Measurement of methane emission from sheep by the sulphur hexafluoride tracer technique and by the calorimetric chamber: failure and success

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(Received 8 March 2007; Accepted 22 August 2007)

The aim of this study was to evaluate the sulphur hexafluoride (SF₆) tracer technique for methane (CH₄) emission measurement in sheep. Ten cryptorchid Romney sheep were involved in two indoor trials (T₁ and T₂), where daily CH₄ emissions were individually measured both by the SF₆ tracer (‘tracer CH₄’) and by the indirect calorimetry chamber (‘chamber CH₄’) techniques while fed on lucerne hay at 1.2 times maintenance requirements. Separate sets of permeation tubes with pre-calibrated permeation rates (‘pre-calibrated PRs’) were used in the two trials (for tracer CH₄) and at the time of T₁ and T₂ these tubes had been deployed in the rumen for 250 and 30 days, respectively. The tracer CH₄ measurements were carried out for 2 (T₁) and 5 (T₂) days in digestibility crates housed within a building (T₁) or a well-ventilated covered yard (T₂). Sheep were transferred to calorimetry chambers for 3 days acclimatisation, followed by measurement of CH₄ emission for 7 (T₁) and 3 (T₂) days. In T₁, samples from the chamber, outflow and inflow (collected over ~22 h) were analysed for CH₄ and SF₆ concentrations using the tracer protocol. Thus, PRs of SF₆ at the time of the trials (‘calculated PRs’) could be inferred and the corresponding CH₄ emissions are then calculated using either the pre-calibrated PR or calculated PR. Permeation tubes were recovered at the end of the animal trials and their ‘post-recovery PR’ determined. In trial T₁, the tracer CH₄ estimates (based on the pre-calibrated PR) were much higher and more variable than the chamber CH₄ values. In this trial, the calculated PR and the post-recovery PR were similar from each other but smaller than the pre-calibrated PR, and when the calculated PR was used in place of the pre-calibrated PR the CH₄ emission estimates agreed well with the chamber CH₄ values. This suggested that the discrepancy was due to a declining PR during the long deployment time of the tubes in T₁, an observation reported elsewhere. When the long intra-ruminal deployment was avoided in T₂, good agreement between the techniques for CH₄ emission measurement was observed.

Keywords: forages, methane, ruminants, SF₆ tracer technique, sheep

Introduction

Measurements of enteric methane (CH₄) emissions from livestock have traditionally been obtained using indirect calorimetry techniques. While these techniques are both accurate and reliable, the expense and need for animal training limit the numbers of animals that can be measured (Johnson et al., 1998). In addition, the extent to which calorimetric results can be extrapolated to free-ranging animals for which diet, behaviour and environment are different from those in enclosed chambers has been questioned and has stimulated the development of measurement techniques suitable for grazing animals (O’Kelly and Spiers, 1992; Johnson et al., 1994a; Lockyer and Jarvis, 1995; Harper et al., 1999; Leuning et al., 1999).

Studies with sheep (Murray et al., 1976; Torrent and Johnson, 1994; Immig, 1996) have established that the rumen accounts for about 87% of the total digestive tract CH₄ production. According to Murray et al. (1976), about 95% of the rumen CH₄ is excreted by eructation, whereas 89% of the lower-gut CH₄ production (about 13% of the total) is exhaled through the lungs. Thus, about 98% of the total tract CH₄ production is excreted through the mouth and nostrils. Based on this knowledge, Johnson et al.
Lassey (2007) summarised attempts to compare calori-
metric and SF6 tracer techniques for CH4 emission mea-
surements. For example, working with cattle, Johnson et al. (1994a) reported good agreement between the SF6 tracer technique and calorimetry chamber CH4 measurements, and more recently Boadi et al. (2002) using cattle with ventilated hoods corroborated that agreement. However, studies conducted in sheep by Pinares-Patino (2000) and Wright et al. (2004) failed to demonstrate agreement between the SF6 tracer and the calorimetric chamber in their CH4 emission estimates. Reasons for the disagreement might be related to the uncertainty of rate of permeation of SF6 from the tracer source deployed in the reticulo-rumen of the experimental animal and poor ventilation leading to increased local concentrations of SF6 and CH4 gases (Pinares-Patino, 2000). The tracer source (permeation tube) is charged at 600 to 900 mg SF6, and at permeation rates (hereafter abbreviated as ‘PR’) of 0.6 to 1.7 mg/day one could expect useful life of permeation tubes beyond a year. Lassey et al. (2001) analysed the performance of SF6 per-
meation tubes and concluded that PR does not remain constant with time, as the technique supposes, but it changes slowly.

