CSIRO PUBLISHING

TECHNICAL REPORT
https://doi.org/10.1071/AN25386

ANIMAL PRODUCTION SCIENCE

# Applying the $SF_6$ tracer gas methodology to measure enteric methane emissions in grazing Brahman heifers

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**Handling Editor:**
James Dougherty

**Received:** 27 October 2025
**Accepted:** 13 November 2025
**Published:** 4 December 2025

**Cite this:** Boulton AC *et al.* (2025) Applying the $SF_6$ tracer gas methodology to measure enteric methane emissions in grazing Brahman heifers. *Animal Production Science* **65**, AN25386. doi:10.1071/AN25386



## ABSTRACT

**Context.** Sulfur hexafluoride ($SF_6$) is a non-toxic, synthetic gas, which is used as a chemical tracer to contextualise the source of other gases. In livestock research, the $SF_6$ tracer gas technique is regaining favour as a useful, viable, and robust approach to measuring enteric methane emissions, especially when respiratory chambers are not an option. This paper outlines the methodology used by the authors as part of a concept exploration study assessing the potential of using the saliva microbiome as a proxy for the rumen microbiome and thus an indicator of methane emissions. In this context, the $SF_6$ technique was implemented to measure enteric methane emissions from tropical beef heifers in a grazing system. **Aims.** To facilitate the use of the $SF_6$ technique by others, this paper describes its implementation for Brahman heifers in a grazing system, emphasising its practical adaptation for long-term use under tropical conditions with *Bos indicus* cattle and methodological insights gained over a full reproductive cycle. **Methods.** This paper describes the methodology applied by the authors, emphasising important lessons learned regarding five critical points of this technique. First, cattle were trained to wear the sampling gear imported from Institute of Animal Science (IZ, Instituto de Zootecnia), São Paulo, Brazil. Second, permeation tubes charged with appropriate $SF_6$ amounts were manufactured, calibrated, and deployed. Third, the sampling gear was optimised for grazing conditions and the size of the cattle. Fourth, the gas sampling procedure, including sampling background $SF_6$ from the environment, was standardised. Fifth, the gas chromatography method for measuring methane and tracer amounts of $SF_6$ was optimised. **Key results.** Training of the animals is an essential step that should always be considered when implementing the $SF_6$ technique. Subsampling the gas from canisters into glass vials for analyses should be conducted open air to avoid contamination issues. Inside the rumen, permeation tubes have a shelf life. **Conclusions.** Having all the components of this methodology in-house facilitated the process of implementing the methodology, supported by collaborations with researchers at IZ and Agriculture Victoria who were already experts in this methodology. **Implications.** Now that the $SF_6$ technique is readily available at Gatton, through QASP and the analytical facility, further research can be conducted on the methane emissions of cattle raised under tropical conditions, expanding the applicability of this approach to grazing *Bos indicus* heifers measured through their first pregnancy cycle.

**Keywords:** *Bos indicus*, bovine, cattle, heifer, livestock, methane, pasture, sulphur hexafluoride, sustainable agriculture, tropical agriculture.

## Introduction

Enteric methane emissions present a significant challenge for the ruminant livestock industry, owing to their substantial contribution to greenhouse-gas emissions and therefore global warming (Gerber *et al.* 2013; Roques *et al.* 2024). Understanding and mitigating

these emissions is crucial for sustainable livestock production. A few methodologies, each with advantages and disadvantages, are available to measure enteric methane emissions from ruminants (Goopy *et al.* 2016; Della Rosa *et al.* 2021, 2023; Ross *et al.* 2024). This paper focuses on the $SF_6$ tracer gas methodology, a flexible platform for grazing systems.

In Australia, the beef breeding sector (i.e. the cow–calf herds) operates mostly under grazing conditions, with approximately 60% of the national herd being located in northern Australia (McCosker *et al.* 2023). Grazing systems represent a significant proportion of the livestock sector globally (Greenwood 2021). Primarily, animal feed is not human food because 86% of the dry matter consumed in livestock feed globally consists of materials that are not suitable for human consumption (Mottet *et al.* 2017). Grazing herds are highly relevant to food security and methane emissions, in Australia and internationally. Therefore, it is important to consider the unique challenges that grazing systems present for measuring enteric methane emissions because of the extensive open nature of these environments. Unlike confined animals, grazing ruminants move freely over large areas, making it difficult to maintain consistent sampling conditions and deploy measurement equipment reliably. Environmental factors such as wind, temperature, and humidity affect gas dispersion, complicating the accuracy and repeatability of measurements. In such systems, only a limited number of methods are feasible for accurately measuring methane emissions (Tedeschi *et al.* 2022). The sulfur hexafluoride ($SF_6$) tracer gas technique is a portable system and thus particularly valuable because it can be used while ruminants are grazing.

