Evaluation of the sulphur hexafluoride tracer technique for methane emission measurement in forage-fed sheep

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ABSTRACT

The aim of this study was to evaluate the sulphur hexafluoride (SF_6) tracer technique for methane (CH_4) emission measurement in sheep. Methane emissions from ten Romney sheep were individually measured both by the SF_6 tracer ('tracer CH_4') and by the indirect calorimetry chamber ('calorimetric CH_4') techniques while fed on lucerne hay. The tracer technique involved the use of permeation tubes with pre-calibrated permeation rates ('pre-calibrated PR') of SF_6. The 'tracer CH_4' measurements were carried out for 5 days in digestibility crates housed within a covered yard. Sheep were transferred to calorimetry chambers for 3 days acclimatisation, followed by measurement of CH_4 emission for 3 days. Permeation tubes were recovered at the end of the animal trial and their 'post-recovery PR' determined after subsequent weighing for a 6-month period. Although the 'tracer CH_4' was slightly lower (by 4%) and had larger variation than the 'calorimetric CH_4' values (18.8±0.4 *vs*. 19.5±0.6), the two measurement techniques did not differ significantly ($P>0.05$) in their CH_4 emission estimates. The 'post-recovery PR' was not different ($P>0.05$) from their 'pre-calibrated PR' values, and they were highly correlated to each other ($r=0.98$, $P<0.0001$). It is concluded that the tracer technique provides a reliable alternative method for CH_4 measurement in forage-fed sheep.

Key words: methane; sheep; forages; SF_6 tracer technique; ruminants.

INTRODUCTION

Measurements of enteric methane (CH_4) emissions from livestock can be accurately measured by indirect calorimetry techniques, but these techniques are expensive and the extent to which calorimetric results can be extrapolated to less controlled systems (*e.g*. grazing) has been questioned and has stimulated the development of measurement techniques suitable for grazing animals (O'Kelly & Spiers, 1992; Johnson *et al*., 1994a; Lockyer & Jarvis, 1995; Harper *et al*., 1999; Leuning *et al*., 1999; Denmead *et al*., 2000).

Studies with sheep (Murray *et al*., 1976; Torrent & Johnson, 1994; Immig, 1996) have reported that most (about 87%) of the enteric CH_4 production arises in the rumen and the study of Murray *et al.* (1976) suggested that almost all (about 98%) of the total tract CH_4 production is excreted through the mouth and nostrils. Based on this knowledge, Johnson *et al.* (1994a) developed the sulphur hexafluoride (SF_6) tracer technique for CH_4 emission measurements on individual animals. Working with cattle, Johnson *et al.* (1994a) reported good agreement between the SF_6 tracer technique and calorimetry chamber CH_4 measurements, and Boadi *et al.* (2002) also using cattle with ventilated hoods corroborated that agreement. More recently, McGinn *et al.* (2006), working with cattle, confirmed more conclusively the agreement between the techniques.

There is some evidence that ruminant species differ in excretion of digestive gases. For example, isotope dilution techniques conducted with sheep (Murray *et al*., 1976) and goats (Dougherty *et al*., 1964) revealed that ruminal absorption of CH_4 in these species is very small (1–5%) compared to that in cattle (20–70%) (Hoernicke *et al*., 1965) and it seems that tracheal inhalation of eructated gases is important in cattle (Colvin *et al*., 1957; Dougherty & Cook, 1962; Hoernicke *et al*., 1965). Further, 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). Thus, even though the tracer technique has been shown to be reliable for CH_4 measurements in cattle, its reliability needs to be confirmed in other ruminant livestock. The present study therefore sought to evaluate the reliability of the SF_6 tracer technique for CH_4 emissions measurement on sheep, pen-fed on forages.

MATERIALS AND METHODS

Experimental design and animals

An indoor trial was conducted during May 1998 with ten cryptorchid Romney sheep (46.9±4.8 kg liveweight) in order to measure their individual CH_4 emissions both by the SF_6 tracer (hereafter referred as 'tracer CH_4') and the indirect calorimetry (hereafter referred as 'calorimetric

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CH_4') techniques while fed on chaffed lucerne hay. Except during the calorimetric measurements, the sheep were housed in a covered yard. All the calorimetric measurements of CH_4 emission used the two-chamber facility at the Animal Physiology Unit, Massey University (Palmerston North, New Zealand). The sheep were prepared with rumen cannulae (65 mm internal diameter) three months before the commencement of the study.

The sheep were managed in random pairs in order to synchronise the availability of the two calorimetry chambers. Thus, management of pairs of sheep were staggered over time. Sheep were brought indoors and put in digestibility crates, where, after an acclimatisation period of 21 days, a 14-day 'tracer CH_4' measurement period took place. Then, sheep were transferred to calorimetry chambers for 3 days of acclimatization followed by 3 days of CH_4 measurement.

