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*Taihoru Nukurangi*

**Ruminant methane measurements:  
Preliminary trials**

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**NIWA Science and Technology Series No. 22**

ISSN 1173-0382

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**August 1995**

Cataloguing-in-publication

Ruminant methane measurements: preliminary trials / K.R. Lassey ... [et al.]. Wellington, N.Z.: National Institute of Water and Atmospheric Research Ltd., 1995. (NIWA science and technology series ; 22)

ISSN 1173-0382  
ISBN 0-478-08355-6

I. National Institute of Water and Atmospheric Research Ltd. II. Title.  
III. Series.

The *NIWA Science and Technology Series* is published by NIWA (the National Institute of Water and Atmospheric Research Ltd.), New Zealand. It supersedes *NIWA Ecosystems Publications* (ISSN 1172-3726; published by NIWA Ecosystems, Hamilton), *New Zealand Freshwater Research Reports* (ISSN 1171-9842; published by NIWA Freshwater, Christchurch) and *Miscellaneous Publications, New Zealand Oceanographic Institute* (ISSN 0510-0054).

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NIWA specialises in meeting information needs for the sustainable development of water and atmospheric resources. It was established on 1 July 1992.

## Introduction

A programme of measuring methane emissions directly from grazing ruminant animals was commenced in FY 1994/95 as a collaboration between two New Zealand Crown Research Institutes, NIWA and AgResearch. Each institute had its own longer-term objectives: NIWA, to provide a scientific basis for assessing New Zealand's anthropogenic emissions of methane (CH<sub>4</sub>), a potent greenhouse; and AgResearch, to provide a basis for investigating both the improved nutritional performance of New Zealand ruminants and a reduction in microbial methane production. The goal of the present trials was to gain experience prior to embarking on a more extensive programme aimed towards those objectives.

New Zealand (NZ) has an unusually high level of methane emissions per capita from human activities, of which ruminant methane production is the dominant component (Lassey et al., 1992). Such production represents a loss of nutritional energy to the animal which is potentially avoidable if rumen microbial activity can be effectively modified (Leng, 1992). The twin goals of better quantifying the ruminant methane source, and better understanding nutritional performance, are advanced by new developments in directly measuring methane emitted at the mouth from individual grazing ruminants.

As part of the NIWA programme, three of the authors (PRZ, HHW and KAJ) came to NZ to transfer the technology their team had developed; two of these were funded by NIWA. Field trials conducted by the combined NIWA-AgResearch-USA team are the subject of this report.

## Method

The basic technique is described by Johnson et al. [1994]. A lightweight collection system is mounted on each animal, and collects an integrated sample of the 'air' from the vicinity of the nostrils and mouth while the animal is grazing. The 'air' comprises an unknown mixture of respired breath (from the lungs), eructed gases (from the rumen), and ambient air.

Throughout this report, the term 'exhaled air' is used to describe the sampled mixture. A knowledge of the actual mixture and of the details of the collection plumbing, is avoided by novel use of an inert tracer (sulphur hexafluoride, SF<sub>6</sub>): an SF<sub>6</sub> source of known strength is placed within the animal's rumen. The production rate of CH<sub>4</sub> in the rumen,  $Q_{CH_4}$ , is then related to the SF<sub>6</sub> source strength,  $Q_{SF_6}$ , by:

$$Q_{CH_4} = Q_{SF_6} \times [CH_4] / [SF_6]$$

where square braces denote gas concentrations in the exhaled air in excess of ambient levels.

This method is dubbed the **ERUCT** technique (**E**missions from **R**uminants Using a **C**alibrated **T**racer).

### The collection system:

The collection system comprises:

- a halter mounted on the animal with suitable plumbing attached; and
- easily removable and interchangeable lightweight cannisters.

