Measuring enteric methane emissions from individual ruminant animals in their natural environment

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Abstract. Ruminant livestock are an important source of meat, milk, fiber and labor for humans. The process by which ruminants digest plant material through rumen fermentation into useful products results in the loss of energy in the form of methane gas from consumed organic matter. The animal removes the methane building up in its rumen by repeated eructations of gas through its mouth and nostrils. Ruminant livestock are a notable source of atmospheric methane, with an estimated 17% of global enteric methane emissions from livestock. Historically enteric methane was seen as an inefficiency in production and wasted dietary energy. This is still the case, but now methane is seen more as a pollutant and potent greenhouse gas. The gold standard method for measuring methane production from individual animals is a respiration chamber, which is used for metabolic studies. This approach to quantifying individual animal emissions has been used in research for over 100 years, however, it is not suitable for monitoring large numbers of animals in their natural environment on commercial farms. In recent years, several more mobile monitoring systems discussed here have been developed for direct measurement of enteric methane emissions from individual animals. Several factors (diet composition, rumen microbial community and their relationship with morphology and physiology of the host animal) drive enteric methane production in ruminant populations. A reliable method for monitoring individual animal emissions in large populations would allow 1) genetic selection for low emitters, 2) benchmarking of farms, and 3) more accurate national inventory accounting.

Keywords: Enteric methane, measurements, methods, normal environment

1 Enteric methane production

Mankind relies on domesticated herbivorous mammals of the Bovidae family (about 3.6 billion worldwide [1], such as ruminants, to produce edible food (e.g. meat and milk), fiber and labor. Importantly, ruminants are efficient convertors of non-human edible plant material into edible energy and protein. A total 37% of the world's terrestrial land area is grassland and provides a natural and potential source of affordable nutrients for animals if managed sustainably [2].

Ruminants are responsible for an estimated 17% of global enteric methane emissions and 3.3% of total global greenhouse gas emissions from anthropogenic sources [3].

Attributes of the ruminant animal and its diet all influence the amount of methane produced (Fig. 1). The main drivers of enteric methane production are diet composition, the rumen microbial community and their symbiotic relationship with the morphology and physiology of the animal's digestive system.