CH4 is a by-product of the feed fermentation process and it is well established that digestion efficiency and digesta kinetics differ between cattle and small ruminants, especially when they are fed on forages (Colucci et al., 1984; De Boer et al., 1984). In addition, there is limited evidence that ruminant species differ in rumen motility and excretion of digestive gases (Colvin et al., 1957; Dougherty and Cook, 1962; Dougherty et al., 1964; Dziuk and McCauley, 1965; Hoernicke et al., 1965; Murray et al., 1976). Hence, reliability of the SF6 tracer technique demonstrated in cattle does not assure comparable reliability in small ruminants.

The present study sought to evaluate the reliability of the SF6 tracer technique for CH4 emissions measurement on forage-fed sheep, with permeation tubes deployed either for long or short terms. The long-term deployment was a non-intended feature.

Material and methods

Experimental design and animals

Two indoor trials (T1 and T2) were conducted with the same group of 10 cryptorchid Romney sheep in order to measure their individual CH4 emissions both by the SF6 tracer (hereafter referred as ‘tracer CH4’) and by the indirect calorimetry chamber (hereafter referred to as ‘chamber CH4’) techniques while fed at 1.2 times their maintenance requirements on chaffed lucerne hay. Trial T1 was conducted during the spring of 1997, when sheep were housed in a naturally ventilated building, whereas T2 was conducted during the autumn of 1998, when sheep were housed in a covered yard. All the chamber measurements of CH4 emission used the two-chamber facility at the Animal Physiology Unit, Massey University (Palmerston North, New Zealand). Before the start of trials and between trials, sheep were maintained at grazing on ryegrass/white clover pasture. In T1, sheep were 14 months old and 40.4 ± 4.2 kg live weight, whereas in T2 they were 22 months old and 46.9 ± 4.8 kg live weight. The sheep were prepared with rumen cannulae (65 mm internal diameter) 3 months before T2 commencement, and foam washers were fitted around the cannulae immediately prior to T2 to avoid loss of gases through the rumen fistula.

In both the trials, management of sheep was staggered over time in order to synchronise with the availability of one chamber dedicated to measurement. Sheep were brought indoors and put in digestibility crates, where, after an acclimatisation period of 21 days, intermittent tracer CH4 emission measurements were conducted over a 7-day (T1) or a 14-day (T2) period. Then, sheep were transferred to calorimetry chambers for 3 days of acclimatisation followed by 7 (T1) or 3 (T2) days of CH4 emission measurement. Figure 1 shows the experimental design for the trials, detailing the specific days when the tracer CH4 measurements took place.

The experimental protocols for both trials of this study were approved by the Animal Ethics Committee of AgResearch Limited.

Feeds and feeding

Chaffed (−5 cm) lucerne hay from one uniform batch was fed during both trials, and the quality of the feed offered was controlled by thorough mixing of the total experimental requirements and weighing the daily allowance for individual animals into plastic bags, prior to the experiment.

The organic matter, crude protein, neutral-detergent fibre, soluble carbohydrate and lipid contents (dry matter (DM) basis) of the hay were 88.6%, 19.1%, 40.1%, 2.7% and 1.8%, respectively, whereas the metabolisable energy (ME) content was 8.4 MJ/kg DM, as determined by near-infrared (NIR) spectroscopic analysis.

The feeding level was restricted to 1.2 times the main-
tenance energy requirements using the feeding standards of the Standing Committee on Agriculture (SCA, 1990). This was to ensure that the animals ate all the feed on offer and also to avoid variation in feed intake between tracer and chamber measurement periods. In trial T1, the sheep were fed twice daily (0800 and 1600 h), in digestibility crates and in the chambers; whereas in T2 sheep were fed automatically 12 times per day when in digestibility crates, but twice daily in the calorimetry chambers. Drinking water was made available ad libitum under all circumstances.
Measurements and sample analyses

Feed intake. The amounts of feed on offer, refused and spilled were weighed daily and samples were taken for DM determinations (65°C, 48 h). Except for small amounts of feed spilled, the sheep ate all of the feed on offer in both the digestibility crates and the calorimetry chambers. Mean ± s.d. daily feed DM intakes in T1 and T2 were 1088 ± 100 and 1139 ± 106 g, respectively.

