monitors or gas chromatography systems (Klein and Wright 2006; Grainger et al. 2007; Goopy et al. 2014b). The other essential measurement is airflow, over a period of either 24 or 48 hours. The accuracy and long-term stability of the measurements are dependent on the sensitivity of the gas analysers used and the precision of their calibration. Chambers are directly calibrated by releasing a certain amount of standard gas of known concentration to estimate recovery values (Klein and Wright 2006). Measurement outcomes are also influenced by the environmental temperature, humidity, pressure, incoming air composition and chamber volume. The larger the chamber the less sensitive the measurements are to spatial fluctuations, as the response time is dependent on the size of the chamber and the ventilation rate (Brown et al. 1984). The calibration of the gas analysers must be accurate and replicable for long-term use.

One constraint of this technique is that normal animal behaviour and movement are restricted in the respiration chambers. Animals benefit from acclimatization in chambers prior to confinement and measurement, in order to minimize alterations in behaviour, such as decreased feed intake (McGinn et al. 2009). However, there is clear evidence that this will happen in a small proportion of animals, regardless of training (Robinson et al. 2014) and this should be borne in mind when interpreting data. Using transparent construction material in chamber design allows animals to have visual contact with the other housed animals.

There are high costs associated with the construction and maintenance of open-circuit respiration chambers. The need for high performance and sensitive gas analysers and flow meters must be considered in design and construction. Only a few animals can be used for measurements within chambers at any one time (Nay et al. 1994). Nevertheless, respiration chambers are suitable for studying the differences between treatments for mitigation strategies, and continue to be regarded as the 'gold standard' for measuring individual emissions.

5.3.2 Ventilated Hood System

The ventilated hood system is a simplification of the whole animal respiration chamber, as it measures the gas exchange from the head only, rather than the whole body. Moreover, it is an improvement on face masks as used by Kempton et al. (1976), because gas measurements can be generated throughout the day and animals are able to access food and water.

Modern ventilated hood systems for methane measurements have been used in Japan, Thailand (Suzuki et al. 2007, 2008), USA (Place et al. 2011), Canada (Odongo et al. 2007) and Australia (Takahashi et al. 1999). Fernández et al. (2012) describes a mobile, open-circuit respiration system.

The ventilated hood system used by Suzuki et al. (2007, 2008) consists of a head cage, the digestion trial pen, gas sampling and analysis, behaviour monitoring and a data acquisition system. Similarly to whole animal chambers, it is equipped with a digestion pen for feed intake and excreta output measurements. An airtight head cage is located in front of the digestion pen and is provided with a loose fitting sleeve to position the animal's head. Head boxes are provided with blowers, to move the main air stream from the inlet to the exhaust. Flow meters

correct the air volume for temperature, pressure and humidity. Air filters remove moisture and particles from the gas samples, which are sent to the gas analysers (Suzuki et al. 2007). The mobile system of Fernández et al. (2012) contains a mask or a head hood connected to an open-circuit respiration system, which is placed on a mobile cart.

The ventilated hood system is a suitable method under some circumstances, especially where open-circuit chambers are not viable. A critical limitation of the hood system is that extensive training is absolutely essential to allow the test animals to become accustomed to the hood apparatus. Thus while it can be used to assess potential of feeds, it is not suitable for screening large numbers of animals. A further consideration is that hoods capture only measurements of enteric methanogenesis and exclude the proportion in flatus.