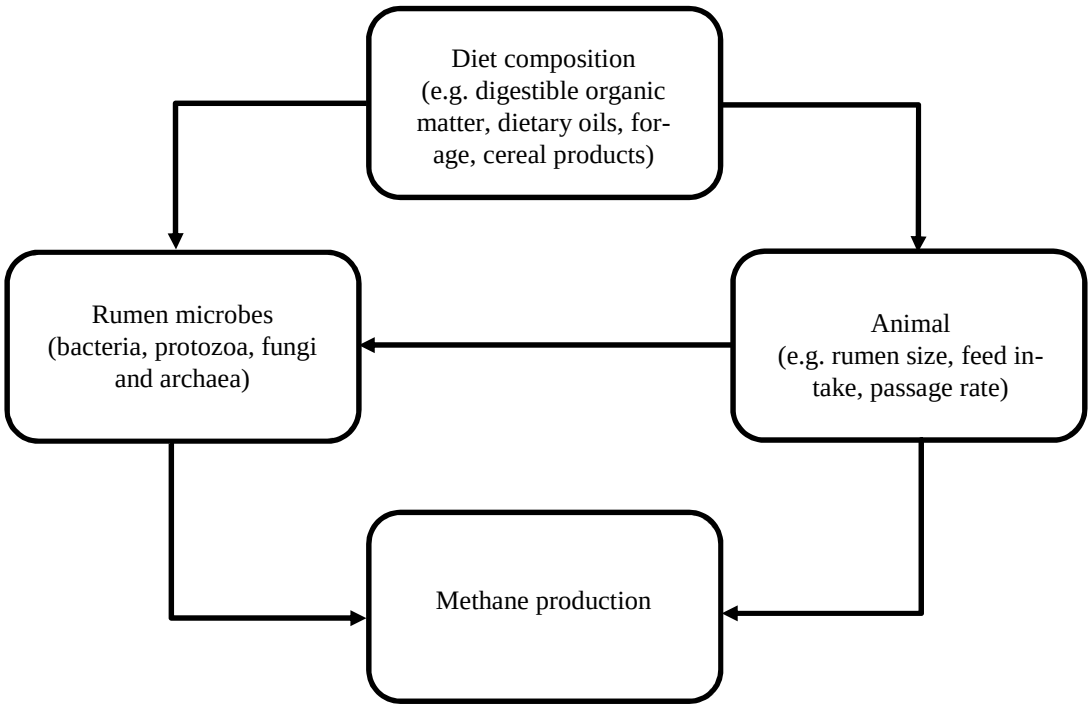

Fig. 1. Main drivers of enteric methane production

1.1 Rumen microbes and methane production

Ruminants have evolved a four-chamber foregut that includes the rumen, which contains bacteria, protozoa and fungi that ferment plant material with a by-product being the production of metabolic hydrogen and its utilization by methanogenic archaea to produce methane gas. Ruminants lack the enzymes needed to degrade plant polysaccharides, and instead rely on a diverse community of rumen microbes. The addition of cellulase and hemicellulase enzymes to a ruminant's diet may also enhance fiber digestion and productivity [4]. The rumen bacteria, fungi and protozoa ferment consumed food to form volatile fatty acids that provides a source of energy for the animal. Eventually the microbial biomass and some unfermented feed components (such as dietary fats and undigested organic matter) pass into the hindgut further providing a potential source of nutrients. In ruminants, enteric methane is produced predominantly in the rumen (87 to 93%) rather than the hindgut [5]. Any methane produced in the hindgut is

largely (i.e. 90%) absorbed and expired through the lungs, with the remainder being excreted through the rectum [6]. The loss of methane from the rectum has been estimated at between 1 and 8% [6-8], with the lower value being associated with sheep and the higher value for dairy cattle.

The process of methane production [3] in the rumen and hindgut is as follows: Glucose equivalents from plant polysaccharides (e.g. cellulose, hemicellulose, pectin, starch, sucrose) are hydrolyzed by microbial enzymes to form pyruvate (1).

$$\text{Glucose} \rightarrow 2 \text{ pyruvate} + 4H \quad (1)$$

The anaerobic fermentation by bacteria, protozoa and fungi of pyruvate produces reduced co-factors such as NADH. Reduced co-factors are then re-oxidized (e.g. NADH to NAD) to complete the synthesis of volatile fatty acids; the principle products being acetate, butyrate and propionate (anions of acetic, butyric and propionic acids).

$$\text{Pyruvate} + H_2O \rightarrow \text{acetate (C2)} + CO_2 + 2H \quad (2)$$
$$2C2 + 4H \rightarrow \text{butyrate (C4)} + 2H_2O \quad (3)$$

The creation of acetate (2) and butyrate (3) provides a source of metabolic hydrogen. Alternatively, the production of propionate (4) can utilize available hydrogen and reduce the potential for methane to be produced.

$$\text{Pyruvate} + 4H \rightarrow \text{propionate (C3)} + H_2O \quad (4)$$

The available metabolic hydrogen is converted to hydrogen gas by hydrogenase-expressing bacteria, and then the hydrogen gas is utilized by methanogens (methanogenesis) to produce methane and water (5).

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (5)$$

Therefore, the diet of the animal influences the production and balance of volatile fatty acids in the rumen. For example, if the ratio of acetate to propionate was greater than 0.5, then hydrogen will accumulate to be used by methanogens [9]. A buildup of hydrogen is potentially detrimental to the animal as it can inhibit microbial growth, forage digestion, and production of volatile fatty acids [10]. While production of methane by methanogens is the main sink for available hydrogen, there are two other lesser but alternative sinks for available hydrogen which are 1) the saturation of unsaturated fatty acids (dehydrogenation) and 2) the production of ammonia from the degradation of amino acids.

1.2 Diet composition and feed intake

Diets high in fiber content promote rumen bacteria that produce acetate. Diets containing more rapidly fermented plant carbohydrates such as starch and sugar, promote rumen bacteria that produce propionate. Changes in diet and available substrate result

either in a shift in the microbial population or a reduction in fermentation rate. For highly fermentable diets, the production of propionate can exceed the current requirement of the animal and its ability to buffer a change in rumen pH (pH below about 5.5). This leads to the production of lactic acid and a change in the microbial population. The ratio of acetate to propionate varies depending on the relative proportions of different rumen bacteria and due to the animal's diet. The rate of rumen microbial fermentation, and availability of metabolic hydrogen, at any given time determine the production of substrates. While both diet composition, morphology and physiology of the host animal influence the microbial community [11-12], diet composition appears to have greater influence than the host animal [13]. In ruminants, archaea (majority being methanogens) have been found to be less diverse than rumen bacteria, reflecting the narrow range of substrates that archaea depend upon [13]. Furthermore, Henderson et al. [13] found the archaeal groups of *Methanobrevibacter gottschalkii*, *Methanobrevibacter ruminantium*, *Methanosphaera sp*. and two Methanomassiliicoccaceae-affiliated groups account for 89% of methanogen communities globally. The diversity of rumen bacteria and interaction with the host animal may explain differences in methane emissions among sheep fed the same diet in a study by Bell et al. [14]. Data from the Rowett Institute in Scotland [14] showed measured metabolizable energy values for paired sheep fed the same diet and amount of feed were highly correlated (Lin's concordance correlation coefficient = 0.93; Fig. 2) but considerable variation existed in methane produced per kilogram dry matter intake between paired sheep on the same diet (Lin's concordance correlation coefficient = 0.22; Fig. 3).

