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A modified sulphur hexafluoride tracer technique enables accurate determination of enteric methane emissions from ruminants

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ABSTRACT

The sulphur hexafluoride (SF_6) tracer technique enables determination of enteric methane emissions from large numbers of individual ruminant animals. The objective of this research was to identify and correct substantial errors within the SF_6 technique. Six experiments were undertaken using respiration chamber, laboratory or SF_6 techniques. Experiment 1 used respiration chambers to demonstrate that the daily pattern of methane emissions from dairy cows was related to their pattern of feed intake. In contrast, the daily emission of SF_6 from these cows was constant and independent of the pattern of methane emission. This finding supports the contention that in order to accurately determine daily methane emissions using the SF_6 technique, it is necessary that gases are collected continuously at a constant rate for 24 h. Since development of the SF_6 technique in 1993, it has been propounded that capillary-tube flow restrictors achieved a constant rate of sample collection into evacuated gas collection canisters. Laboratory experiments 2, 3, 4 and 5 demonstrated that, when capillary-tube flow restrictors are used, the rate of sample collection declined and caused a bias of up to 15.6% in calculated methane emissions. This error was caused by an interaction between the declining sample collection rate and the pattern of an animal's methane emission over 24 h. In contrast, orifice plate flow restrictors maintained a constant sample collection rate at canister pressures <0.31 atm and thereby minimised the decline in sample collection rate. Experiment 5 also demonstrated that sample collection using orifice plate flow restrictors, combined with initial (<0.03 atm) and final (<0.49 atm) canister pressures, substantially reduced measurement error. Accuracy of the modified SF_6 technique, incorporating orifice plate flow restrictors for 24 h sample collection, was validated in Experiment 6. The mean (S.D.) methane yield (g CH_4/kg DMI) of eight cows did not differ (P = 0.135) when determined using the modified SF_6 technique 22.3 (1.44) or chambers 21.9 (1.65). In addition, the between-animal coefficient of variation for methane yield determined using the modified SF_6 technique (6.5%) was similar to that determined using

Abbreviations: atm, atmospheres; cc, cubic centimetre; DM, dry matter; DMI, dry matter intake; ID, internal diameter; OD, outer diameter; sccm, standard cubic centimetres per minute; SF_6, sulphur hexafluoride.

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chambers (7.5%). Consequently the modified SF_6 technique enables the statistical power of experiments to be increased or their size decreased. We conclude that the modified SF_6 technique reduced error associated with SF_6 release, sample collection and analysis. It is recommended that the modified SF_6 technique should be used in preference to the original SF_6 technique for determination of enteric methane emissions from ruminants.

1. Introduction

The emission of enteric methane from ruminants can be estimated using a calibrated tracer technique developed by Zimmerman (1993) and first deployed by Johnson et al. (1994). The tracer technique enables estimation of methane emissions from large numbers of individual ruminant and pseudo-ruminant animals without the need for their confinement.

A requirement of the technique is the release of a tracer gas (sulphur hexafluoride, SF_6) at a known constant rate into the reticulo-rumen of each animal. To achieve this, a slow-release device, known as a permeation tube, is placed in the reticulo-rumen (Zimmerman, 1993). The subsequent pattern of SF_6 emission from a ruminant animal has not been reported. However, it is well known that the rate of methane emission from ruminants can vary considerably throughout the day in relation to feeding (Aguerre et al., 2011; Grainger et al., 2007). Thus, the SF_6 technique requires a representative sample of emitted gases to be collected at a constant rate to ensure that an equal proportion of the sample is collected throughout the typically 24 h sampling period.

An animal's daily enteric methane emission is calculated from the measured gas mixing-ratio (concentration) of eructated and expired methane and SF_6 in a gas sample collected from a point near the mouth and nostrils.

Since the first reported application of the SF_6 technique by Johnson et al. (1994) the collection of gases from the ruminant animal has been achieved using evacuated sample collection canisters. This sampling method is reliant on simple components suited to deployment on livestock halters and harnesses. Gases are drawn into evacuated collection canisters without the use of electronic flow controllers or pumping systems. Instead, gases are typically drawn in by a partial vacuum *i.e.*, a low gas pressure within the sample collection canister (hereafter referred to as canister pressure) of <0.03–0.5 atmospheres (atm) (*e.g.*, Johnson et al., 1994).

The original gas sampling apparatus used for the SF_6 technique restricted the rate of gas inflow into an evacuated canister by placing a length of capillary tubing, with an internal diameter (ID) of 127 μm, between the sample collection point and the canister (Johnson et al., 1994). This capillary-tube created a leak into the canister enabling the canister to slowly fill with gases during a sample collection period of 2–6 h. Since 1994 the capillary-tube has become the standard method of flow restriction used within the SF_6 technique. All of the commonly cited publications describing the SF_6 technique have used capillary-tubes as flow restrictors and have stated that the resulting rate of air flow into evacuated canisters was constant throughout the sample collection period (*e.g.*, Johnson et al., 1994; Johnson and Johnson, 1995; Johnson et al., 2007). However, experimental data supporting this assertion have not been reported in any of the papers dealing with the SF_6 technique. Furthermore, the Hagen–Poiseuille Law (Pfitzner, 1976) states that gas flow through a capillary-tube is related to the driving pressure as described by Eq. (1);

$$Q = \frac{\pi \times \Delta P \times r^4}{8 \times \eta \times l} \quad (1)$$

where Q is gas flow rate through the capillary (m^3/s), ΔP is the pressure gradient across the capillary (Pascal), r is the inner radius of the capillary (m), η is the viscosity of the gas (Pascal-second), l is the length of the tube (m), and π and 8 are constants.

It follows from Eq. (1) that as an evacuated canister fills with collected air, the absolute pressure within the canister will increase and the rate of sample collection will decline. The Hagen–Poiseuille law contradicts the assertion in the context of the SF_6 technique, that the air sampling rate achieved using capillary-tubes is constant. Thus, it would seem that all of the methane measurement experiments conducted since 1994 that have employed a SF_6 technique, have failed to achieve a constant rate of sample collection. The impact this non-constant rate of sample collection has had on the accuracy of the SF_6 technique is difficult to assess. This is because the magnitude of the decline in sampling rate during a typical 24 h sampling period has not been documented. Uncertainty surrounding the magnitude of the potential inaccuracy is further compounded by the fact that the error induced by this phenomenon is almost certainly dependent on the diurnal pattern of methane emission of individual animals.

Since the initial use of the SF_6 technique, several research groups have made modifications to the gas sampling apparatus to improve its robustness. One widespread modification has been to use a short length of crimped capillary-tube as the flow restrictor (*e.g.*, Hegarty et al., 2007; Wims et al., 2010; Williams et al., 2011). Typically a short section of 127 μm ID capillary-tube, between 10 and 20 mm in length, is crimped to reduce its air conductance to the desired rate and it is positioned within a gas tight Swagelok tube fitting. The effect that crimping of capillary-tubes has on the rate of sample collection at different canister pressures has not been reported.

Recently the development of laser technology has enabled mass production of plates containing small holes with diameters less than 20 μm. This technology enables the manufacture of orifice plates from stainless steel, industrial ruby or sapphire, with sufficiently small apertures to be of use as flow restricting devices within the SF_6 technique. First proposed as a potential flow restrictor for the SF_6 technique by Zimmerman (1993), the orifice plate is simply a thin plate with a small, sharp-edged, circular hole at its centre. As gases pass through the small hole they are forced to converge, increasing the pressure and velocity of the gas stream. Air flows through an ideal orifice at the speed of sound when the gas pressure within a sample collection canister is less than 0.53 atm. When this sonic flow state is generated by a downstream vacuum, both the air velocity and mass flow through an orifice become choked or independent of the pressure difference across the orifice plate. Therefore, use of orifice plates may be desirable as a replacement for capillary-tube and crimped-capillary-tube flow restrictors within the SF_6 technique.

The present experiments were undertaken to: (a) determine if the rate of SF_6 emission is related to the daily pattern of methane emission from ruminant animals (b) compare the suitability of capillary-tubes and orifice plates as flow restrictors for the collection of gases within the SF_6 technique, and (c) investigate the accuracy of a modified SF_6 technique, incorporating orifice plate flow restrictors, for determination of enteric methane emissions from ruminants. The following hypotheses were tested: (1) that the rate of SF_6 emission from ruminants is constant and therefore independent of the variable rate of methane emission, (2) that crimped (CAP1.5) and un-crimped (CAP100) capillary-tube flow restrictors generate a diminishing air inflow rate (sample collection rate) into evacuated collection canisters as the canisters fill with collected gases, (3) that implementation of the SF_6 technique using CAP1.5 and CAP100 capillary-tubes results in erroneous estimation of methane emissions from ruminants, unlike the use of OP5 orifice plate flow restrictors that improve accuracy of the SF_6 technique.

2. Materials and methods

2.1. Summary of experimental methods

The 3 hypotheses were tested using a series of 6 experiments. These comprised; a respiration chamber experiment investigating the pattern of methane and SF_6 emission from dairy cows (Experiment 1), three laboratory experiments investigating gas sample collection equipment (Experiments 2, 3 and 4), a computer based simulation (Experiment 5), and an experiment to determine the accuracy of the modified SF_6 technique compared to respiration chambers (Experiment 6).

2.2. Experiment 1: Daily patterns of SF_6 and methane emissions from dairy cows

The daily patterns of SF_6 and methane emission from 6 dairy cows were determined using respiration chambers. This experiment was conducted to test hypothesis 1. The experiment was approved by the Animal Ethics Committee of the Department of Environment and Primary Industries, Victoria. Each cow was dosed with a single SF_6 permeation tube emitting (mean ± S.D.) 4.54 ± 0.342 mg SF_6/d, seven days prior to the measurement day. In this and subsequent experiments cows were familiarised with the experimental facilities and gas sampling equipment (chambers or gas collection apparatus, halters and saddles) prior to commencement of the experiment. Cows consumed, on average, 22.4 kg DM of a diet comprising 45.5% cracked maize grain, 44.6% lucerne hay, 8.9% cold pressed canola meal, 0.5% dried molasses and 0.5% minerals on a DM basis. The respiration chambers were opened twice daily enabling half of the diet to be offered and orts removed at 06:45 and at 15:30 h daily. Each cow had 24 h access to the feed bin and an *ad libitum* water supply. The mean methane and SF_6 flux from each cow was determined every 12 min for 48 h using the respiration chamber method described by Williams et al. (2013). A gas chromatograph (3800; Varian Inc., Palo Alto, CA, USA) was used to measure the SF_6 concentration in air entering and leaving the chamber. Gas streams were automatically selected using a chromatograph control method (Compass CDS 2.0; Bruker Chemical Analysis B.V., Goes, The Netherlands) and an automated 16 position valve (Valco Instruments Co. Inc., Houston, TX, USA).