The cow halter is similar to those commonly used to lead cattle into rings for shows or auctions, and a smaller version was designed for sheep. To the halter is attached a flow-

limiting capillary tube whose length is selected so that a pre-evacuated cannister fills to about 0.5 atmosphere of pressure over the chosen sampling duration. (This choice of pressure ensures a fairly uniform rate of collection). The inlet end terminates in a 15 micron filter and protector attached to a leather nose-patch on the halter; this is designed to sample exhaled air efficiently while at the same time denying entry to the capillary of water, dust or other solid material into which the animal may thrust its nose. At the outlet end of the capillary, a flexible tube is terminated by a Swagelok Quick-Connect stem. This enables quick and easy connection to the Quick-Connect body on the cannister.

The cannister itself is a light-weight pvc yoke. This design supersedes the stainless steel cannister described by Johnson et al., and was developed by two of us (HHW and KAJ) and colleagues at Washington State University. The yokes used in this study were fabricated by NIWA staff from commercial high-pressure-rated pvc tubing. Each was pre-formed from 32mm or 50mm i.d. tubing in the form of two legs joined symmetrically by a 90° elbow and with end caps at the “feet”. This right-angled configuration was shaped towards a “U” after heating to a pliable state at about 130°C. A Swagelok needle valve was tapped into the yoke and a Swagelok Quick-Connect body connected to it.

Each yoke was thoroughly leak tested, and its volume determined manometrically — even though the yoke volume is not required for calculating methane production. Properties of the yokes are shown in Table 1. All 22 cow yokes (C1 to C22) are based on 50mm i.d. tube and have volume around 2.5 litre. Of the 21 sheep yokes (S0 to S20), all but 6 were based on 32mm i.d. tube and these had volumes 800 to 900 ml; the other 6 differed only in i.d. (50mm) and had about twice that volume. As noted in Table 1, a selection of yokes were exposed to 11 days of weather, with no detriment to their performance. Note that actual i.d.s exceed the nominal 32 and 50mm by more than 10%, and these were further increased due to stretching when heated.

With the animal conditioned to wearing the halter, the yokes can be mounted and interchanged quickly by means of velcro straps attached to the halter. The yokes are oriented with the valve pointing along the animal’s back. With the Quick-Connect firmly connected, turning on the valve commences sampling.

The photographs in Fig. 1 illustrate the yokes in place on the animals.

### **The inert tracer:**

The inert SF<sub>6</sub> is released from a permeation tube placed in the animal’s rumen, in advance of the trials. Each tube, fabricated from brass rod of about 12 mm diameter and 3 cm in length, releases SF<sub>6</sub> at a steady rate from an initial charge of about 0.5 g. The SF<sub>6</sub> is released through a permeable teflon (PTFE) membrane retained by a porous frit and Swagelok nut. The permeation rate is determined by regular weighings over several weeks. As permeation is strongly temperature dependent, the tubes are maintained at rumen temperature, 39°C, during this time.

The permeation tubes used in these trials were kindly fabricated and charged with SF<sub>6</sub> by staff of Washington State University (WSU). Table 2 records the properties of the 9 tubes supplied, along with the results of weekly weighings — also by WSU staff — to determine the rate of SF<sub>6</sub> release. Regression fits confirm the uniform loss rate beyond the initial weighing when the tubes may not have equilibrated with their environment.

Typical permeation rates are 1.5 mg/day (1000 ng/min), though two had rates nearly 3 times larger. These two were used for cows, and three others were selected for sheep.

### **The laboratory analyses:**

The field trials were conducted at Palmerston North using animals and pasture managed by AgResearch staff. The gas analyses were conducted by RJM and GWB in NIWA laboratories at Gracefield, Lower Hutt. The logistics of this arrangement required a 48-hour turnaround of yokes, at best.

Analyses were conducted by gas chromatography (GC), using flame ionisation detection (FID) for CH<sub>4</sub>, and using electron capture detection (ECD) for SF<sub>6</sub>. As air samples are collected at ~0.5 atmosphere pressure, each yoke is first over-pressured with 'zero air' (air free of trace gases such as CH<sub>4</sub>, SF<sub>6</sub>) to about 1.8 atmosphere, to provide sufficient sample for GC analyses. This 'dilution factor' is measured manometrically. Each analysis is repeated 3 times, or until stable values are obtained against working standards.