The $SF_6$ tracer gas technique has been regaining favour in recent years. This technique was popular but went out of favour after some variable outcomes were published. For example, variations in the permeation rates affect outcomes for $SF_6$ methodology (Martin *et al.* 2012). It has regained favour since Deighton *et al.* (2014) published their modified version of the technique, which allowed for more accurate measurements of daily methane emissions in dairy cattle. As part of the LESTR project, a large, multidisciplinary initiative funded by Meat and Livestock Australia (MLA), the $SF_6$ tracer gas methodology was implemented in *Bos indicus* beef cattle that were grazing on pasture, with minor modifications made to the equipment and procedures to suit the specific characteristics of the animals and experimental conditions. This technique, meticulously detailed in the $SF_6$ tracer gas manual (Berndt *et al.* 2014), involves using permeation tubes charged with known amounts of $SF_6$ to estimate enteric methane emissions from ruminants. The detailed manual comprises multiple papers that describe various aspects of using $SF_6$ (Berndt *et al.* 2014). Still, its implementation requires adjustments to fit the ruminant group being measured (i.e. consider animal size and behaviour) and the experimental conditions or hypothesis being tested (i.e. consider if the experiment is over a short or lengthy period). The LESTR project includes a pregnancy trial, requiring the animals to be monitored over many months to cover before, during and after pregnancy surveillance of enteric methane emissions. Although the variability reported in $SF_6$ implementation can be a dilemma for researchers, it makes the technique flexible and adaptable.

The LESTR project will examine oral and rumen microbiomes as predictors of methane emissions while collecting a large dataset to represent the Australian beef industry. To this aim, it was relevant to implement a measuring system adjusted for grazing Brahman heifers that represent northern Australia commercial beef herds. Specifically, a cohort of 40 heifers was monitored from before conception through to their first calving, representing the annual cycle of breeding herds, so as to estimate their carbon footprint. Unlike many previous $SF_6$ trials that tested feed interventions over 3 months or less, this LESTR trial required methodological adjustments to measure a complete reproductive cycle under realistic grazing conditions. Pregnancy in bovine females lasts approximately 9 months, meaning that adjustments to the method were required to allow for a long experimental period (just over 12 months).

The purpose of this report is to describe the methodology and practical considerations involved in implementing the $SF_6$ tracer gas technique for measuring enteric methane emissions in cattle. This article is presented as five sections, to discuss each of the critical points identified while implementing the $SF_6$ technique, as follows: (1) training cattle to wear the sampling gear; (2) manufacturing, calibrating, and deploying the permeation tubes charged with an appropriate amount of $SF_6$; (3) optimising the sampling gear for grazing conditions and the size of sampled cattle; (4) standardising the gas sampling procedure, including sampling background $SF_6$ from the environment; (5) optimising the gas chromatography methods to measure methane, $CO_2$, and tracer amounts of $SF_6$. In addition, information about the LESTR experiment (i.e. $SF_6$ quantity charged into permeation tubes, permeation curves, and averaged emissions measured at one timepoint) is provided together with the data reported by other research groups for context (Table 1).

## Training Brahman heifers to wear the sampling gear

Forty commercial Brahman maiden heifers were purchased from two properties in South East Queensland. The cattle were transported and agisted at the cattle research facility at the University of Queensland (UQ), Gatton campus. The heifers were estimated to be between 18 and 24 months of age on arrival. They had minimal previous handling as is common in commercial Australian herds, which are usually mustered once or twice a year. As indicated in recent studies, Australian management of breeding herds is changing (McCosker *et al.* 2023), but it still largely relies on limited