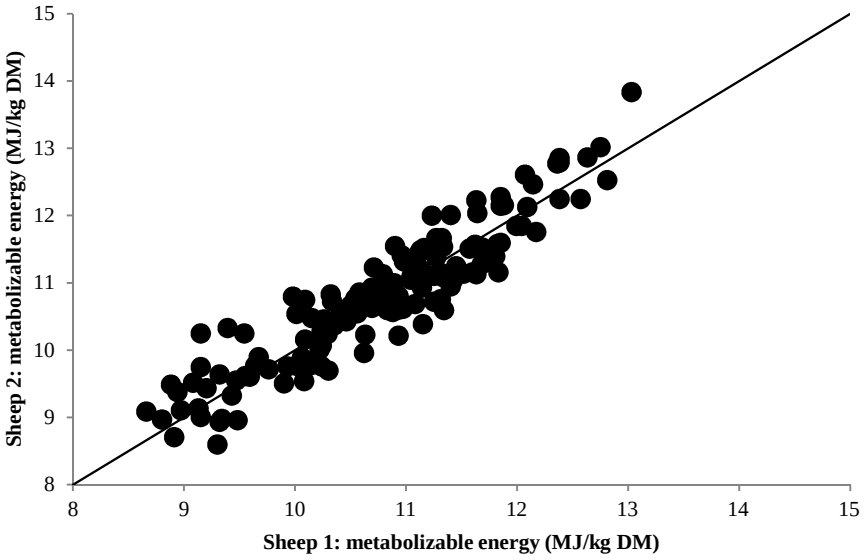

Fig. 2. Observed metabolizable energy values (g/kg dry matter (DM)) for paired sheep (n pairs = 144) fed the same diet and amount of feed. A 45° line through the origin is shown [14].

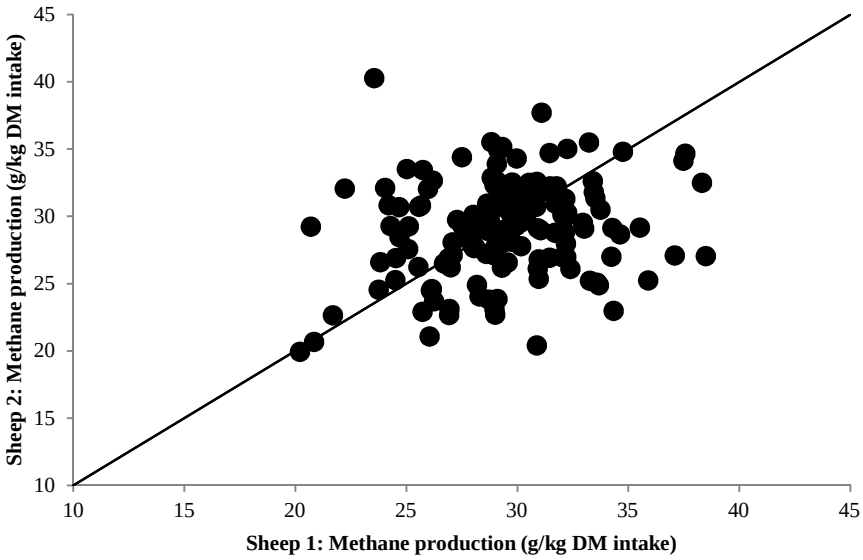

Fig. 3. Observed methane production (g/kg dry matter (DM) intake) for paired sheep (n pairs = 144) fed the same diet and amount of feed. A 45° line through the origin is shown [14].

The effect of diet, i.e., amount of intake and composition, has been found to account for a large proportion of variation in enteric methane emissions from animals [15-16]. It is well recognized that methane production is positively associated with dry matter intake and in particular digestible organic matter intake in ruminant livestock (R^2 = 0.99; Fig. 4).