Measurement of CH4 emissions by the chamber technique. The two chambers (1 and 2) used in this study have been described by Holmes (1973). Briefly, each chamber was made of galvanised sheet steel and rigid steel framework with internal dimensions of 170 cm length × 70 cm width × 150 cm height. Each chamber was insulated externally with polystyrene and temperature maintained at 14°C to 16°C by means of a water-cooled, fan-ventilated heat exchanger controlled thermostatically. Fresh air was drawn in from a height of 5 m above the ground level outside the building and exhausted from each chamber by a rotary pump, allowing a slight negative pressure within the chambers. The outgoing airflow was measured using dry gas meters. Airflow (30 to 40 l/min) was controlled by means of manifolds connected to the pump. Temperature, humidity and pressure of air flowing to the gas meters were measured in order to correct the air volume to standard conditions. An IR gas analyser (Servomex, Crowborough, East Sussex, UK) was used to measure the CH4 concentrations on aliquot representative samples (~7 l) of chamber inflow and outflow air streams. These samples were collected continuously (over ~22 h) in spirometers sealed with liquid paraffin.

The two calorimetry chambers differed in construction, with chamber 1 being older than chamber 2. A gravimetric method was used to test the recovery of CH4 in the chambers. Ultrapure (>99.99%) CH4 (BOC Gases NZ Ltd, Lower Hutt, New Zealand) was metered at about 25 ml/min (17.9 mg/min) into each chamber for about 2.5 h and the dispensing cylinder was weighed at the start and the end of the release periods to determine the weight of CH4 released. The rate of release of CH4 was set to match the average concentration of CH4 (~600 p.p.m.) encountered in the chamber during trials involving sheep. The gas recovery in chamber 2 was 95.5 ± 0.5%, and this factor was used to adjust the CH4 emission data. Chamber 1 had the poorer recovery (88.8 ± 7.0%), and so was used only for acclimatisation prior to CH4 measurements in chamber 2.

In T1, after the daily CH4 measurement by the chamber technique was finished, the remainder (if available) of the aliquot samples from the chamber outflow and inflow gases, which had been collected in the spirometers, were carefully drawn into evacuated PVC containers (hereafter referred to as a ‘yoke’ due to its inverted ‘U’ shape) for gas analysis under the SF6 tracer protocol. Wherever possible, two samples per animal were collected.

Measurement of CH4 emissions by the SF6 tracer technique. The calibrated SF6 tracer source (i.e. the permeation tube), the gas collection system and the subsequent analysis of samples are the three major components of the SF6 tracer technique (Johnson et al., 1994a), and the application of this technique for CH4 measurement in sheep housed in metabolism creases has been described by Pinares-Patiño et al. (2003). Briefly, it involved the use of a calibrated permeation tube charged with SF6 and with a known SF6 PR, which was inserted per os (T1) or per fistula (T2) into the rumen of each animal 250 and 30 days before the commencement of T1 and T2, respectively. The reason for the long pre-deployment of permeation tubes in T1 was that the sheep were selected from a larger flock on the basis of emission experiments that used the same tubes.

PRs of tubes used at each trial were determined individually through serial weighing prior to insertion (hereafter referred as ‘pre-calibrated PR’), and for the two independent sets of permeation tubes used in T1 and T2, the PRs were 1.313 ± 0.440 and 0.691 ± 0.097 mg/day (mean ± standard deviation), respectively. During collection days, a sample of air exhaled by each sheep was drawn continuously (controlled by capillary tubing) from near the mouth and nostrils over ~22 h into a lightweight pre-evacuated yoke suspended above the digestibility crate. Finally, gas chromatography (GC) was used to analyse the concentrations of CH4 and SF6 gases in the collected samples using flame ionisation detection and electron capture.
tracer CH₄ (g/day) = pre-calibrated PR (g/day) 
\times \left[ \frac{[\text{CH}_4]}{[\text{SF}_6]} \right] \times (16/146),

where the multiplier ‘16/146’ is the ratio of molecular weights that converts molarity to mass.