**Table 1.** Bovine enteric methane ($CH_4$) emissions, measured using the $SF_6$ methodology.

| Breed | Category | Number of animals | Indoor/outdoor | Permeation tube calibration (weeks) | Sampling days per period | $SF_6$ release rate (mg/day or PPT) | $CH_4$ (g/day)[A] | $CH_4$ (g $CH_4$/kg DMI) | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Brahman | Heifers | 38 | Outdoor grazing | 8 | 5 | 64.99 ± 7.91 (PPT) | 288.58 ± 17.38 | | Our experiment[B] |
| Brown Swiss | Cows – lactating and dry | 11 | Outdoor grazing and indoor pens | | 7 | 1.40 ± 0.05 to 4.55 ± 0.30 | 358.5 ± 8.1 (lactating) 337.4 ± 7.5 (dry) | 29.0 ± 0.7 28.7 ± 0.4 | Salas-Riega *et al.* (2022) |
| Nellore | Bulls and heifers | 481 | Pens and Paddocks | | 5 | 1.83 ± 0.12 | 179.7 (–RFI[A]) 189.8 (+RFI) | 23.46 21.34 | Sakamoto *et al.* (2021) |
| Nellore | Bulls | 24 | Individual pens | 3 | 5 | 3.11 | 235 (Low RFI) 249 (High RFI) | 25.3 26.2 | Delveaux Araujo Batalha *et al.* (2020) |
| Holstein Friesian | Cows – pregnant, dry | 68 | Outdoor grazing | ≥8 | 6 | 4.97 ± 1.43 (Year 1) 5.78 ± 1.01 (Year 2) 4.26 ± 0.56 (Year 3) | 216 | 25.2 | Ferris *et al.* (2017) |
| Holstein | Cows – lactating | 48 | Indoor | | 3.2 ± 0.1 | 4.38 ± 0.261 | 405 ± 156 | | Hristov *et al.* (2016) |
| Nellore | Bulls and heifers | 46 | Individual pens | | 6 | | 142 ± 3.81 (low RFI) 144 ± 3.64 (high RFI) | 25.1 ± 0.588 22.8 ± 0.054 | Mercadante *et al.* (2015) |
| Holstein × Friesian | Cows – dry | 8 | Indoor | 3 | 5 | 7.25 ± 0.445 | 431 ± 21.1 | 22.3 ± 1.44 | Deighton *et al.* (2014) |
| Holstein | Cows – dry (fistulated) | 6 | Indoor | 10 | 3 | 1.58 ± 0.28 (LRR) 3.15 ± 0.56 (HRR) | | | Martin *et al.* (2012) |
| Holstein × Friesian | Cows – lactating | 24 | Outdoor grazing | 11 | | 1.729 ± 0.158 | 374.5 (control) 369.3 (malic acid) | 19.5 ± 0.45 | Foley *et al.* (2009) |
| Charolais crossbred | Heifers | 11 | Indoor | | | 2.23 ± 0.09 | 207.30–246.10 | | Foley *et al.* (2009) |
| Hereford | Steers | 58 | Outdoor grazing | | 9 | ~3 | 161 ± 20 | | Laubach *et al.* (2008) |
| Friesian × Jersey | Cows – lactating | 302 (2004) 396 (2005) | Outdoor grazing | 12 | 4 (2004) 3 (2005) | 3.81 ± 0.68 (2004) 2.79 ± 0.29 (2005) | 332 ± 42.1 (2004) 289 ± 35.2 (2005) | 19.3 ± 3.0 (2004) 17.4 ± 2.4 (2005) | Cavanagh *et al.* (2008) |
| Holstein × Friesian | Heifers | 7 | Outdoor grazing | 8 | 7 | 1.38–1.70 (2002) 1.15–1.60 (2003) | 223 (LSR)/242 (HSR) 2002 203 (LSR)/200 (HSR) 2003 | | Pinares-Patiño *et al.* (2007) |
| Hereford × Friesian | Steers (fistulated) | 12 | Outdoor feed pads | 10 | 5 | 2.88 ± 0.05 (LRR) 7.34 ± 0.05 (HRR) | 110.8 ± 5.6–157.6 ± 5.1 (LRR) 129.1 ± 3.0–192.5 ± 7.1 (HRR) | 18.3 ± 0.6 (LRR) 21.8 ± 0.4 (HRR) | Vlaming *et al.* (2007) |
| Angus | Steers | 76 | Outdoor feedlot | | ≤10 | 2.75 | 84.4–395.8 (mean 179.6) | | Hegarty *et al.* (2007) |
| Holstein × Friesian | Cows – lactating | 16 | Indoor | 10 | 3 | 3.7 ± 0.7 | 331 ± 74.6 | 17.1 | Grainger *et al.* (2007) |
| Black Angus | Yearling heifers | 10 | Outdoor grazing | 8 | 5 | 3.85–5.24 | 113.5–167.6 144.2 (mean) | 18.7–37.4 (alkane) 14.7–21.3 (CNCPS) | Chaves *et al.* (2006) |
| Angus | Yearling steers | 36 | Outdoor feedlot | | | 0.42–0.95 | 39.165–264.419 | | Guan *et al.* (2006) |

(*Continued on next page*)

**Table 1.** (*Continued*).

| Breed | Category | Number of animals | Indoor/outdoor | Permeation tube calibration (weeks) | Sampling days per period | $SF_6$ release rate (mg/day or PPT) | $CH_4$ (g/day)[A] | $CH_4$ (g $CH_4$/kg DMI) | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Beef | Heifers (spayed) | 8 | Indoor | ≥8 | 3 | 4.30–4.93 | 135.98 ± 9.6 | | McGinn *et al.* (2006) |
| Charolais | Cows – pregnant and dry | 6 | Outdoor grazing | 8 | 7 | 1.53–1.72 | 204.4 ± 28.1–273.3 ± 28.7 | | Pinares-Patiño *et al.* (2003) |
| Crossbred | Yearling heifers | 6 | Indoor | 8 | 3 | 0.36–0.72 | 98.09 ± 2.86 | | Boadi *et al.* (2002*a*) |
| Holstein, Charolais × Simmental | Yearling heifers | 12 | Indoor | 8 | 5 | 0.50–1.01 | 170.4 ± 4.94 (dairy) 163.68 ± 5.58 (beef) | | Boadi and Wittenberg (2002) |
| Red Angus | Yearling steers | 8 | Outdoor grazing | 8 | | 0.50– 1.01 | 183.511 ± 9.165–260.552 ± 8.449 | 15.537 ± 1.074–27.351 ± 1.217 | Boadi *et al.* (2002*b*) |
| Hereford × Simmental | First calving, early lactation | 16 | Outdoor grazing | | | 0.51– 1.84 | 267.64–294.28 | | McCaughey *et al.* (1999) |
| Crossbred | Yearling steers | 16 | Outdoor grazing | | 6 (first rotation) 4 (second rotation) | 0.513– 1.84 | 173.415–219.597 | | McCaughey *et al.* (1997) |
| Friesian | Cows – lactating | 10 | Outdoor grazing | ≥8 | 5 | 1.9–3.5 | 262.8 ± 9.6 (229–313) | | Lassey *et al.* (1997) |

Abbreviations in the table are as follows: RFI, residual feed intake; LSR, low stocking rate; HSR, high stocking rate; LRR, low release rate; HRR, high release rate; CNCPS, Cornell Net Carbohydrate and Protein System model; PPT, parts per trillion.