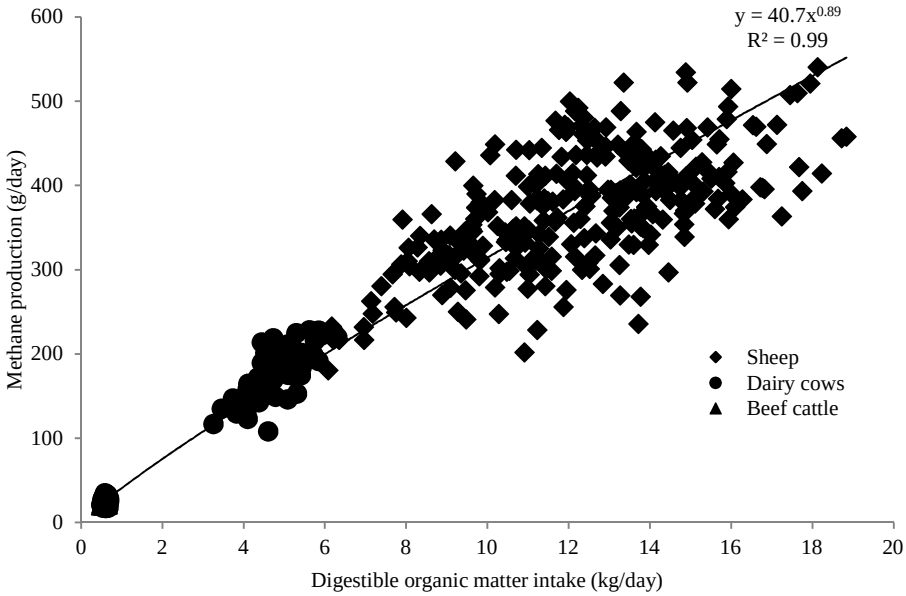

Fig. 4. Relationship between digestible organic matter intake and methane production per day for sheep (♦; n = 288), beef cattle (▲; n = 71) and dairy cows (■; n = 284). The line of best-fit across all values and passing through the origin is shown [14].

Even with the high association between digestible organic matter intake and methane production seen across ruminant livestock, there is notable variation in emissions at a given level of intake, which is particularly noticeable for dairy cows (Fig. 4) and also seen in sheep (Fig. 3). Across cattle and sheep fed diets encompassing a wide range of nutrient concentrations (i.e., 235 to 649 g NDF/kg dry matter, 92 to 251 g crude protein/kg dry matter, 17 to 64 g ether extract/kg dry matter and 9 to 14 MJ metabolizable energy/kg dry matter) and methane emissions (14 to 40 g/kg dry matter), Bell et al. [14] found digestible organic matter, oil (ether extract) and feeding level (metabolizable energy intake expressed as multiples of maintenance requirement) as the important explanatory variables describing methane per kilogram of dry matter intake (6).

$$CH_4 \text{ (g/kg DM intake)} = 0.046 \text{ (s.e. } 0.001) \times \text{digestible organic matter} - 0.113 \text{ (s.e. } 0.023) \times \text{oil (both g/kg DM)} - 2.47 \text{ (s.e. } 0.29) \times (\text{feeding level} - 1) \quad (6)$$

As expected, there is a positive response in methane produced to per unit dry matter intake to increasing digestible organic matter. The positive response to increasing digestible organic matter can be reduced by increasing dietary contents of oil and/or increasing feeding level (Fig. 5). Due to their chemical composition, individual feed ingredients can vary considerably in their methanogenic effect, with distiller's grains resulting in 3.8% of gross energy intake losses as methane and peas 12.8% [17]. Diets that encourage a higher rate of fermentation increase the passage rate of food through the rumen and potential level of feed intake, therefore reducing methane losses per unit

intake [9, 18]. While increased intake of less digestible feeds such as forage and fiber can result in increased acetate and methane production, there appears little effect on methane production per dry matter intake [18]. Whereas an increase in more digestible feeds, such as cereal products in the diet, gives rise to elevated levels of propionate resulting in a curvilinear reduction in methane losses per dry matter intake [19-20]. Easily digestible diets can lose as little as 2 to 3% of gross energy intake as methane, whereas less digestible diets with often more than 50% forage content would be associated with greater than 6% of gross energy intake loss [21]. Also, increasing the dietary oil content in diets inhibits fiber digestion [22-23] and encourages post-ruminal digestion, particularly in the small intestine, which is energetically more efficient with less methane losses than in the rumen.

Animals with the highest feeding levels (4 and 5 times maintenance requirements) and fed diets with high oil content have the lowest emissions per unit intake (Figure 5). Low enteric methane losses per unit intake appear possible by mechanisms that promote the passage of organic matter to post-rumen digestion and reduce rumen fermentation by high intakes of digestible feed and addition of dietary oil.