The 48-hour yoke turnaround has 2 disadvantages over on-site laboratory analysis:

1. it requires a relatively large number of yokes be fabricated per animal, especially if the chosen sampling frequency is high; and
2. without a pressure gauge in the field, it takes too long to detect any problems in the field, such as leaks in the yokes (samples at excessive pressure) or blocked capillaries (no sample at all).

The 150 km separation of field trial venue and analytical laboratory is viewed as interim for these trials only. Future field work would deploy on-site GC.

## **Sampling Strategy**

Given our physical resources, primarily constrained by yoke numbers and separation between field venue and laboratory, a sampling regime of thrice per day (two 6-hour, one 12-hour) on each of 2 sheep and 2 cows was chosen. A third sheep with inserted permeation tube was not used. Sampling was from Monday 20 March through to the following Friday. The sheep were Romney-cross wethers, about 3 years old and weighing 58 and 69 kg. The cows were 6-year old dry (ie, not lactating) Fresians weighing about 600 kg.

The capillary lengths on the halters were selected appropriately as follows:

*Sheep:* The smaller yokes (based on 32mm i.d. tubing) would be used for 6-hour sampling, and the larger yokes for overnight 12-hour samples. Since the volume ratios were 1:2 a common delivery rate was required, and a capillary length of 45cm delivers the required 1.2 ml/min (0.43 litre per 6 hr).

*Cows:* With only one yoke design available, we planned to compare two strategies, one for each cow:

- ◇ *parallel yokes:* deploy two yokes in parallel overnight with no halter change; appropriate "T" plumbing was designed to facilitate this. The two yokes were strapped together in advance and mounted with an unavoidably forward-facing valve.
- ◇ *alternate capillaries:* deploy a different halter for the overnight sample incorporating a capillary tube with half the flow rate.

A capillary length of 15cm delivers the required 3.6 ml/min (1.3 litre per 6 hr), and a 30cm length delivers half this rate for the alternate capillary.

All prepared capillary lengths were equipped with an identifying tag (a farm "ear tag"). Since the capillaries were retained with the host halter, the tag served to identify the halter also.

A 5<sup>th</sup> yoke was also deployed to collect an ambient integrated sample near, and upwind of, the grazing animals. In practice, adjustments due to background CH<sub>4</sub> and SF<sub>6</sub> levels were modest during the week of sustained but light westerly winds. Some compromises were therefore made to minimise yoke deployment: either the sampling duration was extended, or 'grab samples' were taken using a portable pump (NIWA's Thomas pump) to over-pressure stainless steel cannisters (2 atmosphere pressure).

## Results

Tables 3–5 summarise the results for the two cows (identified as #223 and #54), the two sheep (#36 and #38), and for ambient air, respectively.

### Cows:

Results for cow #54 proved to be somewhat unsatisfactory, with few useful samples being collected. This turned out to be due a blockage in capillary tube #273 which developed after Day 1 but which went undetected during the trials. This underlines the need to be able to check yoke pressure quickly after collection, rather than await analysis at a remote lab. This animal was also relatively belligerent, which may have contributed to damaged halter tubing, and/or to leaks which developed in 2 previously leak-free yokes (C7 and C9) — though other yokes also developed leaks.

Cow #223 provided a good time series of measurements, marred only by a slightly leaky yoke (C4) which nevertheless appeared to give a satisfactory CH<sub>4</sub> release estimate, and by the inadvertent deployment of a wrong halter for one overnight sample. Methane production rates varied throughout the week, perhaps related to pasture availability. The duration-weighted average methane production rate during the almost 4 days of sampling was 162 g/day (adjusted for ambient levels), extrapolating to annual production of 59 kg CH<sub>4</sub>. This is quite consistent with estimates for NZ cattle by Lassey et al. (1992) based on physiological energy demand: 80.6 kg/yr (mature dairy cows), 46.1 kg/yr (herd-weighted 'other' dairy cattle), 69.1 kg/yr (breeding beef cows), 51.8 kg/yr (herd-weighted 'other' beef cattle).