CH₄ emission measurements by the SF₆ technique (tracer CH₄) were carried out while animals were kept in digestibility crates, placed 2 to 3 m from each other to minimise cross-inhalation, within a building. The building (Animal Physiology Unit, Massey University) used in T₁ was naturally well ventilated during the working hours (0700 to 1800 h) by opening two doors at each end of the building (one large and one small), but night ventilation was restricted to the small door only. In T₂, the sheep were kept in a well-ventilated covered yard at AgResearch Grasslands (Palmerston North, New Zealand). The tracer CH₄ measurements were carried out for 2 and 5 days in T₁ and T₂, respectively (Figure 1). In both the trials, background air samples were collected from the incoming air stream into two evacuated yokes. Ranges of mixing ratios of SF₆ and CH₄ in background air samples during the trial were 5.1 to 8.1 p.p.t. and 1.9 to 2.8 p.p.m., respectively. Background concentrations of SF₆ and CH₄ were used to correct the concentrations found in the samples.

Estimations of CH₄ emissions from analyses of chamber gases by the tracer protocol in trial 1. In T₁, once analyses of chambers’ inflow and outflow air samples were finished by the calorimetric chamber procedures, the remainder of the aliquot samples collected over ~22 h were drawn into evacuated yokes, and CH₄ and SF₆ molar mixing ratios analysed by GC as per the tracer technique. The molar mixing ratios of the gas SF₆ in these samples were used to calculate the daily amount of this gas released in the chamber from a particular permeation tube in the rumen of a particular animal (hereafter referred to as ‘calculated PR’), according to the formula:

\text{calculated PR (g/day)} = \frac{([\text{out SF}_6] - [\text{in SF}_6]) \times VR \times 6.518} {\text{g/l}},

where [out SF₆] and [in SF₆] are the mixing ratios of SF₆ in the chamber outflow and inflow air, respectively; VR is the standardised ventilation rate (l/day) in the chamber, and 6.518 g/l is the ratio of molecular weight of SF₆ to standard molar volume.

Then, the tracer technique calculation principles were used to estimate CH₄ emissions based on the molar mixing ratios of CH₄ and SF₆ gases in the chamber inflow (in) and outflow (out) air samples (determined as per the tracer protocol) in conjunction with either the pre-calibrated PR or the calculated PR of SF₆ from permeation tubes. Hereafter, CH₄ emission calculation based on chamber gases using the pre-calibrated PR is named ‘pre-calibrated PR in chamber CH₄’, whereas similar calculation using the calculated PR is named ‘calculated PR in chamber CH₄’. The formulas are

pre-calibrated PR in chamber CH₄ (g/day) = \frac{([\text{out CH}_4] - [\text{in CH}_4]) \times ([\text{out SF}_6] - [\text{in SF}_6]) \times 16}{146},

calculated PR in chamber CH₄ (g/day) = \frac{([\text{out CH}_4] - [\text{in CH}_4]) \times ([\text{out SF}_6] - [\text{in SF}_6]) \times 16}{146}.

Post-recovery PR. Permeation tubes used in T₁ and T₂ were retrieved from the rumens of the animals after 397 and 50 days of deployment, respectively, and once cleaned and left to dry, their weight losses were monitored for about 6 months in the laboratory at 39°C and a new post-recovery PR determined (hereafter referred to as ‘post-recovery PR’). The post-recovery PR was not used to re-calculate the CH₄ emission, but it was compared with both the pre-calibrated PR and calculated PR.

In this study, the PR of tubes while in the rumen of the animal is named ‘in-rumen PR’, which cannot be measured directly and is usually equated to the pre-calibrated PR. However, if the recovery of SF₆ in the calorimetry chamber was close to 100%, the calculated PR would be a good estimator of the in-rumen PR. Lassey et al. (2001) have addressed the situation where the pre-calibrated PR cannot be extrapolated with confidence to a protracted trial time.