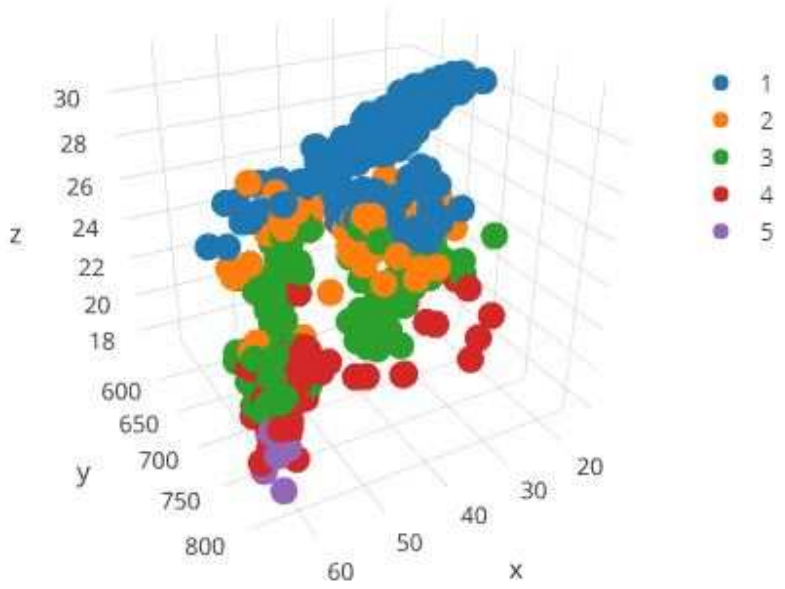

Fig. 5. Effect of diet contents of digestible organic matter (y), oil (x) and feeding level from 1 to 5 (metabolizable energy intake expressed as multiples of maintenance requirement) on methane production (z) by cattle and sheep (n = 643) per kilogram dry matter intake (y values adjusted for the random effect of experiment).

2 Direct measurement of individual animal methane emissions

Several studies have compared different approaches for measuring methane emissions from individual animals [5, 9, 24-25]. Studies with a respiration calorimeter (chamber) investigating the metabolic efficiency of cattle and sheep fed different diet treatments provides a measure of methane output and assessment of variation among animals. However, such an approach is not applicable for population studies on commercial farms. The worldwide interest in measuring methane emissions from individual animals appears warranted given the considerable variation seen among animals fed the same diet (Fig. 3) and the benefit this would bring to advancing our ability to monitor this anthropogenic source of emissions. This has led in recent years to the development of approaches that take repeated 'spot' measurements of methane from the breath of animals in their natural environment.

2.1 Whole animal emissions

Historically most studies assessing methane emissions and energy efficiency of ruminant livestock have been done using a respiration chamber [26-27]. The respiration chamber is recognized as the gold standard method for measuring whole animal methane losses (i.e. mouth, nostril and flatulence; Fig. 6).

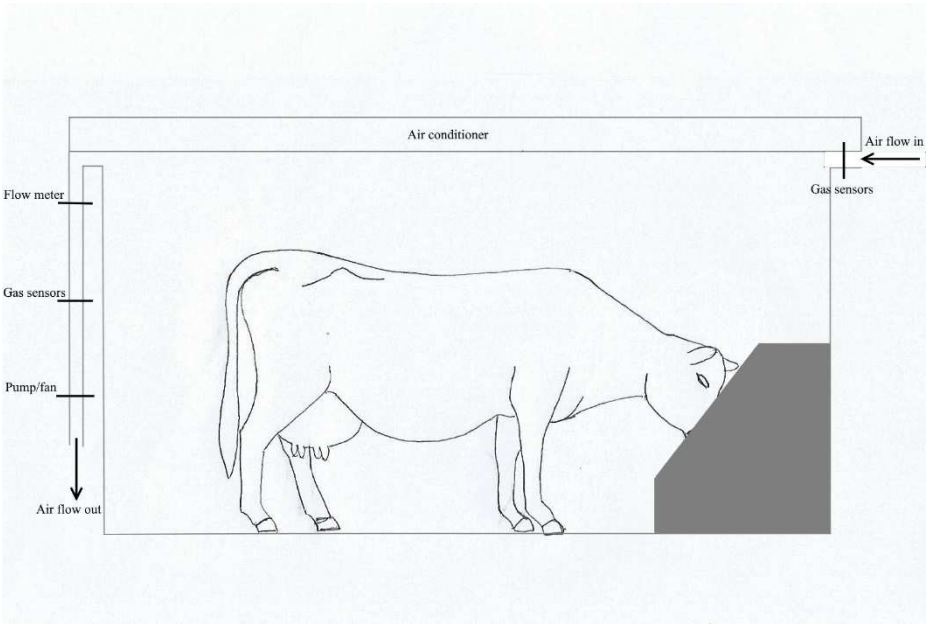

Fig. 6. Illustration of a respiration chamber for measuring whole animal gaseous emissions.

This method involves fresh air flow in and extracted by a pump or fan out of the chamber. The air concentrations (i.e. oxygen, carbon dioxide, hydrogen and methane) in the incoming and outgoing air are measured at intervals using an arrangement of gas sensors to determine the gas emission rate produced by the animal. The gas emission rates are multiplied by airflow to finally derive daily gas production. Chamber temperature, humidity and the mixing of air are often controlled using an air conditioner.