2.3. Description of three gas flow-restrictors tested using laboratory experiments

Three types of inline gas flow restrictors were investigated. Two types were manufactured from capillary tubing and the third type was an orifice plate. All were used to restrict the rate of air flow through Nylon air-sampling tubes with 2.03 mm ID (3.18 mm OD; Ledalon 12; Leda Extrusions NZ Ltd., Upper Hutt, New Zealand). The first type of capillary-tube flow restrictor (CAP100) was constructed from a 100 cm length of stainless steel capillary-tube with 127 μm ID (1.59 mm outer diameter; Supelco, Bellefonte, PA, USA). This tube was fitted at each end with 1.59 mm reducing unions (SS-200-6-1; Swagelok, Solon, OH, USA) and a 15 μm particulate filter at its upstream end (Swagelok; B-2F-15). The second type of flow restrictor (CAP1.5) was made from the previously described stainless steel capillary-tube cut into 1.5 cm lengths. Each CAP1.5 tube was crimped in a single position to restrict its air conductance to the required rate when connected to a canister with internal pressure of 0.03 atm. Once calibrated, each crimped capillary-tube was fitted within a Nylon air-sampling tube attached to a quick connect stem (Swagelok; B-QC4-S-200) using a nut (Swagelok; B-202-1) and ferule set (Swagelok; B-200-SET). A 15 μm particulate filter (Swagelok; B-2F-15) was fitted to the upstream end of the Nylon tube.

The third type of gas flow restrictor (OP5) was made from factory calibrated stainless steel orifice plates with a nominal aperture diameter of 5 μm (SS-1/8″-X-1/8″-NPT, Lenox Laser, Glen Arm, MD, USA). Each orifice plate was fitted between a

Fig. 1. Assembly of the three flow restricting apparatus investigated. Each apparatus comprised: OP5; quick connect stem, orifice plate, 2 μm particle filter, reducing union, sampling tube. CAP1.5; quick connect stem, tube containing a 1.5 cm crimped capillary tube, 15 μm particulate filter, sampling tube. CAP100; quick connect stem, reducing union, 100 cm capillary tube, reducing union, 15 μm particulate filter, sampling tube.

quick connect stem (Swagelok; SS-QC4-D-2PF) and a 2 μm particulate filter (Swagelok; SS-2F4-2). The filter was fitted to a Nylon air-sampling tube by means of a tube connector (Swagelok; SS-200-1-2).

2.4. Description of the measurement apparatus used for simultaneous measurement of absolute canister pressure and air sampling rate during laboratory experiments

In order to simultaneously measure the absolute pressure within a canister and air inflow rate (standard cubic centimetres per min; sccm) the three types of gas flow restrictors (CAP100, CAP1.5 and OP5) were connected to an air flow meter (EW-32908-53; Cole-Parmer, Vernon Hills, IL, USA), digital pressure gauge (XP2i-DP; Crystal Engineering, San Luis Obispo, CA, USA) and a single stainless steel canister with a volume of 800 cubic centimetres (cc). They were connected by means of a four way union cross (Swagelok; SS-200-4) fitted with four on-off valves (Swagelok; SS-41GS2), Nylon air-sampling tube and quick connect couplings (Swagelok; SS-QC4-D-200 and SS-QC4-B-2PF), Fig. 2.

2.5. Experiment 2: Effect of flow-restrictor on sampling rate at different canister pressures

The effect of three types of flow restrictor (CAP100, CAP1.5 and OP5) on the rate of sample collection at different canister pressures were investigated. This experiment tested hypothesis (2) that the rate of air flow through capillary-tube flow restrictors diminishes as canisters fill with collected gases. The air flow rates through six CAP1.5 with mean (S.D.) initial flow

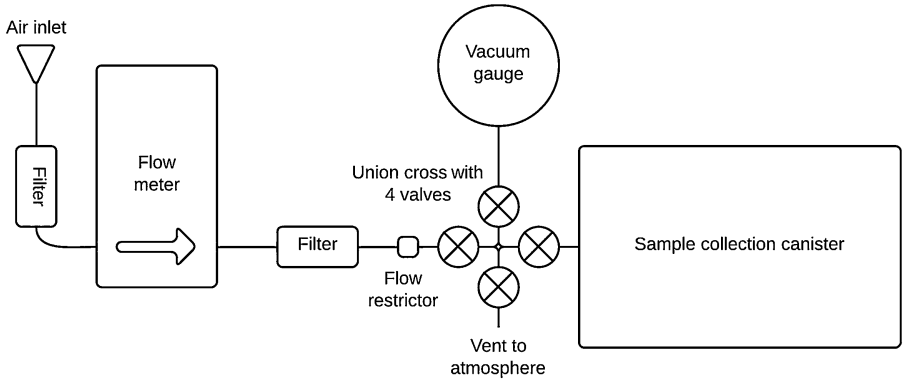

Fig. 2. Configuration of apparatus used to simultaneously measure gas pressure in a canister and air inflow rate (sample collection rate) across different flow restrictors. This apparatus was used in Experiments 2, 3 and 4.

0.27 (0.003) sccm and six OP5 with initial flow of 0.24 (0.011) sccm were measured at a range of different canister pressures using the previously described measurement apparatus (Fig. 2). Measurement began at a mean (S.D.) canister pressure of 0.03 (0.006) atm. The pressure was then increased by venting air into the canister, first to 0.05 atm and then in 0.05 atm increments from 0.05 to 0.95 atm. For comparison the air flow rate through a single CAP100 with initial flow of 1.43 sccm was also measured at the same canister pressures.

2.6. Experiment 3: Effect of flow restrictor on the rate of sample collection over 24 h

The effect of CAP1.5 and OP5 flow restrictors on the rate of sample collection over a 24 h period was investigated. Eight CAP1.5 with mean (S.D.) initial flow of 0.27 (0.009) sccm and 8 OP5 with mean initial flow of 0.24 (0.012) sccm were randomly allocated to one of sixteen 800 cc canisters with initial pressure of 0.03 (0.001) atm to continuously collect air for 24 h. Air flow rate into each canister was measured using the previously described digital flow meter at 0, 12, 18 and 24 h of sample collection. Absolute gas pressure within each sample collection canister was measured using a digital gauge as previously described at 0 and 24 h of sample collection.

2.7. Experiment 4: Relationship between sample collection rate and absolute canister pressure over time for 3 different sample collection apparatus

This experiment was conducted to enable accurate description of the change in sample collection rate that occurs over time using three different gas collection apparatus incorporating either capillary-tube or orifice plate flow restrictors. A detailed description of this relationship was required to test hypothesis 3. The gas collection apparatus used were: (1) CAP100 apparatus incorporating a CAP100 capillary tube connected to a PVC sample collection canister with a volume of 2000 cc, (2) CAP1.5 apparatus incorporating a CAP1.5 capillary tube connected to a stainless steel collection canister with a volume of 800 cc, and (3) OP5 apparatus incorporating an OP5 orifice plate connected to a stainless steel collection canister with a volume of 800 cc. The CAP100 apparatus was designed to represent that generally used by researchers who cite the method of Johnson et al. (1994 or 2007), *e.g.*, Chaves et al. (2006). The CAP1.5 apparatus was designed to represent the sample collection apparatus that incorporates a short crimped capillary-tube, *e.g.*, Wims et al. (2010). The OP5 sample collection apparatus formed the basis of the modified SF_6 technique that was tested in this study. Canister pressure and rate of air inflow into collection canisters, with a common initial pressure of 0.03 atm was measured at approximately hourly intervals for a period of up to 100 h using the measurement apparatus depicted in Fig. 2.

2.8. Experiment 5: Simulation of the effect of gas sample collection bias on the accuracy of daily methane emissions determined using the SF_6 technique

This experiment was conducted to test hypotheses 3 by determining the expected error of methane emissions determined using the SF_6 technique due to a non-constant rate of 24 h gas sample collection by different sampling apparatus.

In order to perform the simulations, methane emission data for 5 different animals was obtained from published and unpublished respiration chamber experiments. These data sets were selected as they provided real data from a variety of animals under a variety of experimental conditions. Thus, these data represent a range of methane emission patterns that occur during implementation of the SF_6 technique. This data provided a daily total methane emission (g CH_4/d) and 24 h pattern of methane emission for each 'animal' with a measurement frequency of between 8 and 60 min depending on the data source. This data was used, within the constraints of the available data frequency, to calculate a minute by minute rate of methane emission (g methane/min) for each animal over 24 h (*i.e.*, 1440 × 1 min periods). This minute frequency data was used as the fixed or known pattern of daily methane emission against which each sample collection apparatus was tested within the simulations performed using Microsoft Excel (Microsoft, 2010). Thus, the simulations enabled calculation of the expected error in daily methane emission (g CH_4/d), that would be caused had the daily methane emission of these animals been determined using the SF_6 technique. Inclusion of three different gas sampling apparatus and three differing initial canister pressures within the simulation tested the effect that these sampling apparatus have on the accuracy of the SF_6 technique.

Respiration chamber data were obtained from the following sources: hourly methane-emission rate data of a Holstein-Friesian cow fed *ad libitum* and emitting 283 g CH_4/d (S1) were obtained by digitising (Plot Digitizer 2.6.3; http://plotdigitizer.sourceforge.net) published data (obtained from Fig. 5 of Sun et al., 2008). The methane emission rate of a Holstein-Friesian cow fed twice daily and emitting 491 g CH_4/d (S2) was obtained at a frequency of 12 min from unpublished data (Fig. 3) measured within the experiment of Moate et al. (2011). Unpublished hourly methane emission rate data of an Angus bull fed once a day (Fig. 3) and emitting 97 g CH_4/d (S3) was measured at an 8 min frequency within the experiment described by Herd et al. (2013), the mean hourly methane emission rate data for 20 control (S4) and 20 nitrate plus sulphate treated (S5) lambs were obtained by digitising (as previously described) published data (obtained from Fig. 1 of van Zijderveld et al., 2010).