[A]Average and, when available, standard errors and range of reported methane emissions using the $SF_6$ methodology. The range is reported in parentheses. Averages were calculated across groups aggregating the data available in each reference.

[B]Our experimental data reported here are based on the aggregated data collected in the second sampling period, when the pregnant group was 4 months pregnant.

mustering events, typically for branding and weaning or pregnancy diagnosis (Fordyce *et al.* 2023). As a result, commercial Brahman heifers often need habituation to humans before research trials can commence.

The initial part of the training involved increasing the exposure of the heifers to research and technical staff so they could get habituated to human contact. Heifers were initially in small quarantine pens that were visited and observed daily by staff. Daily behavioural traits were noted, identifying cohort hierarchy and friendship pairs/groups being formed. Researchers and Queensland Animal Sciences Precinct (QASP) staff used cattle training strategies that consider the animal behaviour principles laid out by Grandin (1989). Staff movement through the two quarantine pens was completed using low-stress stock handling techniques that consider the flight zone of the animals (Stookey and Watts 2014). Over the ensuing weeks, the heifers were put through the cattle handling facilities regularly and a routine of touching and resting hands on the heifers' back and flank, to facilitate good human–cow interactions, was employed. The quarantine pens and extent of the habituation period provided an excellent opportunity to reduce the heifers' flight zone. Noteworthy, the handler adopted a passive body position as soon as the animal allowed being touched on the shoulder (Abramowicz *et al.* 2013). Two heifers were deemed behaviourally unsuitable for the experiment. They raised safety concerns for animals and humans since they never learned to be calm during handling, reducing the training herd to 38 animals.

The equipment used to gather the methane samples included a specially adapted saddle and halter (Fig. 1). The saddle comprised a felt underlay and a canvas material top with nylon straps that pass around the two front legs and beneath the tail, attached to the saddle with metal buckles. The halter included a piece of leather that sits over the bridge of the nose, with nylon straps sitting below the chin and behind the ears. Attached to the leather was a small inlet tube covered in soft plastic that sited above the nostrils. The connective tubing attached to the inlet tube was encased in a further protective tube that ends in a Swagelok® DESO connector that is inserted into the stainless-steel gas canisters used to sample the eructed and respired gases.

Familiarisation with the equipment began with placing the saddles on the heifers for a short time, approximately 10 min, and then removing them again. The duration was extended to overnight. When the heifers were released from the crush following the placement of the saddle, they displayed the typical bucking behaviour of animals not used to saddles. That behaviour occurred for approximately 2–3 min. Afterwards, the heifers settled, accepting the saddle. To ensure animal safety and wellbeing, they were first released into a small empty holding yard until they settled. They were then moved into an adjacent holding yard with the heifers that had already been saddled. Adaptation to the halters began with a soft nylon training halter, which was placed on the heifers for short periods and then increased to overnight use before exposure to the experimental halter and all its tubing. This careful habituation to using saddles and halters was necessary during the training period, which lasted approximately 2 months (between February and April 2023). Afterwards, once heifers were accustomed to using the equipment, they could wear saddles and harnesses and immediately be released back to the herd without problems. In short, the training allowed for methane sampling of commercially sourced Brahman heifers by using the $SF_6$ methodology.

It should be noted that although the main goal of the training was to habituate the heifers to wearing the equipment for 5 days at a time, it also provided the necessary training for

**Fig. 1.** Heifers wearing the saddle and halter equipment designed to link the sampling canister to the animals' noses to collect a sample of respired gas. Note that the coiled tubing is the key under pasture conditions to permit a full range of movement for the grazing animal.

staff in safely putting on and removing the equipment. The main challenge in fitting the equipment is looping the two straps around the forelegs to buckle to the saddle. For safety reasons, it is not advisable to place arms between the animal and the crush to grab the strap. Instead, a long piece of wire with a hooked end was designed to pick up the leg strap from the floor of the crush and bring it upward for the staff member to safely grab it. This is an important safety consideration depending on the design of the saddle and the crush being used. The hooked wire is a very simple tool, but it made the daily process of checking and replacing saddles safer and faster, which was important when sampling methane daily from 38 animals, in 5-day periods.