Housing individual animals in a respiration chamber for usually three days (final two days being used to derive animal gas production) is impractical for large-scale measurements of methane from individual animals. Also, housing an animal in a chamber can affect individual animals differently, and potentially result in depression of appetite [28], which is less of an issue for comparing feed treatments in whole animal metabolic studies than differences among animals. The impact on animal behavior can be minimized by ensuring visual contact with other animals and familiarity with the environment [25].

A more mobile and smaller chamber has been developed for sheep, a portable accumulation chamber (PAC), which measures gas emissions from individual chambers for up to 1 hour [29]. The results appear less repeatable than respiration chamber measurements, presumably partly explained by the contrasting environments when sampling, however the small chamber can be used on commercial farms for short periods and with grazing systems. Other sampling methods measuring whole animal emissions have used an enclosed barn [30], polythene tunnel [28] or simply in the field using a tracer gas [31]. These approaches require careful monitoring of the sampling environment, which makes replicating these techniques consistently on commercial farms difficult.

More invasive methods used in research and not appropriate for commercial farm use, involve injecting radioactively labelled methane (isotope dilution technique) [6, 32] or ethane [33] into the rumen fluid and gas sampled by cannula or within an enclosure such as a chamber.

2.2 Breath sampling

Measurement methods that try to integrate into the natural environment of the animal have been developed that measure solely methane produced from the mouth and nostrils of the animal (since this represents the majority of the animal's emissions). This approach has been found to correlate well with respiration chamber measurements [8, 34]. However, due to the often-higher variability observed with this approach, the number of animals and days needed to assess treatment effects using breath sampling methods are greater than when using respiration chambers. Typically, a minimum of 5 to 7 days of 'spot' measurements are needed. The duration of sampling needed to obtain repeatable measurement that allows assessment of within-cow, between-cow, diet and temporal effects is dependent on the frequency of spot measurements, which can be influenced by visits to the sampling location, and the ability to account for potential sources of error [35].

One such approach is the sulphur hexafluoride (SF_6) tracer method (Fig. 7) which involves collecting breath samples continuously into an evacuated canister over a period of several hours within a day and for 5 to 7 days [7]. The air inlet of a capillary

tube is held close to the nostril of the animal by a head halter. A permeation tube containing a known amount of the inert gas SF_6 is placed in the rumen of the animal and continuously releases the gas over the sampling period. Prior to placing the permeation tube in the animal's rumen, the release rate of SF_6 from each tube is determined by placing the tube in a water bath at 39°C and routinely weighing the tube until an accurate loss rate is obtained. The ratio of concentrations for methane and SF_6 collected in the canister on the animal, and analyzed using gas chromatography, along with the release rate of SF_6 gas (QSF_6) from the permeation tube are used to derive the methane emission rate (QCH_4) and daily methane production (7).

$$QCH_4 = QSF_6 \times [CH_4]/[SF_6] \qquad (7)$$

While the use of the SF_6 technique shows good agreement with methane emissions measured from the same cows in a respiration chamber, the approach appears to produce more variable results [8, 36]. Some of this variability can be attributed to the invasive nature of the equipment, consistency of release/sampling of the SF_6 gas and influence of background gas concentrations. The use of a tracer gas is not always permitted in every country. The method may also be more suited to animals fed a high forage diet and not with diets that result in greater post-ruminal digestion [37].

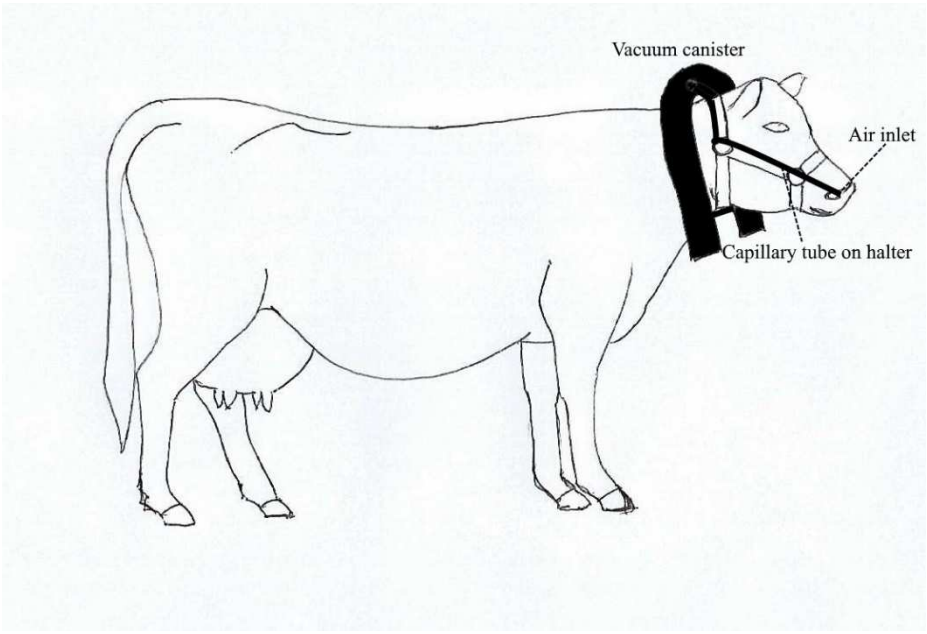

Fig. 7. Illustration of the sulphur hexafluoride (SF_6) tracer method for measuring methane emissions from the nostril of the animal.