5.3.3 Polytunnel

Polytunnels are an alternative to respiration chambers, and operation and measurements are somewhat simpler. Methane emissions from individual or small groups of animals can be acquired under some degree of grazing. This allows test animals to express normal grazing behaviour, including diet selection over the forages confined within the polytunnel space. They have been used in the UK to measure CH_4 emissions from ruminants under semi-normal grazing conditions. Murray et al. (2001) reports CH_4 emissions from sheep grazing two ryegrass pastures and a clover–perennial ryegrass mixed pasture using this methodology. Essentially polytunnels consist of one large inflatable or tent type tunnel made of heavy duty polyethylene fitted with end walls and large diameter ports. Air is drawn through the internal space at speeds of up to 1 m^3 s^{-1} (Lockyer and Jarvis 1995). In general they are used where emissions from fresh forages are of interest because animals can be allowed to graze a confined area of

known quality and quantity. When the available forage is depleted the tunnel is moved to a new patch.

Air flow rate can be measured at the same interval as the CH_4 or can be continuously sampled at the exhaust port (Lockyer 1997). Micropumps may be used to pass the exhausted air to a dedicated gas analyser or a gas chromatograph (GC) (Murray et al. 2001). Data from all sensors can be sent to a data logger, which captures flow rate, humidity and temperature within the tunnel, and gas production from the livestock. Samples of the incoming and exhaust air can be taken as frequently as necessary, depending on the accuracy required. The samples can be either taken manually or by an automatic sampling and injection system.

The polytunnel system requires frequent calibration to assure a good recovery rate, which is performed using the same principle as the chamber technique. Methane measurements can be collected over extended periods of time. Fluctuations occur due to changes in animal behaviour, position relative to the exhaust port, internal temperature, relative humidity and grazing pattern of the animal: eating, ruminating or resting (Lockyer and Jarvis 1995; Lockyer and Champion 2001). The polytunnel is suitable for measuring CH_4 emissions under semi-normal grazing conditions. It has been reported that the polytunnel method gives 15% lower readings of CH_4 concentration compared to the respiration chamber method, suggesting that animals actually consume less in the polytunnel. This requires further investigation. Recovery rate is high in both systems: 95.5–97.9% in polytunnels, compared to 89.2–96.7% in chambers (Murray et al. 1999). With an automated system, measurements can be performed with high repeatability. The system is portable and can be used on a number of pastures or browse shrubs, though again its utility is limited by the inability to capture feed intake.

5.3.4 Sulphur Hexafluoride Tracer Technique

The sulphur hexafluoride (SF_6) technique provides a direct measurement of the CH_4 emission of individual animals. This technique can be performed under normal grazing conditions, but can also be employed under more controlled conditions where intake is measured and/or regulated.

The SF_6 principle relies on the insertion of a permeation tube with a predetermined release ratio of SF_6 into the rumen, orally administered (Johnson et al. 1994). Air from around the animal's muzzle and mouth is drawn continuously into an evacuated canister connected to a halter fitted with a capillary tube around the neck. Johnson et al. (1994) provide a detailed description of the methodology.

The duration of collection of each sample is regulated by altering the length and/or diameter of the capillary tube (Johnson et al. 1994). Several modifications have since been reported with specific applications (Goopy and Hegarty 2004; Grainger et al. 2007; Ramirez-Restrepo et al. 2010). Most recently Deighton et al. (2014) has described the use of an orifice plate flow restrictor which considerably reduces the error associated with sample collection and should be considered in preference to the traditional capillary tube flow restrictors. At completion of sample collection the canisters are pressurized with N_2 prior to compositional analysis by gas chromatography. Enteric CH_4 production is estimated by multiplying the CH_4/SF_6 ratio by the known permeation tube release rate, corrected for actual duration of sample collection and background CH_4 concentration (Williams et al. 2011), which is determined by sampling upwind ambient air concentration. Williams et al. (2011) emphasized the importance of correct measurement and reporting of the background concentrations, especially when the method is

applied indoors. CH_4 is lighter (16 g mol^{-1}) than SF_6 (146 g mol^{-1}) and will therefore disperse and accumulate differently depending on ventilation, location of the animals and other building characteristics.