Cow #223 had her halter changed for overnight sampling (the 'alternate capillaries' sampling strategy), while the more belligerent cow #54 carried 'parallel yokes' overnight (denoted 'twin yokes' in Table 3). The experience gained here favoured the former sampling strategy: the forward-facing valve made inevitable with parallel yokes was prone to damage by a 'careless' cow. It is possible that yokes C7 and C9 were damaged by cow #54 in this way. The 'alternate capillaries' strategy need not require alternate halters; 2 capillaries on the one halter would suffice, provided the idle capillary could be suitably protected.

### Sheep:

Both sheep provided useful time series of measurements, yet the inferred methane production rates were quite dissimilar. The heavier sheep #38 averaged 16.6 g/day (6.06 kg/yr), while sheep #36 averaged 23.4 g/day (8.54 kg/yr). Both animals displayed considerable production

variability during the week, perhaps due to variations in both feed availability and anxiety levels (due to a small flock and the proximity of cows). The average productions compare favourably with estimates for NZ sheep by Lassey et al. (1992) based on physiological energy demand: 11.5 kg/yr (breeding ewes), 6.9 kg/yr (flock-weighted 'other' sheep older than 1 year).

The apparently significant variation in methane production between the 2 sheep is intriguing. A subsequent trial conducted by one of us (GCW) — with lab. analyses by RJM and GWB — involved also the third sheep (#33) with inserted permeation tubes. The 3 sheep in this trial were confined indoors in crates and supplied a succession of 3 dietary feeds over 3 weeks. Again there were significant variations in methane production among the sheep, though #38 was not consistently the lowest. To the extent that there was a consistent pattern between both trials, sheep #38 appeared to react most strongly to a change in environment by reducing feed consumption (and therefore methane production) at those times.

### **Ambient air samples:**

Table 5 shows the results for two overlapping collections of ambient air — also termed background agricultural air (BAA).

Integrated collections earlier in the week deployed a sheep or cow halter mounted on a fence-post near, but not directly downwind of, the grazing animals. Methane concentrations were variable, but not high enough to impact markedly on the calculation of ruminant production rate. Daily 'grab samples' of air, taken from at least 20m further away, showed much better consistency, and suggested lower levels of both CH<sub>4</sub> and SF<sub>6</sub>, than the integrated samples. While this suggests that the integrated samples might be unduly influenced by proximate animals, it is not clear which sample set is the more representative of the air available for inhalation by the grazing animals. We used the daily grab samples as representative of the actual BAA used to adjust the inhaled air samples.

## **Conclusions**

The trials encountered few problems, and so the goal was achieved. From the points of view of both NIWA and AgResearch, immeasurable benefit was obtained from the experience and participation those involved in developing the ERUCT technique (PRZ, HHW, KAJ).

The main strategic recommendations for future campaigns in NZ are:

1. Avoid the use of parallel yokes; rather, for multiple sampling durations, adopt alternate capillaries, either on separate halters or on a common halter.
2. Animals should be acclimatised to the sampling conditions, especially to wearing the halters, in advance of the trials.
3. Ensure pasture availability does not vary throughout the trial, and that grazing is not influenced by avoidable external factors such as small flock/herd size or other anxiety factors.
4. Plan to analyse samples soon after collection. This will (a) enable sampling problems (such as blocked capillary) to be detected quickly, (b) minimise the effect of an introduced leak in a yoke, and (c) greatly reduce the number of yokes needed. It will require that the GC laboratory be located near the trial venue.



5. Preferably fabricate yokes from 50mm i.d. pvc, avoiding 32mm pvc. This is because the former has no deployment disadvantage, while the sample collected by the smaller yoke could prove too small if GC problems necessitated more aliquots be analysed.

### **Acknowledgements**

This work was funded by the NZ Foundation for Research, Science and Technology (FRST), including contract CO1425 with NIWA. Staff at Washington State University kindly provided logistic assistance, including the provision of permeation tubes. The participation of HHW and KAJ in these trials was supported in part by Washington State University, and that of PRZ by the US National Center for Atmospheric Research.

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