Other methods have been developed to sample methane emissions from solely the breath of an animal using a head box [38], mask [39], at a feed bin [34, 40] or with a

laser gun methane detector pointed at the muzzle of the animal [42] (Fig. 8). The use of a head box, mask and laser gun approach may require the animal to be restrained and limit the animal's ability to drink and eat and function normally.

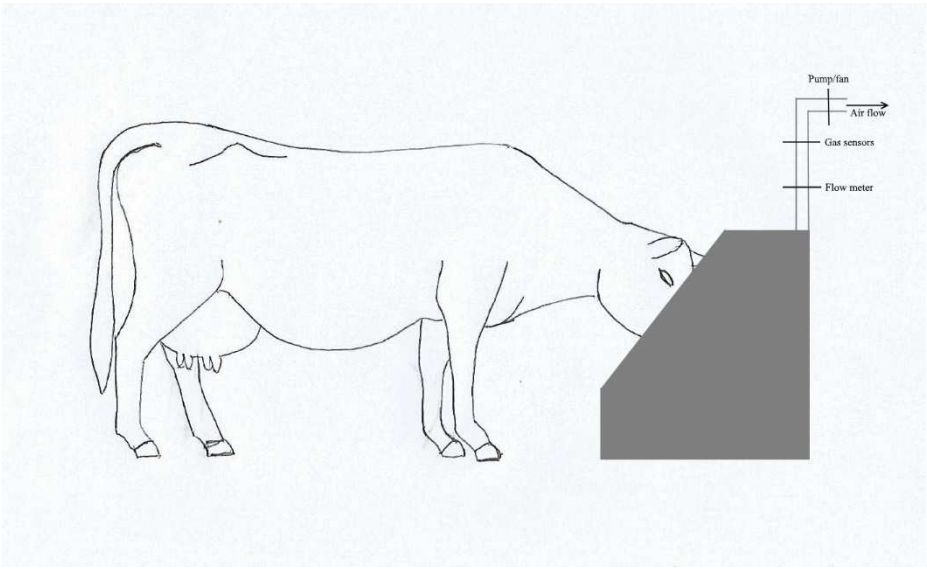

Fig. 8. Illustration of the sniffer method for measuring methane emissions from the mouth and nostrils of the animal at a feed bin.

These methods involve differing levels of complexity (i.e. flow meters, tracer gas, attachments, proximity sensor, filters) and use frequent 'spot' measurements within a day (rather than continuously over 24 hours as with the chamber) to determine methane production. The regular sampling of gas within a day needs to ensure that it accounts for the head position of the animal in close proximity to the sampling tube (i.e. when a peak in gas concentration is observed, Fig. 9) and the diurnal pattern for methane (Fig. 10). The location of the animal's head to the gas sampling tube can be determined using a proximity sensor [40] or filtering the data for eructation peaks of methane [34]. Figure 9 shows eructation peaks for two cows measured during milking at a feed bin, with gas concentration measured every second and with an air flow rate of one liter per minute. Both Cow A and Cow B milked for a similar length of time and consumed a similar amount of a commercial ration (50% forage in the dry matter) during the day (19.7 and 19.1 kg dry matter intake respectively). However, Cow A had a higher eructation rate of 1.3 per minute (mean peak concentration of 728 ppm) compared to Cow B of 1.0 eructation per minute (mean peak concentration of 847 ppm). These differences in mean concentration and frequency of eructations can be combined to derive individual animal methane emission rate. Bell et al. [41] measured the emission rate of 1,964 dairy cows on commercial farms in the UK and found an average individual cow emission rate of 2.9 mg/minute (ranging from 0.6 to 4.8 mg/minute). This equates to an average of 418 g/day per cow and a range of 286 to 526 g/day using the equation by Garnsworthy et al. [34] (methane (g/day) = 252 + (57.2 × emission rate in mg/minute)), which

links on-farm and chamber measurements. This range of values is similar to the range reported for dairy cows of 220 to 480 g/day by Grainger et al. [8]. With a population of about 1.8 million dairy cows in the UK this would amount to approximately 275 thousand tonnes of methane produced each year.