The rate of gas sample collection for the three sampling apparatus from Experiment 4 were calculated at 1 min intervals for a 24 h period using the data presented in Fig. 7. The simulations were run using three different initial canister pressures.

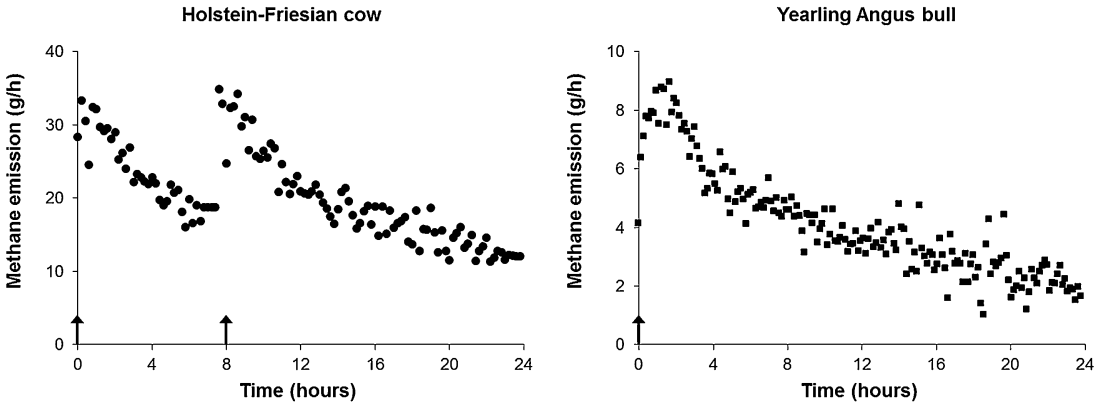

Fig. 3. Diurnal methane emission pattern (g/h) measured using respiration chambers of a single Holstein-Friesian cow fed twice daily (●) from the experiment of Moate et al. (2011) and a single Angus bull fed once daily (■) from the experiment of Herd et al. (2013). Arrows indicate times feed was provided. This data was used for simulations performed in Experiment 5.

These were chosen as the initial pressure typically used at this research institute (0.03 atm), an intermediate pressure of 0.15 atm and the maximal initial pressure reported by Chaves et al. (2006) of 0.26 atm.

The average air flow rate over a 24 h period $\bar{F}$ (g/h) is given by Eq. (2);

$$\bar{F} = \frac{\sum_{i=1}^{24} F_i}{24} \tag{2}$$

where F_i is the measured air flow rate into the canister at hour *i*.

The calculated daily methane emission rate M (g/d) is given by Eq. (3);

$$M = \sum_{i=1}^{24} \left(m_i \times \frac{F_i}{\bar{F}} \right) \tag{3}$$

where m_i is the measured methane production during hour *i*.

2.9. Experiment 6: Validation of a modified SF_6 technique using gas sampling apparatus incorporating orifice plate flow restrictors

The aim of this experiment was to modify the SF_6 technique, incorporating OP5 orifice plate flow restrictors, and to validate the modified method against respiration chambers. The methane emissions of eight Holstein-Friesian cows with mean (S.D.) body weight of 528 (36.4) kg were determined independently using both the modified SF_6 technique and with two respiration chambers.

Measurements were approved by the Department of Environment and Primary Industries Animal Ethics Committee. Five days of SF_6 measurements and two days of respiration chamber measurements were conducted for each cow within an 11 day period.

Each cow was offered 18 kg dry matter (DM/d) of freshly cut perennial ryegrass and 2.2 kg DM/d of concentrate meal (1.0 kg DM cold-pressed canola meal, 1.0 kg DM cracked maize grain and 0.2 kg DM minerals). Half of the daily feed allowance was offered at 06:45 and the remainder at 15:30 h. Feed DM offered and refused were weighed to determine total daily DM intake. During a 14 d pre-experimental dietary adaptation period and during SF_6 measurements, the cows were fed individually in an open sided feed barn described by Williams et al. (2011). After morning and evening feeding, cows were kept outdoors on a woodchip loafing pad.

During respiration chamber measurements, cows were housed individually in one of two respiration chambers for a period of 48 h. Their methane emissions were determined as described by Williams et al. (2013).

Methane emissions from the same cows were also determined using a modified SF_6 technique incorporating the following methodology:

(1) A batch of 20 permeation tubes, as described by Deighton et al. (2011a) and containing mean (S.D.) SF_6 of 2.28 (0.076) g, were incubated at 39.0 °C using a laboratory incubator (Heratherm Advanced Protocol; Thermo Fisher Scientific, Waltham, MA, USA) located within an air conditioned room with a constant temperature of approximately 21 °C. A constant incubation temperature was maintained to prevent temperate fluctuation affecting the rate of SF_6 release (Deighton et al., 2014). Incubation temperature was verified using a NIST calibrated total immersion thermometer with 0.1 °C scale (Extreme Precision; ICL Calibration Laboratories Inc., Stuart, FL, USA) and recorded every 10 min throughout the calibration period using data logging thermometers (TidbiT v2; Onset Computer Corp., Bourne, MA, USA). Permeation tubes were weighed twice weekly at recorded times. The mass of individual tubes was determined

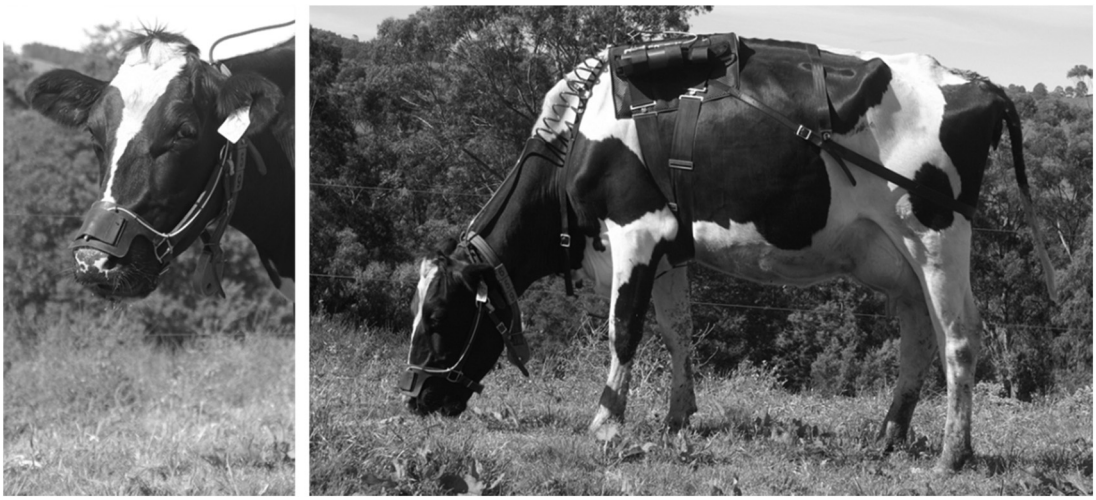

Fig. 4. Halter and saddle used to mount gas collection apparatus on each cow during Experiment 6. The sample collection point is located in a fixed position on the muzzle by means of the leather halter. A flexible airline coil was used to protect the gas sampling tube between the halter and saddle. The evacuated sample collection canister and orifice plate flow restriction apparatus are securely located on the cow's back. Adjustable girth and rump straps prevent dislodgement of the mesh saddle.

using a calibrated analytical balance with 0.1 mg resolution. Measurements were performed following verification of calibration using a standard 50 g weight.

(2) The rate of SF_6 emission from each tube was determined by linear regression of mass over 21 d. Eight permeation tubes with similar SF_6 release rates were selected in order to minimise the range of SF_6 release rate within the experiment. The mean (S.D.) SF_6 release rate of the selected tubes was 7.25 (0.445) mg/d and the regression coefficient (r^2) for each selected tube was greater than 0.999. This ensured that each animal within the experiment herd received a similar daily dose of SF_6 and therefore emitted a similar amount of SF_6.

(3) Immediately after the 21 d determination of SF_6 release rate, the permeation tubes were administered to cows, per os, 7 days before commencement of the gas collection period. This was done in order to ensure that the decline in SF_6 release rate from permeation tubes over time, as reported by Deighton et al. (2013), would have a negligible effect on their predicted rate of SF_6 release during the subsequent 5 d gas collection period.

(4) Methane and SF_6 gas remaining in each canister from its previous use was removed using the procedure described by Deighton et al. (2011). Care was taken to prevent entry of ambient air into canisters during this process. Canisters were then evacuated to 0.03 atm in preparation for sample collection.

(5) Gases were collected into evacuated 800 cc canisters during 5 consecutive 24 h collection periods using OP5 flow restrictors (Fig. 1). It was important that the external side of each orifice plate was exposed to atmospheric pressure in order to achieve the maximum duration of constant sample collection during each 24 h collection period. Particulate filters were cleaned prior to use to ensure they did not restrict airflow and thereby disrupt the pressure difference across each orifice plate. Each filter was flushed with air at a pressure of 6 atm in the reverse direction of sample flow, soaked in ethanol for 24 h, flushed with ethanol and dried overnight at 65 °C.

(6) The air inflow rate (0.24 sccm) through factory calibrated 5 μm orifice plates (Lenox Laser, Glen Arm, MD, USA) at a minimum canister pressure of 0.03 atm was matched to the volume of the canisters (800 cc). This was done in order to maintain a post-collection canister pressure <0.49 atm. to minimise sample collection bias throughout a 24 h sample collection period.