## Manufacturing, calibrating, and deploying permeation tubes charged with $SF_6$

Permeation tubes are brass capsules engineered to slowly and consistently deploy the tracer $SF_6$ gas. The components of the permeation tubes are important in determining the release rate of the tracer gas where the size of the tube body limits the amount of tracer gas available and, subsequently, the duration of the trial. The guidelines for using $SF_6$ (Berndt *et al.* 2014) include comprehensive information on the properties of permeation tubes. At the UQ, Faculty of Science workshop, permeation tubes were manufactured on the basis of the specifications in the guidelines for the Instituto of Animal Science (Instituto de Zootecnia, IZ) 'long-term' permeation tubes (Berndt *et al.* 2014). The lead time for acquiring some components is an important point for researchers undertaking the manufacture of permeation tubes for the first time. There was a delay of several months in the arrival of parts from outside Australia because of the non-standard sizing of frits and membranes required to seal the permeation tubes.

Calibrating permeation tubes is a relatively simple procedure. It requires available and laboratory-trained staff to weigh the tubes regularly throughout the 6–10-week period following filling (Berndt *et al.* 2014). Following deployment, the surveillance tubes must continue to be weighed weekly until the trial is completed. In the LESTR trial, an analytical electronic balance and a drying oven at 39°C were used for calibration for 8 weeks and surveillance for just over 12 months. Access to an −80°C freezer for storage of permeation tubes is critical if they are not employed immediately following calibration (Deighton *et al.* 2011). Calibration curves for tubes used in the trial and for sentinel tubes are provided as Supplementary material (Table S1).

Cattle permeation tubes are normally 50–60 mm long and include a Swagelok® nut. They do not have the smooth surface of other orally administered products, such as trace elements and deworming bolus. It is important to ensure that the bolus gun or similar applicator has a small retention cup to prevent the permeation tube from slipping down below the plunger and getting trapped in the cup, resulting in a prolonged procedure and greater stress for the animal. Alternatively, it is possible to use a gelatine capsule to cover the tube and facilitate the process (Williams *et al.* 2024). Another recommendation is to place the individual animal in a small holding pen for 15 min following the insertion of the permeation tube, to ensure the tube is not regurgitated. In case the permeation tube is regurgitated, it can be easily collected and reinserted into the animal if needed. In the current experiment, two heifers regurgitated their permeation tubes, which were then reinserted. In this context, permeation tubes must have unique number identifiers so they can be matched back to the correct animal. Both the IZ and the UQ tubes had unique number identifiers engraved in their metal bodies.

## Optimising and maintaining the sampling gear for grazing Brahman heifers

The design of the saddles and halters imported from IZ, São Paulo, Brazil, is fit for purpose and has been used in previous studies (Sakamoto *et al.* 2021). However, one adaptation was necessary to measure methane under grazing conditions. The original fixed-length tube was replaced with a coiled tube that allows a full range of movement so that the animal can feed on grass, with less chance of breaking the equipment (Fig. 1).

The original halters comprised the capillary plastic connective tubing (i.e. the ⅛ sampling tubing) encased in a larger-diameter straight length of tubing wrapped in duct tape (Sakamoto *et al.* 2021). Towards the halter is the flow restrictor and, on the opposite end, a Swagelok® DESO connector. In the first pre-trial sampling, there was a high rate of equipment failure because of an inadequate length of the tubing, resulting in breakages mainly at the end of the tubing where it connected to the stainless-steel canisters. The straight protective tubing was therefore replaced to include a length of recoil tubing as used by the Agriculture Victoria Research team (Deighton *et al.* 2014). The recoil tubing provided greater flexibility to the equipment to allow for the growth of the animal and changing grazing conditions. However, even though this reduced the number of breakages, they occurred throughout the sampling period, with the main point of weakness being the end where the tubing connects to the canisters. Breakage of the connection leads to data loss. When sampling *Bos indicus* heifers, a minimum of 16 animals per treatment group for five consecutive days is recommended, so that, accounting for data loss in the sampling process, researchers can collect enough individual cattle data to represent each treatment or experimental condition. In the LESTR experiment, a minimum of 3 days with valid data per animal yielded enough data when averaged to represent a sampling period, because the individual averages were not different when 3 days were

compared with 5 days. Still, the $SF_6$ manual recommends a minimum of 4 days per sampling period.

Despite equipment breakage, 30 of the 38 heifers had useful data measured in all five consecutive days, six animals had data on 4 days and only one heifer had just 2 days of data, in the first measurement period. We fixed equipment as often as needed, which contributed to the successful sampling. Overall, we collected 162 individual methane measurements across five measurement periods that represented before, during and after pregnancy. The number of animals measured in each sampling period and the number of methane measurements successfully obtained is detailed in Table 2.

## Standardising the gas sampling procedure: sampling the cattle and their environment

The duration of gas sampling varies across studies, with a 5-day period being commonly used in *Bos indicus* research (Mercadante *et al.* 2015; Delveaux Araujo Batalha *et al.* 2020). This study aimed to collect five 24-h representative samples per animal and their environment, although field conditions often led to equipment failures, such as clogged or damaged tubing and connectors, necessitating frequent repairs. To maintain consistent gas flow, a mass flow meter was employed between sampling periods to assess and calibrate flow restrictors, detect blockages, and identify equipment damage. To mitigate disruptions, a surplus of equipment is recommended; at least 10% extra saddles and halters were always readily available for equipment changes. Collecting multiple samples over 5 days increases the chance of obtaining a minimum of three valid 24-h samples per animal, enabling reliable statistical analyses and group comparisons.