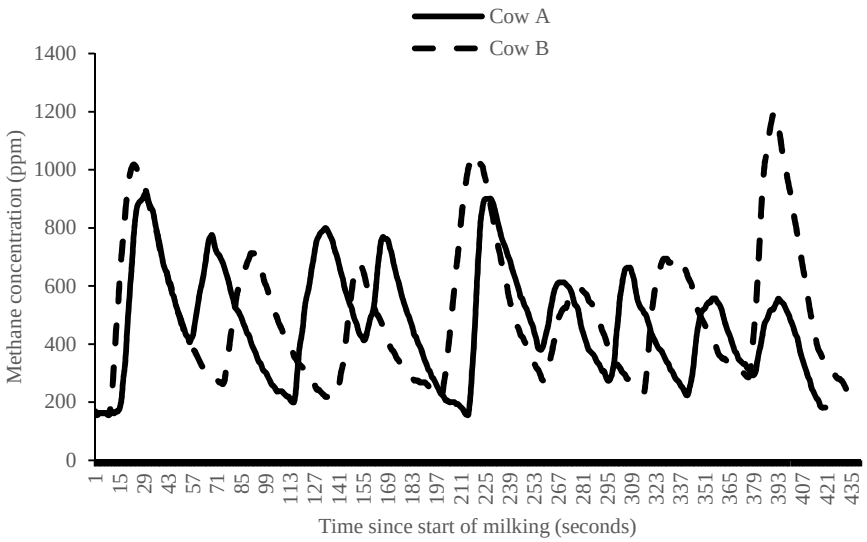

Fig. 9. Methane measured continuously during milking from a feed bin showing a profile for Cow A and Cow B.

Within periods of 'spot' measurements, the frequency of eructations and gas concentration of eructations varies among cows (Figure 9). Also, methane emissions over a 24-hour day are characterized by a diurnal pattern [43-45], with a peak in emissions after feeding being followed by a gradual decline until the next consumption of feed. The average diurnal pattern across a group of animals often shows a peak in methane production soon after feed is allocated due to this activity stimulating most animals to feed (Fig. 10). However, in reality there is considerable variation in diurnal patterns among animals due to the time when animals choose to eat [45].

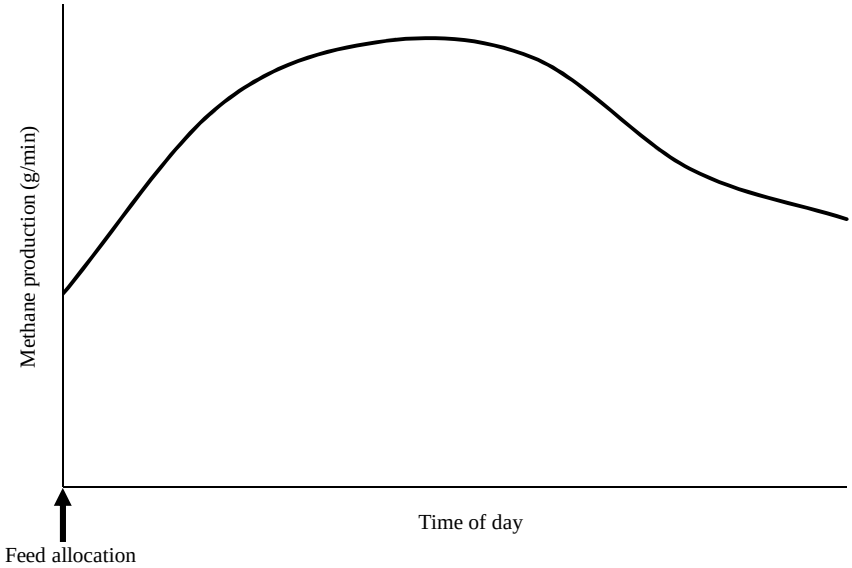

Fig. 10. Average diurnal pattern of methane emissions during a 24-hour day for cows allocated food once per day.

The diurnal pattern is affected by feed allowance and feeding frequency [43], and does not appear to change over time or with a change in diet [45]. The frequent 'spot' sampling of breath methane emissions has come about due to the need to measure methane from commercial animals. Methods that are more mobile, non-invasive to the animal and can fit into the animal's natural environment are of great interest, but bring challenges in application and data processing. Taking 'spot' measurements of methane (expressed in various units of concentration, emission rate, ratio with carbon dioxide or grams/day) has been found to be a repeatable measure [40, 46], however, to be a reliable measure the data requires processing to account for sources of error such as cow head position [40], number and timing of measurements [24, 35] and potential changes in the sampling environment. Overall, this approach and development can provide useful insight into quantifying methane emissions on commercial farms as illustrated and explore sources of variation in large populations of animals.

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