(7) The sample collection apparatus and collection canister were fitted to each cow using a specially designed leather halter and lightweight saddle (Fig. 4). Collection of gases commenced immediately prior to feeding at approximately 06:30 h each day. Sample collection was initiated by connection of the quick connect stem of the orifice plate assembly (Fig. 1) to the quick connect body of the sample collection canister (B-QC4-B-2PF; Swagelok, Solon, OH, USA). Gases were collected from a sampling point on the muzzle of each cow positioned to minimise its blockage with feed particles (Fig. 4). Collected gases were drawn through a 15 μm particulate filter and then through a 325 cm length of tube with 1.0 mm ID (3.18 mm OD; Ledalon 12; Leda Extrusions N.Z. Ltd., Upper Hutt, New Zealand) attached to the orifice plate flow restriction assembly. Between the halter and saddle the sampling tube was protected within a flexible airline coil (2 m × 8 mm OD; RS Components Pty Ltd., Wetherill Park, NSW, Australia). Use of saddles rather than neck mounted equipment reduced damage to sampling apparatus and enabled cows to enter feed stalls and head bales. The light weight saddles were constructed using an anti-gall equine saddle mesh to prevent skin abrasion or sweat accumulation in hot weather.

(8) The time weighted background gas concentration to which each cow was exposed was calculated using the procedure described by Williams et al. (2011). Background air samples were collected into evacuated 800 cc canisters using orifice

plate flow restrictors. As insufficient canisters were available to enable an individual background sampler to be placed on the saddle of each cow separate samples were collected indoors and outdoors. To achieve collection of sufficient sample volume during periods less than 24 h an increased sample collection rate was used for background gas samplers. Indoor samples were collected for 6 h using 8 μm orifice plates, outdoor samples were collected for 18 h using 6 μm orifice plates. Eight canisters were used to determine the concentration of gases at each stall location within the feed barn as described by Williams et al. (2011). Four canisters were used to determine the concentration of background gases outdoors. One canister was placed on each side of the loafing pad, approximately 1 m above the ground.

(9) After the 24 h sample collection period, each 800 cc canister contained gas at a pressure of approximately 0.47 atm. As a sample pressurising pump was unavailable at the time of this experiment ultra-pure nitrogen carrier gas was added to each canister to increase the pressure to approximately 1.5 atm to enable pressurised delivery of samples onto the gas chromatograph (GC; 3800; Varian Inc., Palo Alto, CA, USA). Addition of nitrogen to each canister resulted in an approximately 3.2-fold dilution of the collected gas sample.

(10) Sampled gases and gas standards were introduced to the GC using an automated valve controlled by the sample analysis program. Gases were passed through a Nafion dryer (MD-110-24F-2, Perma Pure, Toms River, NJ, USA) to remove moisture prior to analysis to improve column and detector performance (Pleil et al., 1987). The 7 cc chromatograph sample loop was then flushed with sample gas for approximately 30 s. To ensure a consistent standard and sample gas pressure within the sample loop prior to injection the sample loop was momentarily equalised to atmospheric pressure prior to gas injection onto the columns.

(11) The dilution of collected gases prior to their analysis was determined to enable post-analysis calculation of methane and SF_6 concentration within collected gas samples. To enable this correction to be performed accurately the canister pressure was recorded on three occasions: before sample collection ($Pressure_1$), after sample collection ($Pressure_2$) and after pressurisation of canisters with ultra-pure nitrogen ($Pressure_3$). All measurements were made using a digital pressure gauge (XP2i-DP; Crystal Engineering, San Luis Obispo, CA, USA). The post-analysis correction for sample dilution was performed using Eq. (4);

$$\text{Collected [gas]} = \text{Measured [gas]} \times \frac{\text{Pressure}_3}{\text{Pressure}_2 - \text{Pressure}_1} \quad (4)$$

where Collected [gas] is the concentration of methane (ppm) or SF_6 (ppt) within a gas sample as collected, Measured [gas] is the concentration of methane (ppm) or SF_6 (ppt) within a gas sample as measured by the GC, $Pressure_1$ is the canister pressure at the start of the collection period (atm), $Pressure_2$ is the canister pressure at the end of the sample collection period (atm), $Pressure_3$ is the canister pressure as analysed following addition of ultra-pure nitrogen (atm).

(12) The concentration of methane and SF_6 within diluted samples was measured using the GC as previously described and control software (Compass CDS 2.0; Bruker Chemical Analysis B.V., Goes, The Netherlands). Gas separation and analysis was achieved by means of an electron capture detector (ECD) with a 1.8 m column (Molecular sieve, 5A 80/100, 3.18 mm OD × 2 mm ID) for the measurement of SF_6 and a flame ionisation detector (FID) with a 3.7 m column (Porapak Q, 80/100, 3.18 mm OD × 2.16 mm ID) for measurement of methane. Ultra-pure nitrogen (999.99 g/kg N_2; Coregas, Yennora, NSW, Australia) was used as the carrier gas together with a column temperature of 80 °C. The ECD was operated at 300 °C with a column flow of 30 cc/min and the FID was operated at 250 °C with a column pressure of 3.06 atm. The sample injection volume was 5 cc for the ECD and 2 cc for the FID. Columns were conditioned prior to each day of sample analysis. The molecular sieve column was conditioned for 12 h to a maximum 250 °C. The porapak Q column was conditioned for 4 h to a maximum 105 °C. A temperature ramp rate of 5 °C per minute was used during column conditioning. The GC was calibrated daily prior to sample analysis. Four standards were used to generate a linear response relationship ($r^2 > 0.998$). Each standard contained a different concentration of methane and SF_6 in ultra-pure nitrogen, these were; (i) Ultra-pure nitrogen carrier gas and three NIST certified gas mixtures (Scott-Marrin Inc., Riverside, CA, USA), (ii) 20.37 ppm ± 1% methane, 18.66 ppt ± 10% SF_6, (iii) 60.6 ppm ± 1% methane, 57.5 ppt ± 5% SF_6, (iv) 255.4 ppm ± 1% methane, 244.4 ppt ± 5% SF_6. Samples collected were analysed during the day following their collection. In the present experiment the GC was recalibrated each morning prior to commencing sample analysis.

(13) The methane emission of each animal was calculated using the background-adjusted concentrations of methane and SF_6 within gases collected each day and the predicted SF_6 release rate from each animal's permeation tube as described by Eq. (5) (after Williams et al., 2011).

$$R_{CH_4} = R_{SF_6} \frac{[CH_4]_M - [CH_4]_{BG}}{[SF_6]_M - [SF_6]_{BG}} \times \frac{MW_{CH_4}}{MW_{SF_6}} \times 1000 \quad (5)$$

where; R_{CH_4} is the rate of methane emission (g/d), R_{SF_6} is the rate of SF_6 release from a permeation tube (mg/d), $[CH_4]_M$ and $[SF_6]_M$ are the concentration of methane and SF_6 within an animal's sample, $[CH_4]_{BG}$ and $[SF_6]_{BG}$ is the concentration of methane within concurrently collected samples of background air. Concentrations of methane are expressed in parts per million (μmol/mol) while those of SF_6 are expressed in parts per trillion (pmol/mol) of the collected gas sample. The molecular mass of methane (MW_{CH_4}) is 16, and that of SF_6 (MW_{SF_6}) is 146. The multiplier of 1000 accounts for the disparate units of $[CH_4]$ and $[SF_6]$ and R_{SF_6} so that R_{CH_4} is expressed as g CH_4/d.

2.10. Statistical analysis

In Experiment 1, the methane emission rate (g/h), and the SF_6 emission rate (mg/h) data were each analysed using a linear mixed model in ReML software in GenStat 16. The mixed model included linear regression with time fixed effects for each cow and random splines (Verbyla, 1995; Verbyla et al., 1999) over time for each cow. This was to account for any non-linear trends in gas emission rates over the 48 h period. These were tested by change in deviance chi-square when the spline terms were excluded from the model. Linear trends were tested by F-tests.

In Experiment 2, the percentage flow data for canister pressures were analysed by ANOVA, including factorial treatment structure, device-type by pressure, and split-plot (repeated measures) blocking structure, with canister split for pressure. Linear and quadratic contrasts were embedded in the ANOVA testing the effect of pressure. Single degree of freedom comparisons were performed between CAP1.5 and CAP100 capillary-tubes, and between CAP1.5 and CAP100 capillary-tubes and OP5 orifice plates. Analysis assumptions of normality and constant variance were checked graphically using histograms, quantile plots of residuals, and by graphing residuals against fitted values. The analysis was performed using GenStat 16 statistical software (VSN International, 2013).

In Experiment 3, the percentage decline in flow rate, relative to the initial flow rate, was calculated at 12, 18 and 24 h for each canister. Data were subjected to a repeated measures ANOVA with blocking structure of canister split for time and factorial treatment structure of device-type by time. Linear and quadratic orthogonal polynomial contrasts for the effects of time and time within device-type, were embedded within the ANOVA. The ANOVA assumptions of distributional normality and constant variance were checked and the analysis performed as described for Experiment 2.

In Experiment 4, polynomial regression curves were fitted to the data using Microsoft Excel (Microsoft, 2010). Equations describing the relationship between sample collection rate and time for each apparatus, were required for the simulations run in Experiment 5.

In Experiment 6, data (methane g/d or methane g/kg DMI) were averaged for each method (modified SF_6 and chamber) within each cow and a paired t-test conducted for the difference between method means.

3. Results

3.1. Experiment 1

The mean (S.D.) within-cow SF_6 emission rate was 0.207 (0.0424) mg/h. There was no evidence of a diurnal pattern in SF_6 emission and no relationship between the pattern of SF_6 emission and the pattern of methane emission. Both linear (regression) and non-linear (spline) trends with time were highly significant ($P < 0.001$) for methane emission over 24 h. For the 24 h SF_6 emission data a linear trend, both for individual cows and combined over cows ($n = 6$), was not significant ($P = 0.587$). Similarly there were no significant spline effects (non-linearity with time) detected for SF_6 emission ($P = 0.99$). The pooled correlation between hourly SF_6 and methane within cows was 0.056 (S.E. = 0.105), so that the rate of SF_6 emission was seen to be effectively independent of the rate of methane emission. The rate of SF_6 emission displayed a mean hourly coefficient of variation of 20.5%, with no appreciable auto-correlation (−0.065 SE = 0.069 at lag 1, and −0.037 SE = 0.076 at lag 2). For illustrative purposes the methane and SF_6 emission profiles for an individual cow are shown in Fig. 5.

3.2. Experiment 2

The rate of air flow into evacuated canisters through the three types of flow restrictors declined in response to increasing canister pressure between 0.05 and 0.95 atm.