It is necessary to collect samples from each animal as well as control environmental samples because atmospheric methane and $CO_2$ vary and background contamination is possible (Williams *et al.* 2011). Some authors use sentinel animals to collect the environmental sample. In the LESTR trial, four canisters were used for environmental sampling.

**Table 2.** Number of heifers measured versus number of $CH_4$ measurements obtained per sampling period.

| Measurement period | Heifers measured | Number of methane measurements |
|---|---|---|
| (1) Pre-pregnancy | 18 | 16 |
| (2) At 4 months | 38 | 35 |
| (3) Second trimester | 37 | 37 |
| (4) Third trimester | 37 | 37 |
| (5) Post-calving | 37 | 37 |
| Grand total | 167 | 162 |

Each individual measurement represents an average per animal obtained from the five consecutive days of sampling.

These canisters were fixed on the fences around the paddock where the animals were grazing, and identified by their exact geographic location (Fig. 2). The four environmental canisters were used only for collecting background gases and were identified by their L-shaped Swagelok® fitting. This fitting made it easier to attach the sampling hose when resting the canister in the holding basket attached to the paddock fencing. When sampling from the environmental canisters, specific Swagelok needle nose connector, stopcock with Luer Lock connection, 50-mL syringe and polytetrafluoroethylene-faced 10-mm septa were used to ensure that the risk of contamination was minimal. In short, the laboratory consumables used for environmental sampling were entirely separated from the ones used for animal sampling. The data obtained from the environmental sampling were used as prescribed by earlier $SF_6$ publications, as a correction factored into the reported animal emissions.

To collect a subsample from each canister, 10-mL crimp top headspace glass vials were used. This was recommended by UQ analytical services as being the most appropriate for the type of gas chromatograph being used. The headspace vials were capped with a 20-mm bimetal PTFE/silicon septa. During the pilot trial, the vials were capped with PTFE/butyl septa; however, the headspace vials did not hold the vacuum well, and as such it is important to analyse the samples as soon as possible after collection. A recommendation would be to discuss the best collection vessels with the analytical team that will be doing the gas chromatography, because this can reduce the preparation steps for the laboratory personnel and streamline analysis. Samples were stored at the analytical

**Fig. 2.** Environmental canister with tubing and inlet attached to paddock fence at the University of Queensland Gatton campus, Gatton, Queensland. This picture also showcases the pasture where the animals were kept.

laboratory from the collection day onward, at room temperature, and were processed within 1 week of collection.

During the pilot sampling period, the cannisters removed from the animals were taken to the Central Analytical Laboratory (CAL) at the UQ Gatton Campus. In this laboratory, the individual gas subsamples were collected in triplicates from each canister into the glass vials that were then transported to the UQ St Lucia campus for analysis. When the samples were analysed, the $SF_6$ concentrations were extremely high. Following discussion with the analytical team, it was determined that because the permeation tubes had been filled with $SF_6$ in the Gatton laboratory previously, there were contamination problems. The background concentration of $SF_6$ in the Gatton laboratory was very high, affecting all results. It was recommended that future subsampling from canisters to glass vials be conducted in an open space to ensure good airflow. In the subsequent trial periods, canisters were subsampled crush side in the cattle yards, where air flow was adequate and no $SF_6$ contamination issues occured. Results from these trials were within expected value ranges (Table 1). The contamined samples were all discarded and their results were not used in the calculation of average methane emissions reported here.

## Gas chromatography analysis of $SF_6$, $CH_4$, and $CO_2$

The presence of $SF_6$ gas was analysed on an Environmental Shimadzu QP2010 Ultra gas chromatography–electron capture detector (GC-ECD) system (Kyoto, Japan), coupled with a Shimadzu HS20 autosampler. The pressure of gas samples and the atmosphere were manually equalised before loading the glass vials in the HS20 autosampler. The glass vials were pressurised by heating to 200°C before being injected directly into the GC-ECD system. Sample line temperature and transfer line temperature were set at 150°C. Ultrapure nitrogen (999.99%) was used as carrier gas at a total flow of 8 mL/min. The separation of gas components was performed on a ShinCarbon ST Micropacked gas chromatography column, with specifications of 100/120, 2 m, 1 mm ID (Restek, USA). The gradient program started at 145°C for 1.5 min, then the temperature was ramped up to 150°C at a rate of 0.4°C/min. The ECD detector was set at 300°C with 10 mL/min makeup gas (ultrapure nitrogen, 999.99%). The $SF_6$ gas was eluted at 8.7 min (Fig. 3).