The experimental protocol for this study was approved by the Animal Ethics Committee of AgResearch Limited.

Feed and feeding

Chaffed (~5 cm) lucerne hay was fed at 1.2 times the maintenance energy requirements using the feeding standards of the Standing Committee on Agriculture (SCA, 1990). This was to ensure that the animals ate nearly all the feed on offer, and to avoid variation in feed intake between tracer and calorimetric measurement periods. Sheep were fed automatically 12 times per day while in digestibility crates, but twice daily in the calorimetry chambers. Drinking water was made available *ad libitum* at all times.

Except for small amounts of feed spilled, the sheep ate almost all of the feed on offer in both the digestibility crates and the calorimetry chambers. Mean (±S.D.) daily feed dry matter intake (DMI) was 1139±106 g. The organic matter, crude protein, neutral detergent fibre, soluble carbohydrate and lipid contents (dry matter basis, DM) 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.

Measurement of CH_4 emissions by the calorimetric technique

The two calorimetry chambers (1 and 2) used in this study have been described by Holmes (1973). Temperature in the chambers was maintained at 14–16 °C. An infrared gas analyser (Servomex, UK) was used to measure the CH_4 concentrations on aliquot representative samples (c. 7 l) of chamber inflow and outflow airstreams. These samples were collected continuously (over ~22 hours) in spirometers sealed with liquid paraffin. The two calorimetry chambers differed in construction, with chamber 1 being older than chamber 2. All the 'calorimetric CH_4' measurements were conducted in chamber 2, following sheep acclimatisation in chamber 1.

Measurement of CH_4 emissions by the SF_6 tracer technique

The calibrated SF_6 tracer source (*i.e.* the permeation tube), the gas collection system and the subsequent analysis of samples are the three major components of the SF_6 tracer technique (Johnson *et al.*, 1994a), and the application of this technique for CH_4 measurement in sheep housed in metabolism crates has been described by Pinares–Patiño *et al.* (2003). Briefly, it involved the use of a calibrated permeation tube charged with SF_6 with a known SF_6 'permeation rate' (hereafter abbreviated as 'PR'), which was inserted via fistula into the rumen of each animal 30 days before the commencement of the study. The PR of each individual tubes were determined through serial weighing prior to insertion (hereafter referred as 'pre-calibrated PR'). The 'pre-calibrated PR' of the tubes used (mean ± standard deviation) was 0.691±0.097 mg/day. 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 hours into a lightweight pre-evacuated yoke suspended above the digestibility crate. Finally, gas chromatography (GC) was used to analyse the concentrations of CH_4 and SF_6 gases using flame ionisation detection and electron capture detection, respectively.

The daily 'tracer CH_4' emission was calculated using the ratio of molar mixing ratios, CH_4 to SF_6 (each corrected for mixing ratios in background air) in the yoke-borne sample, in conjunction with the pre-calibrated PR of SF_6 (Johnson *et al.*, 1994a):

$$\text{'Tracer } CH_4\text{'} \text{ (g/day)} = \text{'Pre-calibrated PR'} \text{ (g/day)} \times [CH_4]/[SF_6] \times (16/146)$$

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

Methane emission measurements by the SF_6 technique ('tracer CH_4') were carried out while animals were kept in digestibility crates placed 2–3 m from each other within a well-ventilated covered yard at AgResearch Grasslands (Palmerston North, New Zealand). The tracer CH_4 measurements were carried out for 5 days distributed over a 14-day period. Background air samples were collected into two evacuated yokes facing the prevailing

direction of the incoming air stream.

At the end of the comparative CH_4 emission study (after 50 days deployment) the permeation tubes were retrieved from the rumen of the animals, and once cleaned and let 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 as 'post-recovery PR').

Statistical analysis

The mean CH_4 emission estimates (g/day) derived from the two CH_4 measurement techniques (calorimetric and tracer) were compared by the paired t-test (Cody & Smith, 1991). For each measurement technique, coefficients of variation were calculated by dividing the root mean square error by the overall mean value. In addition, these mean emission estimates were subject to correlation analysis. Similarly, the 'pre-calibrated PR' and the 'post-recovery PR' of SF_6 from the permeation tubes were compared by paired t-test. For this the 'post-recovery PR' was expressed as a percent of the corresponding 'pre-calibrated PR'. Coefficients of correlation between these relative PR values were also calculated.