As a percentage of gas inflow rate at a canister pressure of 0.05 atm, the gas inflow rate through both CAP1.5 and CAP100 capillary-tubes declined as the gas pressure in canisters increased from 0.05 to 0.95 atm (Fig. 6). In contrast, OP5 orifice plates maintained a constant rate of gas inflow at all canister pressures less than 0.31 atm and declined thereafter (Fig. 6). Consequently the OP5 maintained a significantly lower ($P<0.001$) decline in gas inflow rate compared to CAP1.5 and CAP100. At a canister pressure of 0.5 atm the mean rate of gas inflow, as a percentage of flow at 0.03 atm, was 73% for CAP100 and CAP1.5 and 97% for the OP5 flow restrictor. The rate of decline in gas inflow with increasing canister pressure did not differ between CAP100 and CAP1.5 ($P = 0.374$). The relative decline in gas inflow rate through CAP100 and CAP1.5 capillary-tubes were coincident, with a Lin's concordance coefficient of 0.999.

3.3. Experiment 3

The decline in the rate of collection of gases at the end of a 24 h continuous sampling period was greater for CAP1.5 (22%) than for OP5 (8%; $P<0.001$). The rate of air collection through OP5 remained constant for at least the first 12 h of sample collection and declined from its initial rate at some time between 12 h and 24 h of the sample collection period (Table 1).

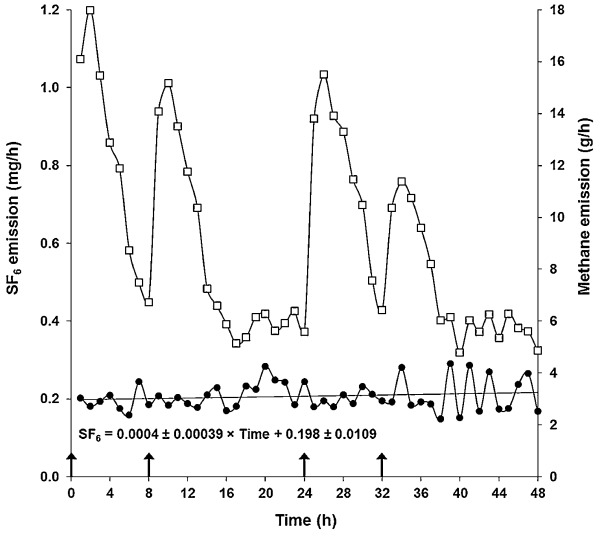

Fig. 5. An example of the typical hourly sulphur hexafluoride (●) and methane (□) emission patterns from a single Holstein-Friesian cow measured within a respiration chamber over 48 h during Experiment 1. The slope of the linear regression of SF_6 emission does not differ from zero (P = 0.336). Arrows indicate the times feed was provided.

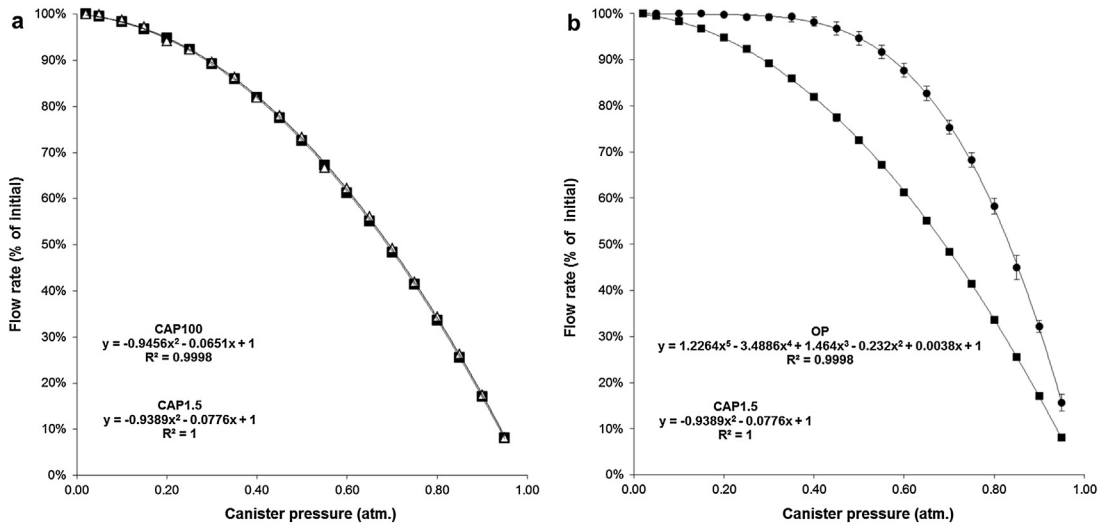

Fig. 6. Air flow rate (mean ± S.D.) into evacuated canisters as a percentage of inflow rate at an initial canister pressure of 0.03 atm measured in Experiment 2. (a) The flow rate through CAP100 (△) and CAP 1.5 (■) are coincident and decline with increasing gas pressure in canisters. (b) Flow rate through the 5 μ orifice plates (●) ($n = 6$) was independent of canister gas pressure at pressures < 0.31 atm. The air flow rate through 1.5 cm of 127 μm crimped capillary-tube (■) ($n = 6$) declined with increasing gas pressure in canisters. (NB. The S.D. error bars for the CAP1.5 data are so small as to be imperceptible).

Table 1
Mean air flow rate into canisters and gas pressure within canisters at specific times during a 24 h continuous collection of air into 800 cc evacuated canisters during Experiment 3.

Time	CAP1.5				OP5			
(h)	Flow (sccm)		Pressure (atm)		Flow (sccm)		Pressure (atm)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
0	0.273	0.009	0.025	0.0007	0.244	0.012	0.025	0.0017
12	0.257	0.006	n.d.	n.d.	0.244	0.012	n.d.	n.d.
18	0.238	0.010	n.d.	n.d.	0.235	0.005	n.d.	n.d.
24	0.213	0.003	0.462	0.0094	0.226	0.007	0.441	0.0235

CAP1.5 = 1.5 cm long crimped capillary-tube.
OP5 = orifice plate with 5 μm aperture.
atm = atmospheric pressure.
sccm = standard cubic centimetres per minute.
n.d. = not determined.

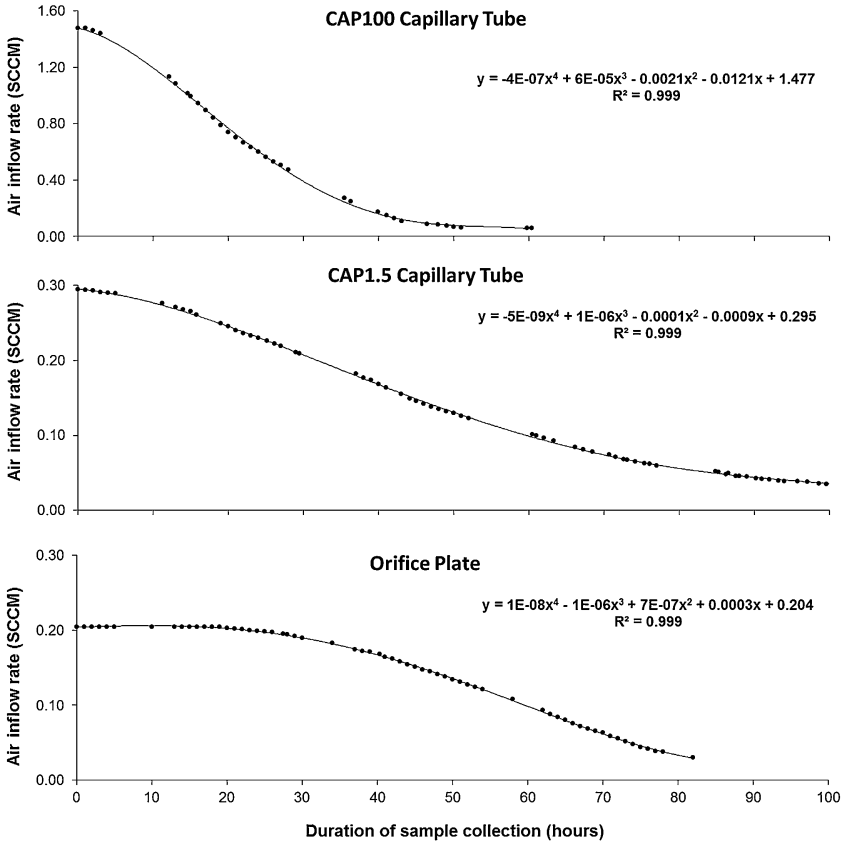

Fig. 7. The rate of air flow into evacuated canisters over time for three different sample collection apparatus tested in Experiment 4. CAP100 apparatus consisted of a 100 cm capillary-tube with 127 μm ID and 2000 cc canister. CAP1.5 apparatus consisted of a 1.5 cm crimped capillary-tube and 800 cc canister. The OP5 apparatus consisted of an orifice plate with a 5 μm orifice diameter and 800 cc canister. All canisters had an equal initial pressure of 0.03 atm at 0 h.

3.4. Experiment 4

The gas sample collection rate achieved using both CAP100 and CAP1.5 gas collection apparatus declined throughout the 100 h measurement period. In contrast the OP5 gas collection apparatus maintained a constant rate of gas sample collection for 19 h and declined thereafter (Fig. 7).

3.5. Experiment 5

The simulations revealed error in calculated daily methane emissions caused by the declining rate of sample collection generated by CAP100, CAP1.5, and to a substantially lesser extent, OP5 sample collection apparatus. Error in calculated methane emission caused by CAP100 sampling apparatus and an initial canister pressure of 0.03 atm ranged from −1.4 to 11.4%. Error was considerably reduced by matching the flow rate of CAP1.5 flow restrictors to the collection canister volume. Error generated using the CAP1.5 sampling apparatus and 0.03 atm initial canister pressure ranged from −0.3 to 3.2%. Use of OP5 sampling apparatus and 0.03 atm initial canister pressure effectively eliminated error in calculated methane emissions with a range from 0 to 0.3% (Table 2). Simulations were also performed for pre-sampling canister pressures of 0.15 and 0.26 atm. In general the error in calculated methane emission was increased by increasing the initial canister pressure (Table 2).