The presence of $CH_4$ and $CO_2$ gases on each sample was analysed on an Environmental Shimadzu Nexis 2030 gas chromatography–flame ionisation detector (GC-FID) system (Kyoto, Japan), coupled with a Shimadzu HS20NX autosampler. The pressure of gas samples and the atmosphere were manually equalised before being loaded in the HS20NX autosampler. The gas vials were pressurised by heating to 120°C before being injected directly into the GC-FID system. Sample line temperature and transfer line temperature were set at 120°C. Ultrapure helium was used as carrier gas at a total flow of 15 mL/min. The separation of gas components was initially performed on HP-PlotQ column (30 m, 0.53 mm, 40 μm). From zero to 2.5 min, a valve directed $CH_4$ and other gases through an RT-MS5A column (30 m, 0.53 mm, 50 μm), where $CH_4$ was eluted and detected by FID at 6.9 min. After 2.5 min, the valve directed $CO_2$ to a heated nickel catalyst where $CO_2$ was methanised to $CH_4$ and then detected by FID at 3.1 min (Fig. 4). The gradient program started at 40°C for 7.5 min, then the temperature was ramped up to 60°C at a rate of 4.0°C/min and held for 1.5 min. The FID detector was set at 400°C with 15 mL/min makeup gas (ultrapure nitrogen, 999.99%). The $CO_2$ gas was eluted at 3.1 min and the $CH_4$ was eluted at 6.9 min (Fig. 4). Three calibration curves were established to quantify $SF_6$, $CH_4$, and $CO_2$ concentrations in the samples. The calibration ranges were 3–500 ppt for $SF_6$, 1.12–500 ppm for $CH_4$, and 200–1000 ppm for $CO_2$. These calibration curves were utilised to accurately determine the concentrations of $SF_6$, $CH_4$, and $CO_2$ in the samples.

Detection of $CH_4$, $CO_2$, and $SF_6$ in the gas sampled from the studied animals allowed the calculation of individual methane emissions and $CO_2$ on a per-day basis. The individual daily estimates of emissions are based on the average of five

