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

C.S. PINARES-PATIÑO, C.W. HOLMES1, K.R. LASSEY2 and M.J. ULYATT

Rumen, Nutrition & Welfare Section, AgResearch Limited, Grasslands Research Centre, Tennent Drive, Palmerston North, New Zealand

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
CH₄) 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 CH₄ 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 CH₄’ measurement period took place. Then, sheep were transferred to calorimetry chambers for 3 days of acclimatization followed by 3 days of CH₄ 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 CH₄ 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 CH₄ 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 CH₄’ measurements were conducted in chamber 2, following sheep acclimatisation in chamber 1.

**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 crates has been described by Pinares–Patiño et al. (2003). Briefly, it involved the use of a calibrated permeation tube charged with SF₆ with a known SF₆ ‘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 CH₄ and SF₆ gases using flame ionisation detection and electron capture detection, respectively.

The daily ‘tracer CH₄’ emission was calculated using the ratio of molar mixing ratios, CH₄ to SF₆ (each corrected for mixing ratios in background air) in the yoke-borne sample, in conjunction with the pre-calibrated PR of SF₆ (Johnson et al., 1994a):

\[
\text{‘Tracer CH}_4\text{’ (g/day)} = \text{‘Pre-calibrated PR’ (g/day)} \times \frac{[\text{CH}_4]}{[\text{SF}_6]} \times (16/146)
\]

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

Methane emission measurements by the SF₆ technique (‘tracer CH₄’) 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 CH₄ 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 T2. 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 CH₄ estimates were 4% lower than the corresponding calorimetric estimates, but with no significant difference between the methods. Tracer CH₄ emission estimates could be expected to be slightly smaller than those measured in respiration chambers because the flatus CH₄ excretion, which accounts for approximately 2% of the total emission of CH₄ (Murray et al., 1976), is not accounted for by either the SF₆ 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 CH₄ emissions were measured while animals were in chambers, revealed that although the tracer technique CH₄ 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 CH₄ yields (CH₄ 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 CH₄ measured in the same animal calorimetry facility. The extremely high estimates of CH₄ 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 CH₄ 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 SF₆ tracer technique provides an effective method for CH₄ 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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REFERENCES


De Boer, J.J.; Cottyn, B.G.; Bouque, C.V.; Aerts, J.V.; Buyssse, F.X. 1984: Comparative digestibility by sheep and cows and consequences on energy value. Canadian Journal of Animal Science 64 (suppl.): 175–176.


Dougherty, R.W.; Allison, M.J.; Mullenax, C.H. 1964: Physiological disposition of C¹⁴-labeled rumen gases...