Data calculation and analysis
For each trial, the mean values and the CV of CH₄ emissions per animal (g/day) were calculated. The two CH₄ measurement techniques (chamber and tracer) were compared by the paired t-test (Cody and Smith, 1991). In addition, the mean CH₄ emission estimates were subject to correlation analysis. Similar statistical analyses were carried out to compare the daily CH₄ emissions measured by the chamber technique and the emissions estimated based on chamber’s gas molar mixing ratios (T₁ only). For the latter, only the chamber CH₄ emission values corresponding to the days when the chamber gases were withdrawn and analysed by GC (as per the tracer protocol) were computed.
Methane emission from sheep

Table 1 Mean ± s.e. and coefficient of variation (CV) of methane (CH₄) emissions from sheep (g/day) at the two trials (T₁ and T₂) as measured by the sulphur hexafluoride (SF₆) tracer technique (tracer CH₄ in digestibility crates) and by the calorimetry chamber (chamber CH₄), and test of the difference of their means

<table>
<thead>
<tr>
<th>Trials (g/day)</th>
<th>Tracer CH₄ (g/day)</th>
<th>Chamber CH₄ (g/day)</th>
<th>Tracer–chamber CH₄ (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± s.e.</td>
<td>CV</td>
<td>Mean ± s.e.</td>
</tr>
<tr>
<td>T₁</td>
<td>24.7 ± 1.6 (20)</td>
<td>18.4</td>
<td>17.8 ± 0.3 (70)</td>
</tr>
<tr>
<td>T₂</td>
<td>18.8 ± 0.4 (40)</td>
<td>7.8</td>
<td>19.5 ± 0.6 (24)</td>
</tr>
</tbody>
</table>

1CV calculated by dividing the root mean square error by the mean value.

2Paired t-test of Ho: tracer CH₄ – chamber CH₄ = 0. Superscripts indicate that within a particular trial, the difference of means is significantly different (**P < 0.01) or not different (n.s. P > 0.05) from 0. Values in parenthesis indicate number of observations.

Finally, where data were available, pre-calibrated PR, calculated PR and the post-recovery PR of SF₆ from the permeation tubes were compared by paired t-tests. For this purpose, each individual calculated PR and post-recovery PR was expressed as a percentage of the corresponding pre-calibrated PR. Coefficients of correlation between these relative PR values were also calculated.

In T₂ two animals could not adapt to the chamber conditions. So, data for chamber measurements in this trial were collected from eight animals.

Results

Tracer and chamber CH₄ emissions

In trial T₁, the tracer technique yielded significantly greater CH₄ emission (by 39%) than the chamber technique, whereas in T₂ the two measurement techniques did not differ significantly from each other in their CH₄ emission estimates (Table 1). In both the trials, the chamber technique produced less variable CH₄ emission estimates (by 0.65 and 0.45 in T₁ and T₂, respectively) than the tracer technique (Table 1). In trial T₁, the CH₄ emission estimates by the tracer and the chamber techniques were not significantly correlated with each other (r = 0.53, P > 0.09), but in trial T₂, a significant correlation (r = 0.93, P = 0.001) between the two techniques was observed (Figure 2).

Estimations of CH₄ emissions based on analyses of chamber gases by the tracer protocol in trial 1

Table 2 presents for trial T₁ the CH₄ emission estimates (g/day) based on the molar mixing ratios of chamber gases analysed by the tracer protocol in conjunction with either the pre-calibrated PR or calculated PR as well as the chamber CH₄ emission values determined by the chamber technique. The CH₄ emission estimated by the pre-calibrated PR in chamber CH₄ procedure was much higher (by 38%) than the chamber CH₄ value, whereas the calculated PR in chamber CH₄ estimates and chamber CH₄ values were not significantly different from each other (19.5 and 18.6 g/day, respectively). Overall, the chamber CH₄ values had smaller variation than those of pre-calibrated PR in chamber CH₄ and calculated PR in chamber CH₄ estimates (Table 2).

Table 2 Mean ± s.e. and coefficient of variation (CV) of methane (CH₄) emissions (g/day) for trial T₁ as estimated based on analyses of chamber gases by the tracer routine (‘pre-calibrated PR’ in chamber CH₄ and ‘calculated PR in chamber CH₄’) or measured by the calorimetry chamber (chamber CH₄), and tests of the difference of their means (each value represents the mean of 28 simultaneous observations)

<table>
<thead>
<tr>
<th>CH₄ emission estimates (g/day)</th>
<th>Mean ± s.e.</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-calibrated PR in chamber CH₄ (1)</td>
<td>27.0 ± 1.1</td>
<td>20.1</td>
</tr>
<tr>
<td>Calculated PR in chamber CH₄ (2)</td>
<td>19.5 ± 0.6</td>
<td>13.8</td>
</tr>
<tr>
<td>Chamber CH₄ (3)</td>
<td>18.6 ± 0.4</td>
<td>5.5</td>
</tr>
<tr>
<td>(1)–(3)</td>
<td>9.5 ± 1.1**</td>
<td></td>
</tr>
<tr>
<td>(2)–(3)</td>
<td>1.3 ± 0.6**</td>
<td></td>
</tr>
</tbody>
</table>

1CV calculated by dividing the root mean square error by the mean value.