This method enables gas concentrations in exhaled air of individual animals to be sampled and takes into account the dilution factor related to air or head movement. The high within- and between-animal variation is significant limitation of this method. Grainger et al. (2007) reported variation within animals between days of 6.1% and a variation among animals of 19.7%. Pinares-Patiño et al. (2011) monitored sheep in respiration chambers simultaneously with the SF_6 technique. They reported higher within (x 2.5) and between (x 2.9) animal variance compared to the chamber technique, combined with a lower recovery rate (0.8±0.15 with SF_6 versus 0.9±0.10 with chambers). These sources of variation need to be taken into account in order to determine the number of repeated measures necessary to ensure accurate results. Moate et al. (2015) describes the use of Michaelis–Menten kinetics to better predict the discharge rate of capsules, which should reduce error associated with estimating discharge rates. It should also prolong the useful life of experimental subjects through the improved predictability of discharge rates over much longer intervals.

The SF_6 technique allows animals to move and graze normally on test pastures. This makes the method suitable for examining the effect of grazing management on CH_4 emissions (Pinares-Patiño et al. 2007) but it does so at a cost. The SF_6 method is less precise, less physically robust (high equipment failures) and more labour intensive than respiration chamber measures.

5.3.5 Open-path Laser

The use of open-path lasers combined with a micrometeorological dispersion method can now be used to measure enteric methane emissions from herds of animals. It therefore facilitates whole-farm methane measurements across a number of pastures.

The open-path laser method for whole-farm methane measurements is already in use in Canada (McGinn 2006; Flesch et al. 2005, 2007), Australia (Loh et al. 2008; McGinn et al. 2008; Denmead 2008; Tomkins et al. 2011), New Zealand (Laubach and Kelliher 2005) and China (Gao et al. 2010). Methane concentration measurements are performed using one or more tuneable infrared diode lasers mounted on a programmable and motorized scanning unit (Tomkins et al. 2011). The tuneable infrared diode laser beams to a retro reflector along a direct path, which reflects the beam back to a detector. The intensity of the received light is an indicator of the CH_4 concentration (ppm) along the path. In an optimal situation there should be at least one path for each predominant wind direction: one path upwind (background CH_4) and multiple paths downwind (CH_4 emission) of the herd. This method assumes that the herd acts as a surface source or, when individual animals can be fitted with GPS collars, individual animals are treated as point sources.

Regardless of application, the CH_4 concentration is calculated as the ratio of the external absorption to internal reference-cell absorption of the infrared laser beam as it travels along the path (Flesch et al. 2004, 2005). Methane concentration and environmental indicators such as atmospheric temperature, pressure, and wind direction and speed are continually measured and recorded using a weather station (Loh et al. 2008, 2009). Data -- including GPS coordinates of the paddock or individual animals from a number of averaging time periods -- can be merged

using statistical software. After integrating, WindTrax software (Thunder Beach Scientific, Nanaimo, Canada) uses a backward Langrangian Stochastic (bLS) model to simulate CH_4 emissions (g day^{-1} per animal), by computing the line average CH_4 concentrations with atmospheric dispersion conditions.

The data integrity of the open-path laser method is highly dependent on environmental factors and the location of test animals. Flesch et al. (2007) described several criteria to determine data integrity using the open-path laser method. These criteria are based on wind turbulence statistics, laser light intensity, R^2 of a linear regression between received and reference waveforms, surface roughness, atmospheric stability and the source location (surface or point source). Invalid data can be generated as a result of misalignment of the laser, unfavourable wind directions, surface roughness or periods in which the atmospheric conditions (rain, fog, heat waves, etc.) are unsuitable for applying the model (Freibauer 2000; Laubach and Kelliher 2005; Loh et al. 2008). To optimize the positioning of the equipment, these meteorological and physical aspects of the experimental site must be taken into account (Flesch et al. 2007; Loh et al. 2008, 2009). Moreover, the measurement area is restricted by the length of the laser paths when using a surface source approach. It is important to define the herd location, as uneven distribution of the herd results in miscalculations of the CH_4 concentration. Tomkins et al. (2011), comparing open-circuit respiration chambers with the open-path laser technique, reported estimated CH_4 emissions using the bLS dispersion model of 29.7±3.70 g kg^{-1} dry matter intake (DMI), compared to 30.1±2.19 g kg^{-1} DMI measured using open-circuit respiration chambers.