The simulation also enabled prediction of the decline in sample collection rate that occurs using each type of flow restrictor with any given combination of initial sample collection rate and canister volume. When CAP100 or CAP1.5 capillary tubes were used, the rate of sample collection declined by 27% with respect to the initial rate of sample collection. This occurred in association with an increase in canister pressure from 0.03 to 0.5 atm. However, when OP5 orifice plate flow restrictors were used, the rate of sample collection declined by only 5% given the same change in canister pressure. Similar differences between these types of flow restrictors were predicted for canister pressure increases from 0.03 to 0.8 atm (Table 3). CAP100 and CAP1.5 capillary tubes had the same change in flow in response to declining canister pressure as shown in Fig. 6.

Table 2
The expected error in calculated daily methane emissions caused by biased gas sample collection within the tracer gas technique. The percentage error is presented for three different gas sampling apparatus and three differing initial canister pressures. Simulations within Experiment 5 were performed at a 1 min frequency for 24 h using 5 differing methane emission patterns obtained from respiration chamber data.

Sampling apparatus	Canister volume	Initial air flow rate	Flow decline over 24 h	Error (%) in calculated daily methane emissions				
Type	cc	sccm	%	S1	S2	S3	S4	S5
Initial canister pressure = 0.03 atmospheres								
CAP100	2000	1.477	64	+6.8	+6.5	+11.4	+8.9	−1.4
CAP1.5	800	0.273	25	+2.0	+1.9	+3.2	+2.5	−0.3
OP5	800	0.244	3.8	+0.2	+0.2	+0.3	+0.3	0
Initial canister pressure = 0.15 atmospheres								
CAP100	2000	1.429	69	+7.7	+7.4	+15.6	+10.0	−1.9
CAP1.5	800	0.264	31	+2.6	+2.5	+4.4	+3.3	−0.5
OP5	800	0.244	10	+0.7	+0.7	+1.0	+0.9	0
Initial canister pressure = 0.26 atmospheres								
CAP100	2000	1.354	73	+8.5	+8.2	+15.2	+10.9	−2.4
CAP1.5	800	0.250	37	+3.2	+3.0	+5.4	+4.0	−0.7
OP5	800	0.244	20	+1.5	+1.4	+2.3	+1.9	−0.2

S1. Cow with emission of 283 g methane per d (Fig. 5; Sun et al., 2008).
S2. Cow with emission of 491 g methane per d (Fig. 3).
S3. Bull with emission of 97 g methane per d (Fig. 3).
S4. Mean emission of lambs, 17 g methane per d (Fig. 1; van Zijderveld et al., 2010).
S5. Mean emission of lambs, 9 g methane per d (Fig. 1; van Zijderveld et al., 2010).
sccm = standard cubic centimetre per minute.

Table 3
The calculated percentage decrease in rate of air inflow (sample collection rate) through CAP100, CAP1.5 or OP5 flow restrictors for various final (post-collection) canister pressures within Experiment 5. An initial canister gas pressure of 0.03 atm was assumed in all cases.

Flow restrictor type	Final canister pressure (atm)				
	0.4	0.5	0.6	0.7	0.8
CAP1.5 or CAP100	18.1	27.4	38.4	51.5	66.3
OP5	1.86	5.27	12.1	24.0	42.2

3.6. Experiment 6

The mean methane yield (g CH_4/kg DMI) of cows ($n = 8$) determined by the modified SF_6 technique incorporating OP5 orifice plates and an initial canister pressure of 0.03 atm did not differ from that determined using a respiration chamber (P = 0.135, Table 4). The mean (S.D.) methane emission during the SF_6 measurement period was 431 (21.1) g CH_4/d. During respiration chamber housing the emission was 455 (20.4) g CH_4/d. The between-cow coefficient of variation in daily methane yield was 6.5% for the modified SF_6 technique and 7.5% for the respiration chamber technique.

4. Discussion

4.1. Pattern of methane and SF_6 emission

In the original patent describing the SF_6 technique it was stated that the release rate of SF_6 from the permeation tube must be "known and constant" (Zimmerman, 1993). However, in the twenty years since the development of the technique, there have been no published reports to confirm that the release rate of SF_6 within the reticulo-rumen is constant. Whilst Experiment 1 did not measure the release rate of SF_6 from permeation tubes within the reticulo-rumen, it measured the hourly release rate of SF_6 from cows. Analysis of data collected in Experiment 1 did not detect any systematic trend in the pattern of SF_6 emission from cows over a 48 h period. There was no correlation between emissions of SF_6 and methane

Table 4
The mean (±S.D.) methane yield of Holstein-Friesian dairy cows determined using both the modified SF_6 technique or respiration chambers within Experiment 6.

	SF_6 technique	Respiration chamber	SED	P
n	8	8		
DMI (kg/d)	19.3 ± 0.54	20.9 ± 1.08	0.41	0.005
CH_4 (g/kg DMI)	22.3 ± 1.44	21.9 ± 1.65	0.36	0.135
CV (%)	6.5	7.5		

CV = coefficient of variation.

within animals at an hourly time scale therefore we accept hypothesis 1. These results demonstrate that the pattern of SF_6 emission from a cow is consistent with a constant rate of release from the permeation tube throughout the day. In contrast, there was a substantial diurnal pattern in methane emissions that was related to the pattern of feed intake, as has been observed in previous studies (*e.g.*, Moate et al., 1997). The finding that the daily rate of SF_6 emission was constant underpins the validity of the simulations described in Experiment 5 due to the independence of the rates of release of SF_6 and methane.

Hammond et al. (2009) speculated that "a contributor to variance associated with SF_6 measurements is an inconsistent equilibration of the SF_6 gas with rumen gases, for example, if it is entrapped and later released from particulate matter in the rumen". Analysis of the pattern of SF_6 emission from animals in Experiment 1 does not support this speculation. There was no evidence of a diurnal pattern in SF_6 emission as the slope of the linear regression of SF_6 emission does not differ from zero ($P = 0.336$). Hence the daily pattern of SF_6 emission from animals is unlikely to contribute substantially to the variance associated with estimated daily methane emissions.

An hour to hour variation in SF_6 emission rate of 21% supports the assertion of Deighton et al. (2014) that sample collection periods less than 24 h may increase measurement error of the SF_6 technique. We estimate that error due to hourly variation in SF_6 emission rates will contribute to the within-animal, between-day coefficient of variation (CV) by approximately $(0.2/\sqrt{t})$ where t is the duration of the sample collection period in hours. Hence hourly variation of SF_6 emission rates implies a 4% within-animal CV between days when sample collection periods of 24 h are used.

The modified SF_6 technique implemented in Experiment 6 incorporated 5 consecutive 24 h gas collection periods. The daily methane emission of ruminants is substantially influenced by feed intake during the day of gas measurement and also during the previous day as described by Moate et al. (2012), Eq. (6);

$$\mathrm{TME} = (77.1 \pm 28.34) + (19.2 \pm 1.41) \times \mathrm{TDMI} + (8.1 \pm 1.07) \times \Delta\mathrm{DMI} \quad (6)$$

where TME is today's methane emission (g/d), TDMI is today's dry matter intake (kg) and ΔDMI = yesterday's DMI − TDMI (kg).

Moate et al. (2012) reported that "yesterday's DMI is responsible for about 30% of today's methane emission". Therefore if DMI on the day prior to measurement differs from that on the day of methane measurement, an animal's estimated methane yield can be substantially over or under estimated, dependant on the change in DMI between days. Incorporation of 5 consecutive days of gas collection within the modified SF_6 technique ensures that the maximum error in mean methane emission from this source is limited to approximately 6%.

4.2. Sample collection rate

Since inception of the SF_6 technique in 1993 capillary tubes have been the standard method used to restrict the rate of sample collection into evacuated canisters. Their use has relied upon an assumption that their rate of air conductance remains constant throughout the sample collection periods used within the technique. When first deployed, capillary tubes were used to collect samples for short periods of 2–6 h (Johnson et al., 1994). However, due to the diurnal variation in enteric methane emission, researchers routinely use sample collection periods of 24 h in order to collect samples representative of the daily methane emission of each animal. Experiments 2, 3 and 4 demonstrated that the rate of sample collection is not constant over any period when CAP100 or CAP1.5 capillary tube is used to restrict the flow of gas into evacuated canisters. The rate of gas inflow through capillary tube declined by approximately 27% as canister pressure increased from 0.05 to 0.5 atm (Experiment 2). This effect was also observed when sampling-rate and canister-volume combinations reported in the literature were tested in Experiment 4 (*e.g.*, Chaves et al., 2006; Wims et al., 2010). Between 0 and 24 h of collection, the rate of gas collection declined by 22% (Experiments 3 and 4) enabling acceptance of hypothesis 2. The present experiments revealed a curvilinear decline in the rate of flow of air through capillary tubing as canister pressure increases (Fig. 5). However, the rate of air flow through capillary tubes within gas sampling apparatus did not fully conform to the Hagen-Poiseuille Law, which predicts a linear decline in gas flow through a capillary tube as the pressure difference across the tube declines (Eq. (2)). A decline in sampling rate was also observed by Hegarty et al. (2007) who reported an 18% decline in sampling rate over a 48 h period. In contrast, several researchers have stated that the rate of air collection achieved using capillary-tube flow restrictors is constant (*e.g.*, Zimmerman, 1993; Johnson et al., 1994, 2007) but they did not present any data to support their assertions. The observed decline in flow rate when a capillary tube is used means that gases collected early in a collection period will comprise a greater proportion of the total sample than gases collected late in the period, thereby causing sampling bias. Therefore, on the basis of the results obtained from Experiments 2, 3 and 4 we recommend that use of capillary tube flow restrictors be discontinued.