2PR = permeation rate.

3Paired t-test of Ho: difference of means = 0. Superscripts indicate that the difference of means is significantly different (**P < 0.01) or not different (n.s. P > 0.05) from 0.

The chamber CH₄ values did not correlate with the pre-calibrated PR in chamber CH₄ estimates (r = 0.44, P = 0.18) but they correlated positively (r = 0.64, P = 0.03) with the calculated PR in chamber CH₄ estimates. A high and significant correlation was observed between the tracer CH₄ and the pre-calibrated PR in chamber CH₄ estimates (r = 0.88, P = 0.001).
Table 3 Mean ± s.e. pre-calibrated permeation rate (PR), the in chamber calculated PR and the post-recovery PR of sulphur hexafluoride ($SF_6$) from permeation tubes* used in each trial and tests of difference between their means

<table>
<thead>
<tr>
<th>Trials</th>
<th>Pre-calibrated PR</th>
<th>Calculated PR</th>
<th>Post-recovery PR</th>
<th>Means difference test$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1)</td>
<td>(2)</td>
<td>(3)</td>
<td>(2) − (1)</td>
</tr>
<tr>
<td>$T_1$</td>
<td>100</td>
<td>79.6 ± 3.4</td>
<td>77.8 ± 3.1</td>
<td>−20.4 ± 3.4***</td>
</tr>
<tr>
<td>$T_2$</td>
<td>100</td>
<td>99.1 ± 1.0</td>
<td></td>
<td>−0.9 ± 1.0n.s.</td>
</tr>
</tbody>
</table>

All values (except columns (2) and (3)) expressed as percent of the pre-calibrated PR (100%). For columns (2) and (3), the calculated PR expressed as percent of post-recovery PR. Each value represents the mean of 8 ($T_1$) or 10 ($T_2$) observations.

$*^{Independent set of tubes deployed at each trial.}$

$^a$Paired t-test Ho: difference of means = 0. Subscripts indicate that within a trial, the difference of means is significantly different (***) $P < 0.001$ or not different (n.s. $P > 0.05$) from 0.

Figure 3 Calibration data from serial weighing of tube 344 deployed in sheep no. 2 during trial $T_2$. The pre-calibrated permeation (PR) and post-recovery PR of sulphur hexafluoride ($SF_6$) are deduced by linear regression from the data of days 13 to 126 and days 189 to 352, respectively (day 0 is the date of the tube fill). The post-recovery PR was only 0.007 mg/day less than the pre-insertion pre-calibrated PR (0.737 vs. 0.744 mg/day). This figure is similar to Figure 2a of Lassey et al. (2001).

PR of $SF_6$: relationships between the pre-calibrated PR, calculated PR and the post-recovery PR

Table 3 shows the mean PRs and tests of differences of means among the pre-calibrated PR, the calculated PR (based on chamber gases) and the post-recovery PR of tubes deployed at each trial. Data for calculated PR was not available for trial $T_2$. The calculated PR and post-recovery PR of tubes used in $T_1$ were significantly ($P < 0.001$) lower (by 20% and 22%, respectively) than their respective pre-calibrated PR. However, the post-recovery PR of tubes used in this trial ($T_1$) was not different ($P > 0.05$) from the calculated PR. In this trial ($T_1$), the pre-calibrated PR was highly correlated with both the calculated PR ($r = 0.93, P < 0.0001$) and the post-recovery PR ($r = 0.97, P < 0.0001$). Similarly, the calculated PR and post-recovery PR were also highly correlated ($r = 0.90, P = 0.0009$).

In contrast to $T_1$, in $T_2$ the post-recovery PR was not different ($P > 0.05$) from their pre-calibrated PR values (Table 3), but they were highly correlated with each other ($r = 0.98, P < 0.0001$). The latter observation is illustrated in Figure 3, where the pre-calibrated PR and the post-recovery PR of tube 344 deployed in sheep no. 2 typically represents the permeation behaviour of the set of tubes used in $T_2$, i.e. there was very little change in PR between the pre-insertion and the post-recovery stages.