The open-path laser method does not interfere with the normal grazing behaviour of the cattle and is non-invasive. Spatial variability is taken into account in these measurements, as the method can simulate gas fluxes over a large grazing area. Moreover, the tuneable diode laser is highly sensitive and has a fast response to changes in CH_4 concentration, with detection limits at a scale of parts per trillion (McGinn et al. 2006). The labour intensity is low, although the equipment requires continuous monitoring. This method is expensive, which reflects not only the requirement for sensitive and rapid-response instruments to analyse CH_4 concentration, but also the requirement to capture micrometeorology data. Diurnal variations due to grazing and rumination pattern, pasture composition and individual variation need to be considered in planning experimental protocols to prevent over- or under- calculation of the total emission. Furthermore, DMI determination is not very accurate as this is based on predictive models using the relationship between LW and LW gain, following assumption of the ARC (1980).

5.4 Short-term measurement

While most assessments of enteric methane emissions are focused on daily methane production (DMP), or the derivative, daily methane yield (MY), there is increasing impetus to estimate the emissions of large numbers of animals in their productive environment. This is driven both by the demand for data to establish genetic parameters for DMP and to verify mitigation strategies or GHG inventories. This area is discussed only briefly here, as there is currently limited scope for the application of these technologies in sub-Saharan Africa. The area has been ably reviewed by Hegarty (2013).

5.4.1 Greenfeed® emission monitoring apparatus

Greenfeed® is a patented device (Zimmerman & Zimmerman 2012) that measures and records short-term (3–6 minute) CH_4 emissions from individual cattle repeatedly over 24 hours by attracting animals to the unit using a 'bait' of pelleted concentrate. By being available 24 hours per day potential sampling bias is reduced and the technique has been shown to provide comparable estimates to those produced both by respiratory chamber and SF_6 techniques (Hammond et al. 2013). However, a significant limitation of the technique is the requirement to supply an 'attractant' to lure the animal to use the facility, consisting of up to 1 kg of concentrate pellets per day. This will certainly affect DMP and may also alter volatile fatty acid profiles or the overall digestibility of the diet. Attempts to use energy neutral attractants, such as water have proven equivocal (J Velazco, pers. comm.).

5.4.2 Portable Accumulation Chambers (PAC)

A PAC consists of a clear polycarbonate box of approximately 0.8m^3, open at the bottom and sealed by achieving close contact with flexible rubber matting. Methane production is

measured by the increase in concentration that occurs while an animal is in the chamber for approximately 1 hour. PACs were designed to screen large numbers of sheep, variously to identify potentially low and high emitting individuals and to develop genetic parameter estimates in sheep populations. This technique initially showed close agreement with respiratory chamber measurements (Goopy et al. 2009; Goopy et al. 2011). Subsequent investigations demonstrated such measurements to be moderately repeatable in the field and to have potential for genetic screening of animals (Goopy et al. 2015). Longer-term comparisons of PAC measurements and respiratory chamber data, however, suggest that these two methods may be measuring quite different traits and further investigation is required before committing significant resources to PAC measurements (Robinson 2015).

5.4.3 Application of CH4:CO2 Ratio

Madsen et al. (2010) proposed using the ratio of CH_4:CO_2 in exhaled breath to assess enteric methane production in ruminants. This method requires knowledge about the intake, energy content and heat increment of the ration consumed. Haque et al. (2014) applied this method, using a fixed heat increment factor. Hellwing et al. (2013) regressed open-circuit chamber measurements of DMP in cattle against estimates calculated using CH_4:CO_2 ratios and found them to be only moderately correlated (R^2 = 0.4), which suggest this method is unsuitable for precision measurements.