4.3. Erroneous estimation of ruminant methane emissions

Use of capillary tube to restrict the rate of gas inflow into canisters results in erroneous estimation of enteric methane emissions from ruminant animals. This error can be substantial, particularly when animals are fed only once per day or where experimental treatment leads to a different daily pattern of methane emission between treatment groups. The measured pattern of sample collection rate over 24 h for CAP100 sampling apparatus (Fig. 7) was used to mathematically 'sample' the known methane emissions from ruminants, at one minute intervals over 24 h. These simulations demonstrated that error in calculated methane emission could range from at least −2% to +16% enabling acceptance of hypothesis 3. The negative

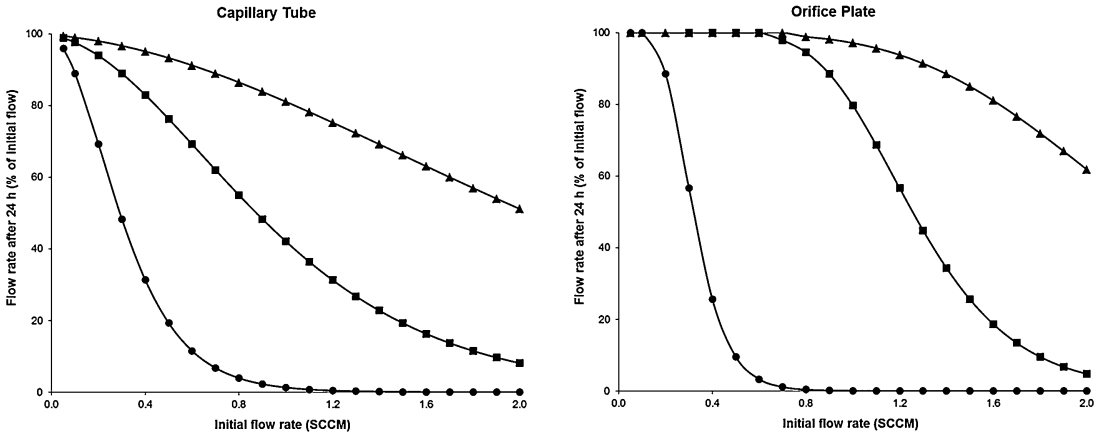

Fig. 8. The relationship between the initial flow rate of air through capillary-tube or orifice plate flow restrictors and the final flow rate after a 24 h sample collection period. Data was simulated during Experiment 5 using the flow vs. pressure relationship described in Fig. 6 for canisters with volume of 500 cc (●), 1500 cc (■), or 3500 cc (▲) using a pre-collection gas pressure in canisters of 0.03 atm.

error calculated for sheep (S5, Table 2) is noteworthy because for that particular treatment, the error generated was caused by a change in the pattern of daily methane emission associated with the experimental treatment. For this reason identical sampling protocols must be applied to all treatment groups within an experiment. Different feeding times or different gas sampling start times can generate differing errors in calculated methane emissions unless a constant rate of sample collection is achieved throughout the collection period.

It has been claimed that declining sampling rate would not bias the final methane to SF_6 ratio of collected gases as gases would always be drawn into canisters in the ratios in which they existed at the sampling site in any instant (Hegarty et al., 2007). While it is correct that the instantaneous ratios represent the relative concentrations of gases at the time they are collected, this does not prevent bias because the rate of methane emission varies throughout the day.

Erroneous estimates of methane emission resulting from the interaction between sampling bias and the pattern of daily methane emission has the potential to either exaggerate or cancel true differences in methane emission that exist between animals and experimental treatment groups. The present simulations have demonstrated that treatment induced differences in the pattern of methane emission in the experiment of van Zijderveld et al. (2010) would cause a different error in the measurement of methane emissions from each treatment group. If this experiment had used the SF_6 technique with CAP100 sampling apparatus the mean methane emissions from untreated control lambs would have been overestimated by 10.9% and those from treated lambs underestimated by 2.4% (S4 & S5, Table 2). The marked differences in sampling bias revealed by the present simulations demonstrates the importance of selecting a suitable gas inflow rate for a collection canister of a given volume. The greater the rate of air collection, the greater the decline in sample collection rate over a 24 h period for any given canister volume (Fig. 8).

The SF_6 technique has been reported to overestimate daily methane emissions compared to respiration chambers due to erroneous estimation of SF_6 release rate from permeation tubes used months after their calibration (Deighton et al., 2013). However, overestimation also occurred when measurements were undertaken within 7 d of insertion of permeation tubes into the reticulo-rumen (*e.g.*, Boadi et al., 2002; Grainger et al., 2007; Pinares-Patiño et al., 2011). In these studies, gas collection for the SF_6 technique used capillary tubes. We speculate that the overestimation of methane emissions of 6% by the SF_6 technique in these studies may be due to biased sample accumulation, coincident with hours of greatest feed intake and methane emission.

4.4. Improving the accuracy of the SF_6 technique

The accuracy of estimating methane emissions can be improved by use of a modified SF_6 technique incorporating orifice plates, instead of capillary tube, to restrict the rate of sample collection. Implementation of this modified technique can maintain the rate of gas inflow into canisters within 5% of the initial inflow rate throughout a 24 h collection period. In contrast, CAP100 and CAP1.5 capillary-tube flow restrictors tested within the present experiments failed to maintain a constant rate of sample collection. Our modified SF_6 technique differs from that described by Johnson et al. (1994) and Johnson & Johnson (1995) in that it is specifically designed to measure methane emissions over a 24 h period with a near constant rate of sample collection. We recommend that the modified SF_6 technique, incorporating orifice plates and 24 h sample collection periods, should be used in preference to the original implementation of the technique.

In summary, the modified SF_6 technique incorporates the following methodology:

(1) Determination of SF_6 release rate from a uniform set of permeation tubes at 39.0 °C over 21 d to achieve a mass-loss regression coefficient of greater than 0.999.

Table 5
The number of replicates (animals) required to detect as significant (at the 1% or 5% levels) a 5%, 10% or 15% treatment difference in methane yield, with a probability of detection (power) of 80%, using a one-tailed, two-sample *t*-test, given between-animal methane emission coefficients of variation ranging between 4% and 20%.

Coefficient of variation%	5% treatment difference		10% treatment difference		15% treatment difference	
	P = 0.01	P = 0.05	P = 0.01	P = 0.05	P = 0.01	P = 0.05
4	15	9	5	3	4	3
6	31	19	9	6	5	3
8	53	33	15	9	8	5
10	82	51	22	14	11	7
12	117	72	31	19	15	9
14	159	98	41	25	19	12
16	207	128	53	33	25	15
18	262	161	67	41	31	19
20	323	199	82	51	38	23

(2) Accurate prediction of the SF_6 release rate from each permeation tube at the time of gas sample collection. This can be achieved either by insertion of tubes into the reticulo-rumen immediately after determination of their release rate and 7 d prior to sample collection, or by correction for the time related decline in SF_6 release rate using Michaelis–Menten kinetics (Moate et al., 2013).
(3) Minimising sample cross-contamination by purging canisters of residual methane and SF_6 gas.
(4) Minimising bias, due the interaction between sample collection rate and diurnal fluctuation of methane emission, by collection of gas samples using orifice plate flow restrictors, an initial canister pressure <0.03 atm, and a final canister pressure <0.49 atm.
(5) Minimising bias due to the daily methane emission contributed by DMI on the day prior to the sample collection period (Moate et al., 2012). This can be achieved by repeating measurement of methane emissions for a minimum of five consecutive days.
(6) Collection of background gas samples representative of the gases that each animal was exposed to during the sample collection period (Williams et al., 2011; Lassey, 2013). This being distinct from the ambient background gas concentration at the trial site.
(7) Daily calibration of the GC to produce a linear standard gas response with regression coefficient greater than 0.999 using a minimum of three certified standard gas mixtures and ultra-pure nitrogen carrier gas.
(8) Use of a uniform GC column injection pressure for all standard and sample gases.
(9) Correction for the effect of sample dilution and the contribution of background gas to the gas concentration of each sample using Eqs. (4) and (5).

4.5. Increasing statistical power of SF_6 experiments

The statistical power of experiments measuring methane emissions can be increased by reducing the between-cow CV of methane emission (Table 5). Within Experiment 6, biased gas sample collection was minimised by implementation of the modified SF_6 technique. The between-animal CV in methane yield, measured using the modified SF_6 technique, was 6.5%. This was comparable with the CV for methane yield of the same cows determined using respiration chambers (7.5%, Table 4). This variation is much less than the previously published CV between-cows reported to range from 11 to 21.5% (Boadi and Wittenberg, 2002; Hammond et al., 2009; Vlaming et al., 2008) and up to 24.5% CV between-sheep (Hammond et al., 2009).

Implementation of the modified SF_6 technique, for five consecutive days using orifice plate flow restrictors, and with a pre-sampling canister pressure <0.03 atm and a post-sampling canister pressure <0.49 atm, has substantially reduced the between-animal CV for methane emission compared with the original SF_6 technique. As a result, when the modified SF_6 technique is employed only 1/3 of the number of animals are required, compared with the original technique, to detect as significant a treatment difference of 10% in methane yield (Table 5). Therefore experiments can be conducted with fewer animals and cost of labour, feed, permeation tubes, gas sampling equipment and analysis of gas samples should be reduced.

4.6. Future applications of the SF_6 technique

Orifice plates are easily used as they produce a gas flow rate dependant on their aperture size, and create a robust installation with standard fittings. While further research is required to optimise the selection and use of orifice plates or similar 'sonic nozzle' flow restrictors, the present implementation offers a useful improvement to the original SF_6 technique. We consider that the present modifications should be implemented as the method of choice for future applications of the SF_6 technique. These modifications will increase statistical power or enable reduction of the size and associated cost of livestock experiments.

The SF_6 technique can be used to determine the methane emissions from large numbers of animals simultaneously. Given the accuracy of the modified SF_6 technique, it can be used to screen large numbers of cattle for methane emissions and should become the method of choice to identify individual animals genetically predisposed to be low methane emitters.

5. Conclusion

The present research has demonstrated for the first time that the rate of methane and SF_6 emission from ruminant animals (dairy cows) is independent. While the rate of methane emission fluctuated in response to feed intake, the rate of SF_6 emission remained relatively constant throughout the day. As a result the ratio of methane:SF_6 within emitted gases varied throughout the day.

The present research has also demonstrated that the gas inflow rate through capillary tubes into evacuated sample collection canisters is not constant but declines over time. This decline in sample collection rate is consistent with the Hagen-Poiseuille law and results from the declining vacuum within canisters as they fill with collected gases. The simulations presented demonstrated that the declining rate of sample collection caused by capillary-tube flow restrictors has the potential to result in errors in estimated methane emissions of up to 15.6%. The magnitude of the error, for a given animal, being dependent on the decline in sample collection rate and the pattern of methane emission from the animal during the sample collection period. Therefore, we conclude that capillary tubes are unsuitable for use as flow restrictors for the SF_6 technique.