Discussion

Johnson et al. (1994a) examined the validity of the $SF_6$ tracer technique for CH$_4$ measurement in cattle by comparing 55 measurements made with the tracer (in pens) to those obtained from 25 measurements using open-circuit respiration chambers and found that whereas the tracer mean estimates were 93% of those in the chambers, these differences were not significant. A more recent validation test of the tracer technique against ventilated hoods also using penned cattle (Boadi et al., 2002) showed that although the techniques did not differ significantly, the tracer estimates were slightly higher (by 5%) than the calorimetric measurements. Results of trial $T_2$ of the present study, in which the PRs were more precisely known due to the more recent calibration, agreed with those found by Johnson et al. (1994a) in showing that the calorimetry chamber CH$_4$ values were 4% higher than the corresponding tracer estimates, but with no significant differences between the methods. Tracer CH$_4$ emission estimates could be expected to be slightly smaller than those measured in respiration chambers because the flatus CH$_4$ excretion, which accounts for approximately 2% of the total emission of CH$_4$ (Murray et al., 1976), is not accounted for by either the $SF_6$ tracer technique or by partial enclosure calorimetry (ventilated hoods).

Results of this study also support earlier reports (Johnson et al., 1994a and 1994b; Ulyatt et al., 1999; Boadi et al., 2002; Clark et al., 2005) that the tracer technique is associated with larger variation than the standardised calorimetry techniques. However, a recent study conducted by McGinn et al. (2006), where the influence of environment on estimations were cancelled by conducting all measurements in chamber, reported that the tracer technique was more accurate and precise than the calorimetric technique, especially when forage diets were used. Since estimates of total CH$_4$ emissions using the $SF_6$ tracer technique depend on the assumption that CH$_4$ and $SF_6$ are predominantly excreted via the mouth and nose (Ulyatt et al., 1999), it can be conjectured that the proportion of
CH₄ excretion in flatus is not only dependent on feeding conditions, but also highly variable between animals.

Results of T₂ of the present study, however, are in disagreement with those of Wright et al. (2004), who working with sheep fed on a low-quality tropical grass hay found emissions expressed as a percentage of gross energy intake (%GEI, hereafter termed 'CH₄ yield') of 16% to 37% and 8% to 12% GEI for tracer and chamber methods, respectively, with the chamber estimates being similar to previous observations (Kurihara et al., 1999) on cattle fed on similar diets, and CH₄ measured in the same chambers. However, the extremely high estimates of CH₄ yield for the tracer method observed by Wright et al. (2004) have no precedent in the literature. The production of CH₄ is related to the agreement with those of Wright et al. (2004), who working the extremely high estimates of CH₄ yield for the tracer suspect in trial T₁ is probably traceable to an inappropriate of SF₆ from permeation tubes remains constant until the SF₆ discused. First, the tracer CH₄ estimates observed in trial T₁, CV similar to those (1965). In contrast to T₁, the tracer CH₄ estimates in T₂ chamber measurements (e.g. Blaxter and Clapperton, showed much lower variability. Second, that the estimates of CH₄ emissions from ruminants fed on temperate forages show CH₄ yields up to 8.3% GEI. Thus, the results of Wright et al. (2004) are difficult to explain with regard to tracer CH₄ emission estimates.

In contrast to trial T₂ and expectations based on CH₄ excretion dynamics (Murray et al., 1976), results of trial T₁ of the present study showed that the tracer CH₄ estimates (based on pre-calibrated PR) were 1.4 times greater than the corresponding chamber CH₄ values. Arguments to support our hypothesis that the tracer rather than the chamber CH₄ estimates lacked accuracy in trial T₁ are now discussed. First, the tracer CH₄ estimates observed in trial T₁ were associated with large variability (CV = 18%), whereas the chamber CH₄ values were more consistent, with a CV similar to those (~7%) more commonly observed in chamber measurements (e.g. Blaxter and Clapperton, 1965). In contrast to T₁, the tracer CH₄ estimates in T₂ showed much lower variability. Second, that the estimates of CH₄ emission by the tracer technique appear to be suspect in trial T₁ is probably traceable to an inappropriate usage of PR. Evidence for this is the anomalously high estimate of CH₄ emission rate for this case (Table 1), together with the systematic disagreement between pre-calibrated PR and calculated PR (Table 3). In contrast, the facts that the chamber CH₄ and the calculated PR in chamber CH₄ estimates were not only similar, but were also highly correlated suggest that the most likely in-rumen PRs at the time of T₁ were close to the calculated PRs rather than to the pre-calibrated PRs.