5.4.4 Spot Sampling with Lasers

Spot measurements of methane in the air around cattle's mouths have been made using laser devices to provide short-term estimates of enteric methane flux (Chagunda et al. 2009;

Garnsworthy et al. 2012). These estimates are then scaled up to represent DMP – requiring an impressive number of assumptions to be met to satisfy such scaling. Chagunda and Yan (2011) have claimed correlations of 0.7 between laser and respiratory chamber measurements, but this claim is based on the laser apparatus measuring methane concentrations in the outflow of the chambers, rather than from the animals themselves.

5.5 Emerging and Future Technologies

5.5.1 Blood Methane Concentration

This methodology relies on enteric methane being absorbed across the rumen wall, transported in the blood stream to the pulmonary artery and respired by the lungs. The jugular (vein) gas turnover rate of enteric SF_6, (introduced by an intraruminal bolus) and CH_4 has been used to determine the respired concentrations and solubility of these gases (Ramirez-Restrepo et al. 2010). The solubility coefficients and CH_4 concentrations are determined by gas chromatography, comparing the peak area of the sampled gases with standards. Variances in CH_4 and SF_6 blood concentrations may be related to the methodology, or may occur because these gases are not equally re-absorbed. This requires further investigation. Sampling can be logistically challenging and labour intensive and it is important to recognize that this method provides little more than a 'snapshot' of methane concentration at time of sampling.

5.5.2 Infrared Thermography

Montanholi et al. (2008) have examined the use of infrared thermography as an indicator for heat and methane production in dairy cattle. No direct relationship was reported, however, between temperature in any specific part of the body and methane production.

5.5.3 Intraruminal Telemetry

The use of a rumen bolus to measure methane in the liquid phase is logistically possible and small changes (<50 μmol L^{-1}) in CH_4 concentrations could be detectable (Gibbs 2008). Low pH and redox potential have been correlated with decreased CH_4 concentrations, and a pH and redox sensor has been developed to suit a rumen bolus by eCow Electronic Cow Management at the University of Exeter, UK (www.ecow.co.uk). This technology is still in its exploratory stages but the application of a rumen bolus to measure CH_4 in the rumen headspace has been patented (McSweeney pers. comm.) and could theoretically provide accurate CH_4 concentration estimates for large numbers of free grazing animals.

5.5.4 Quantitative Molecular Biology

Gibbs (2008) examined the correlation between the numbers of methanogens and CH_4 production in short time intervals. Results from real-time polymerase chain reaction (PCR) suggest that increased CH_4 production is related to increased methanogen metabolic activity rather than increased population size.

Summary

EMP is a complex trait, involving animal physiology and behaviour, plant factors and animal management. Although there are many techniques available to estimate EMP, all have limitations. The appropriateness of a technique is strongly influenced by its intended purpose and the degree of precision required. It is important to recognize that while more sophisticated in vitro techniques can provide robust information about the fermentative, and hence, methanogenic potential of feeds, they do not truly represent in vivo fermentation, nor do they account for feed intake, and will be of limited predictive use for animals grazing heterogeneous pastures. If intake is unknown it will diminish the utility of established models, especially when

assumptions regarding *ad libitum* intake are violated. Lasers, infrared and SF_6 techniques can all be used to measure EMP of animals at pasture. However, all are technically fastidious and in situations where intake is unknown, cannot be used to determine emissions intensity. Respiration chambers, while requiring significant capital to construct and technical skill to operate, provide precise and accurate measurements of EMP on known feed intake. Whilst there are justified criticisms surrounding reproducibility of EMP at pasture and evidence of changed feeding behaviour in some cases, respiration chambers remain the most accurate method of assessing EMP in individual animals.

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Table 1 Techniques for estimation of methane emission from livestock