The present research has also demonstrated that the decline in sample collection rate over time can be substantially reduced by the use of stainless steel orifice plate flow restrictors. The simulations presented demonstrated that use of orifice plate flow restrictors can effectively eliminate bias in gas sample collection and thereby minimise error in estimated methane emissions. Therefore, we conclude that orifice plate flow restrictors should be used to control the rate of gas sample collection into evacuated canisters used within the SF_6 technique. We speculate that orifice plates made from non-ferrous materials such as industrial ruby or sapphire will also be suitable for use in gas collection apparatus.

We describe a modified SF_6 technique developed for 24 h sample collection periods that enables determination of enteric methane yield with similar accuracy as achieved using respiration chambers. Use of the modified SF_6 technique reduces between-animal variation in estimated methane emissions by reducing errors associated with: (1) the rate of SF_6 tracer gas release *in vivo*, (2) gas sample collection and (3) sample analysis. Consequently the statistical power of SF_6 experiments can be increased or their size and associated cost decreased. It is recommended that the modified SF_6 technique described herein be used in preference to the original SF_6 technique for determination of enteric methane emissions from ruminants.

In addition to research involving gas emissions from ruminants, we have not overlooked the potential for the orifice plate sampling apparatus described here to be useful as a low cost and robust tool for constant rate collection of air samples in atmospheric research and monitoring of airborne pollutants.

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References

Aguerre, M.J., Wattiaux, M.A., Powell, J.M., Broderick, G.A., Arndt, C., 2011. Effect of forage-to-concentrate ratio in dairy cow diets on emission of methane, carbon dioxide, and ammonia, lactation performance, and manure excretion. J. Dairy Sci. 94, 3081–3093.

Boadi, D.A., Wittenberg, K.M., 2002. Methane production from dairy and beef heifers fed forages differing in nutrient density using the sulphur hexafluoride (SF_6) tracer gas technique. Can. J. Anim. Sci. 82, 201–206.

Boadi, D.A., Wittenberg, K.M., Kennedy, A.D., 2002. Validation of the sulphur hexafluoride (SF_6) tracer gas technique for measurement of the methane and carbon dioxide production by cattle. Can. J. Anim. Sci. 82, 125–131.

Chaves, A.V., Thompson, L.C., Iwaasa, A.D., Scott, S.L., Olson, M.E., Benchaar, C., Veira, D.M., McAllister, T.A., 2006. Effect of pasture type (alfalfa vs. grass) on methane and carbon dioxide production by yearling beef heifers. Can. J. Anim. Sci. 86, 409–418.

Deighton, M.H., O'Loughlin, B.M., Buckley, F., Boland, T.M., 2011a. A simple method for pre-calibration storage of sulphur hexafluoride permeation tubes. Anim. Feed Sci. Technol. 166–167, 198–200.

Deighton, M.H., O'Loughlin, B.M., Boland, T.M., Buckley, F., 2011. Gas sampling error in the ERUCT technique: effect of sample cross-contamination. In: Proceedings of the Agricultural Research Forum, Tullamore, Co. Offaly, Ireland, 14–15th March, p. 62.

Deighton, M.H., O'Loughlin, B.M., Williams, S.R.O., Moate, P.J., Kennedy, E., Boland, T.M., Eckard, R.J., 2013. Declining sulphur hexafluoride permeability of polytetrafluoroethylene membranes causes overestimation of calculated ruminant methane emissions using the tracer technique. Anim. Feed Sci. Technol. 183, 86–95.

Deighton, M.H., Williams, S.R.O., Lassey, K.R., Hannah, M.C., Boland, T.M., Eckard, R.J., Moate, P.J., 2014. Temperature, but not submersion or orientation, influences the rate of sulphur hexafluoride release from permeation tubes used for estimation of ruminant methane emissions. Anim. Feed Sci. Technol. 194, 71–80.

Grainger, C., Clarke, T., McGinn, S.M., Auldist, M.J., Beauchemin, K.A., Hannah, M.C., Waghorn, G.C., Clark, H., Eckard, R.J., 2007. Methane emissions from dairy cows measured using the sulfur hexafluoride (SF_6) tracer and chamber techniques. J. Dairy Sci. 90, 2755–2766.

Hammond, K.J., Muetzel, S., Waghorn, G.G., Pinares-Patino, C.S., Burke, J.L., Hoskin, S.O., 2009. The variation in methane emissions from sheep and cattle is not explained by the chemical composition of ryegrass. Proc. N. Z. Soc. Anim. Prod. 69, 174–178.

Hegarty, R.S., Goopy, J.P., Herd, R.M., McCorkell, B., 2007. Cattle selected for lower residual feed intake have reduced daily methane production. J. Anim. Sci. 85, 1479–1486.
Herd, R.M., Bird, S.H., Donoghue, K.A., Arthur, P.F., Hegarty, R.F., 2013. Phenotypic associations between methane production traits, volatile fatty acids and animal breeding traits. In: Proc. of the 20th Association for the Advancement of Animal Breeding and Genetics Conference, Napier, New Zealand, 20–23rd October, pp. 286–289.
Johnson, K., Huyler, M., Westberg, H., Lamb, B., Zimmerman, P., 1994. Measurement of methane emissions from ruminant livestock using a SF_6 tracer technique. Environ. Sci. Technol. 28, 359–362.
Johnson, K.A., Johnson, D.E., 1995. Methane emissions from cattle. J. Anim. Sci. 73, 2483–2492.
Johnson, K.A., Westberg, H.H., Michal, J.J., Cossalman, M.W., 2007. The SF_6 tracer technique: methane measurement from ruminants. In: Makkar, H.P.S., Vercoe, P.E. (Eds.), Measuring Methane Production From Ruminants. Springer, Dordrecht, Netherlands, pp. 33–67.
Lassey, K.R., 2013. On the importance of background sampling in applications of the SF_6 tracer technique to determine ruminant methane emissions. Anim. Feed Sci. Technol. 180, 115–120.
Microsoft, 2010. Microsoft Excel ver. 14.0.6129.5000. Microsoft Corporation, Redmond, WA, USA.
Moate, P.J., Clarke, T., Davis, L.H., Laby, R.H., 1997. Rumen gases and bloat in grazing dairy cows. J. Agric. Sci. 129, 459–469.
Moate, P.J., Deighton, M.H., Hannah, M.C., Williams, S.R.O., 2012. Intake effects on methane emissions from dairy cows. In: Proceedings of the CCRSPI conference, Melbourne, Australia, 27–29 November.
Moate, P.J., Williams, S.R.O., Deighton, M.H., Hannah, M.C., 2013. Rate of release of SF_6 from permeation tubes is described by Michaelis–Mentin kinetics. Adv. Anim. Biosci. 4, 420.
Moate, P.J., Williams, S.R.O., Grainger, C., Hannah, M.C., Ponnampalam, E.N., Eckard, R.J., 2011. Influence of cold-pressed canola, brewers grains and hominy meal as dietary supplements suitable for reducing enteric methane emissions from lactating dairy cows. Anim. Feed Sci. Technol. 166, 254–264.
Pfitzner, J., 1976. Poiseuille and his law. Anaesthesia 31, 273–275.
Pinares-Patiño, C.S., Lassey, K.R., Martin, R.J., Molano, G., Fernandez, M., MacLean, S., Sandoval, E., Luo, D., Clark, H., 2011. Assessment of the sulphur hexafluoride (SF_6) tracer technique using respiration chambers for estimation of methane emissions from sheep. Anim. Feed Sci. Technol. 166–167, 201–209.
Pleil, J.D., Oliver, K.D., McClenny, W.A., 1987. Enhanced performance of Nafion dryers in removing water from air samples prior to gas chromatographic analysis. J. Air Waste Manage. 37, 244–248.
Sun, H., Trabue, S.L., Scoggin, K., Jackson, W.A., Pan, Y., Zhao, Y., Malkina, I.L., Koziel, J.A., Mitloehner, F.M., 2008. Alcohol, volatile fatty acid, phenol, and methane emissions from dairy cows and fresh manure. J. Environ. Qual. 37, 615–622.
van Zijderveld, S.M., Gerrits, W.J.J., Apajalahti, J.A., Newbold, J.R., Dijkstra, J., Leng, R.A., Perdok, H.B., 2010. Nitrate and sulphate: effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. J. Dairy Sci. 93, 5856–5866.
Verbyla, A.P., 1995. A Mixed Model Formulation of Smoothing Splines and Testing Linearity in Generalised Linear Models. Department of Statistics research report 95/5, University of Adelaide, Adelaide, Australia.
Verbyla, A.P., Cullis, B.R., Kenward, M.G., Welham, S.J., 1999. The analysis of designed experiments and longitudinal data using smoothing splines (with discussion). Appl. Stat. 48, 269–311.
VSN International, 2013. GenStat for Windows 16th Edition. VSN International, Hemel Hempstead, UK http://www.genstat.co.uk
Vlaming, J.B., Lopez-Villalobos, N., Brookes, I.M., Hoskin, S.O., Clark, H., 2008. Within- and between-animal variance in methane emissions in non-lactating dairy cows. Aust. J. Exp. Agric. 48, 124–127.
Williams, S.R.O., Clarke, T., Hannah, M.C., Marett, L.C., Moate, P.J., Auldist, M.J., Wales, W.J., 2013. Energy partitioning in herbage-fed dairy cows offered supplementary grain during an extended lactation. J. Dairy Sci. 96, 484–494.
Williams, S.R.O., Moate, P.J., Hannah, M.C., Ribaux, B.E., Wales, W.J., Eckard, R.J., 2011. Background matters with the SF6 tracer method for estimating enteric methane emissions from dairy cows: a critical evaluation of the SF6 procedure. Anim. Feed Sci. Technol. 170, 265–276.
Wims, C.M., Deighton, M.H., Lewis, E., O'Loughlin, B., Delaby, L., Boland, T.M., O'Donovan, M., 2010. Effect of pregrazing herbage mass on methane production, dry matter intake, and milk production of grazing dairy cows during the mid-season period. J. Dairy Sci. 93, 4976–4985.
Zimmerman, P.R., 1993. System for Measuring Metabolic Gas Emissions from Animals. In: U.S. Patent and Trademark Office (Ed.), United States, Pat. No. 5, 265,618, p. 11.