RESULTS

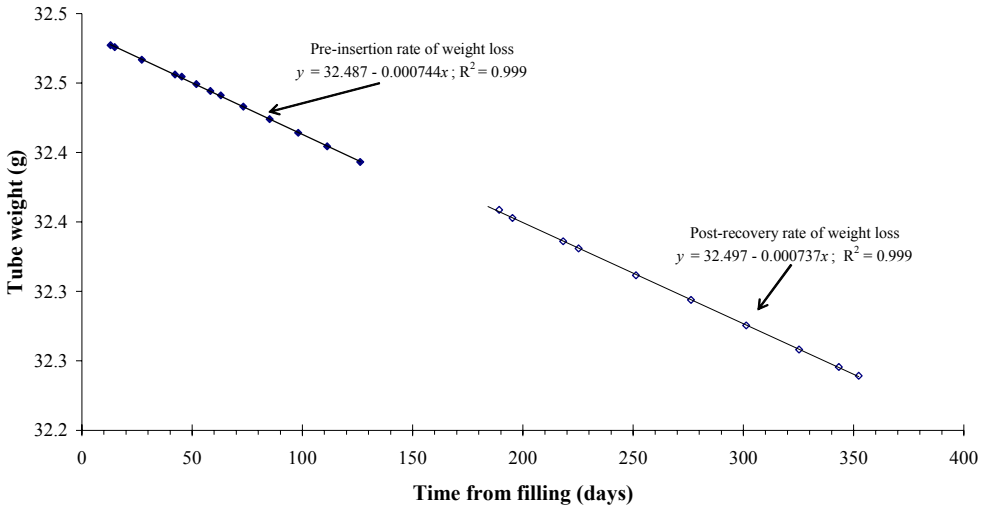
The CH_4 emission estimate (mean ± standard error, over 10 animals) by the tracer technique was 18.8±0.4 g/day, whereas the corresponding value for the calorimetric technique was 19.5±0.6 g/day; these values were not significantly different from each other (P>0.05). The tracer estimates had a larger coefficient of variation than the calorimetric values (7.8 *vs*. 4.3%). A high and significant coefficient of correlation (r=0.93, P=0.001) between the estimates by the two techniques was observed.

The mean 'post-recovery PR' of permeation tubes used in this study was not significantly different (P>0.05) from their 'pre-calibrated PR' values (99.1 *vs*. 100%) and these PRs were highly correlated with each other (r=0.98, P<0.0001). The latter observation is illustrated in Figure 1, 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, *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 made using open circuit respiration chambers and found that, although the tracer mean estimates were 93% of those in the chambers, this difference was not significant.

Figure 1: Calibration data from serial weighing of Tube 344 deployed in Sheep No. 2 during trial T_2. The 'pre-calibrated PR' and 'post-recovery PR' of SF_6 are deduced by linear regression from the data of days 13–126 and days 189–352, 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 Fig. 2a of Lassey *et al*. (2001).



A subsequent 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 the present study, in which the performance of permeation tubes were closely monitored, agreed with those found by Johnson *et al.* (1994a) in showing that the tracer CH_4 estimates were 4% lower than the corresponding calorimetric estimates, but with no significant difference 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). In fact, a more comprehensive study recently conducted by McGinn *et al.* (2006), where both tracer and calorimetric CH_4 emissions were measured while animals were in chambers, revealed that although the tracer technique CH_4 emission estimate was slightly lower than the calorimetric value (by 4%), this difference was not significant.

The results 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 equivalent to CH_4 yields (CH_4 energy expressed as percent of gross energy intake, %GEI) of 16–37 and 8–12 %GEI for tracer and calorimeter chamber methods, respectively, with the chamber estimates being similar to previous observations (Kurihara *et al*., 1999) for cattle fed on similar diets, and CH_4 measured in the same animal calorimetry facility. The extremely high estimates of CH_4 yield for the tracer method observed by Wright *et al.* (2004) have no precedent in the literature.

The results of the present study and those of our previous studies with sheep also fed on forages (Pinares-Patiño *et al*., 2000), in which the 'failure' of agreement between the tracer and calorimetric techniques for CH_4 emission measurement was attributed to the long tube residence in the rumen (~1 year), suggest that permeation tubes should not be deployed for too long as the 'pre-calibrated PR' cannot be extrapolated with confidence to a protracted trial time (Lassey *et al*., 2001).

Results of this study support earlier reports (Johnson *et al*., 1994a,b; Ulyatt *et al*., 1999; Boadi *et al*., 2002; Clark *et al*., 2005) that the tracer technique is associated with larger variation than indirect calorimetry. However, the 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.

In conclusion, when long time elapse between tube insertion and measurement time (evidence from our previous studies) is avoided, the SF_6 tracer technique provides an effective method for CH_4 emission estimations by individual forage-fed sheep. Nevertheless, the source of the extra variation associated with this technique remains to be established.

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