It is commonly assumed (Johnson et al., 1994a) that PR of SF₆ from permeation tubes remains constant until the SF₆ non-gaseous load is exhausted, resulting in a declining SF₆ headspace pressure in the tube (Lassey et al., 2001). In this study, the permeation tubes used in trial T₁ were retrieved after 397 days of deployment, with the trial taking place on days 250 to 270. Although the post-recovery PRs for these tubes were 78%, on average, of their pre-calibrated PRs, these two estimates of PR for each tube were highly correlated with each other. Moreover, post-recovery PRs and calculated PRs were indistinguishable, suggesting that changes in PR through time were systematic across the set of tubes used in this trial. These findings are in agreement with the observations by Lassey et al. (2001) who monitored loss of mass from permeation tubes recovered from sheep or maintained in laboratory as controls for periods up to 750 days, and found that the rate of mass loss fell slowly over time in a manner well captured by a quadratic mass v. time curve, with non-linearity being ignorable for about the first 3 months after calibration. Lassey et al. (2001) sugges- that the most likely cause of non-linearity was the interaction between the SF₆ gas and the surfaces of Teflon pores (the permeation medium), and accordingly, correction of PRs based on the performance of sibling tubes (main- tained in the laboratory) was proposed when tube recovery was unavailable (Lassey et al., 2001).

One could argue that collection efficiencies for CH₄ and SF₆ might differ. While CH₄ production and excretion from the rumen is associated with the feeding pattern (Johnson et al., 1998), and therefore varies throughout the day, the excretion rate of the released tracer gas (SF₆) is presumed to remain constant throughout the day. As a result, the CH₄/ SF₆ ratio in gases excreted through the mouth and nostrils could vary markedly throughout the day. However, because gas-mixing processes within the rumen headspace as well as during expulsion through the throat and the mouth or nose are highly turbulent, we would not expect such mixing to discriminate between CH₄ and the nine-fold heavier SF₆ molecules. Therefore, unless collection efficiency varies systematically throughout the day in sympathy with feeding patterns, we would expect the relative collection abundances of CH₄ and SF₆ to closely represent their relative emissions entrained in the ‘breath’. Nevertheless, it is possible that background sampling could be compromised in the very still air of an unventilated room in which ‘gravitational settling’ of heavier gases might introduce some stratification. A bias could then be introduced for those animals whose inefficient breath-collection provided CH₄ or SF₆ concentrations little higher than the measured background. However, our sample acceptance criteria would normally reject ‘breath’ samples that are close to background. Thus, while the building used in trial T₁ had limited nocturnal ventilation, we do not believe that a bias was thereby introduced in our results.

Conclusions

The first trial (T₁) of this study showed that the SF₆ tracer technique produced much higher and less-consistent CH₄ emission estimates than the calorimetry chamber technique. This was almost certainly due to the long time elapse between tube calibration and the trial in conjunction with a slow decline in SF₆ PR. The post-recovery calculations of
PRs of SF$_6$ (post-recovery PR) and the estimation of PRs of SF$_6$ based on chamber gas concentrations (calculated PR) support this deduction. However, when this long time elapse was avoided in trial T$_2$, a good agreement between the two techniques for CH$_4$ emission measurement was observed. The SF$_6$ tracer technique is reliable for use in sheep provided that the trial takes place within about 4 to 6 weeks of pre-calibrating the permeation tubes. For tubes that have an unavoidable long residence in the rumen, a post-trial tube recovery and post-recovery PR would provide a more reliable estimator of PR at the time of trial. Whether the tracer technique is associated with extra variability or not remains to be established.

Acknowledgements

The authors acknowledge Mr J Purchas (Massey University) for his skilled assistance on the calorimetric chamber measurements, and Ms CF Walker and Mr AM McMillan (NIWA) who assisted with sample collection and GC analysis. This research was funded by the New Zealand Foundation for Research, Science and Technology and by the New Zealand Ministry of Agriculture and Forestry.

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