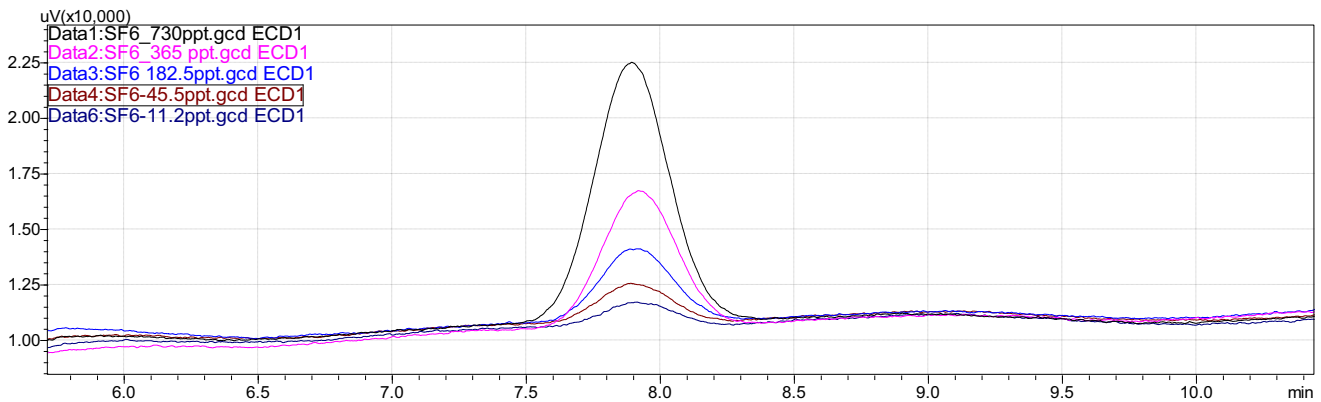


**Fig. 3.** A gas chromatography–electron capture detector was used to measure the $SF_6$ gas.

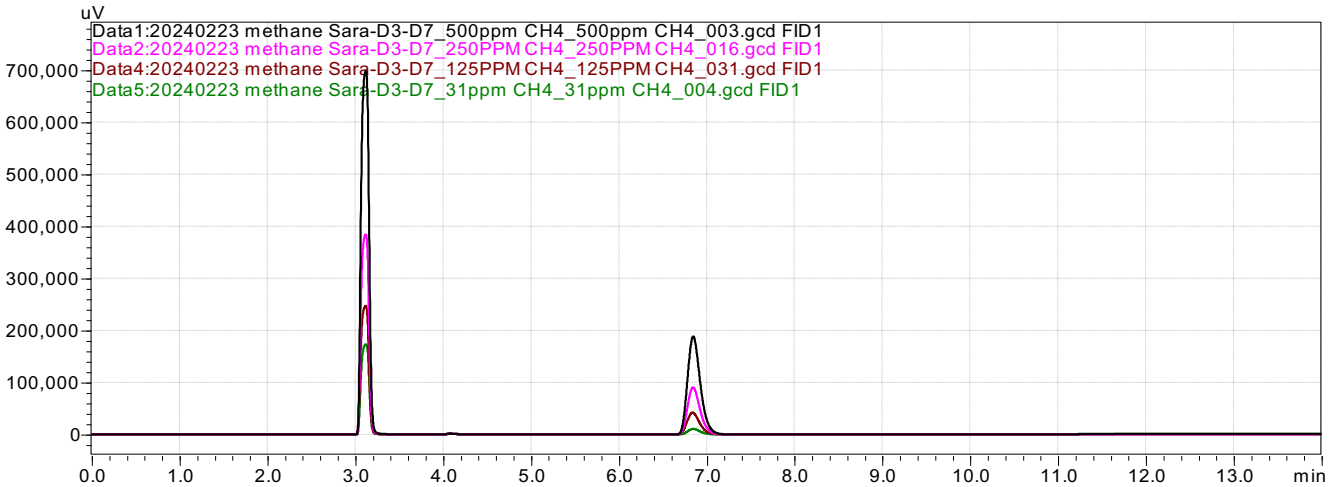


**Fig. 4.** A gas chromatography–flame ionization detector was used to measure $CH_4$ and $CO_2$.

samples, including five consecutive 24-h periods of sampling. Although some missing data probably are inevitable, it was possible to average over at least three 24-h periods of sampling for each animal.

## Estimated enteric methane emissions

After implementing the $SF_6$ technique, the estimated amounts of enteric methane emissions for the 38 Brahman heifers measured were within the reported ranges for cattle (Table 1). The data reported here were captured at the first sampling period, when 38 animals were measured successfully, which occurred when the pregnant group of heifers ($n$ = 20) was 4 months pregnant (the control group was not pregnant, $n$ = 18). For all 38 animals, we were able to collect data. Having both pregnant and non-pregnant heifers in a cohort is a representation of the reality of breeding herds, especially for heifers in their first season. These 38 heifers emitted 289 g/day of methane (s.e. ±17), on average, across all sampling periods. To calculate this average, we had 162 individual methane measurements, as we followed these heifers throughout their first reproductive cycle.

Brahman heifers were genotyped to estimate their *Bos indicus* content. DNA genotypes from the Illumina Bead Chip assay commercialised by NEOGEN Australasia were generated and used to estimate the breed composition. Their *Bos indicus* content was on average 87%, as estimated using reported methods (Hayes *et al.* 2023). This range of *Bos indicus* content is expected and representative of the genetic makeup of commercial Brahman herds, typical of northern Australia (Hayes *et al.* 2023).

A challenge for grazing experiments is the fact they cannot measure dry-matter intake (DMI). There are methods for estimating DMI such as using the metabolic weight of animals or using external markers, such as yttrium, for example, and performing fecal sample analyses (Davies and Gouveia 2006). Estimating DMI provides an opportunity to report methane emissions per DMI, so that grazing experiments might be compared with feedlot trials by using the same emissions metric. Still, estimated DMI will not be identical to measured DMI.

## Final considerations

Most research has focused on short-term monitoring of methane emissions in response to dietary interventions or at specific stages of production. However, extended-duration measurement is essential to capture temporal variation in emissions across key physiological states, such as pregnancy and lactation. This report has described a practical and robust approach for using $SF_6$ methodology to measure methane emissions for extended durations under grazing conditions, which is critical for global beef enterprises. Additionally, this report has highlighted the critical importance of also considering practical aspects when planning research using these techniques in real-world research settings. In particular, careful consideration of the duration of the experiment is recommend, because it directly influences the required $SF_6$ charge for the permeation tubes. Longer experiments require either a higher amount of $SF_6$ charge within each permeation tube, or a slower release rate produced by adapting the permeation tube specifications. Thorough preparation, including developing a detailed standard operating procedure (SOP), and ensuring comprehensive training of the research team, is also strongly recommended. Consulting with experienced technicians is also advised to enhance the effectiveness of the preparation. Ensuring team members gain hands-on experience with all equipment, such as halters and saddles, and understand gas sampling techniques, contamination risks and equipment readiness, is essential before data collection begins.

The methodology described here can be readily replicated in other tropical environments. The calibration and permeation tube preparation steps can be reproduced in most laboratory settings equipped with liquid nitrogen, an analytical balance, an oven, and basic gas-handling tools. Accurate methane quantification requires access to a gas chromatograph, an equipment often available in research institutions. Samples can be transported from farms to research institutions, either in the original canisters or as subsamples in glass vials; in the latter case, analysis within 1 week of collection is recommended to ensure precision. Important determinants of successful implementation are ensuring animal habituation and appropriately scaling the equipment to the animal's size and management system. With these considerations, the $SF_6$ tracer gas technique can be successfully adapted for a wide range of tropical production systems, from extensive grazing to more intensive operations, provided that animals can be handled daily at a crush during the measurement period.

Ultimately, the $SF_6$ technique provides an excellent approach for measuring methane in grazing conditions, which is essential for understanding cow–calf operations globally, because breeding is not carried out in feedlots. Once $SF_6$ measurement capability is established, it enables the investigation of more nuanced and biologically relevant questions related to enteric methane emissions in extensively managed cattle.

The $SF_6$ technique and its application to grazing systems will be ideal for understanding the impact of the cows' reproductive cycle, including pregnancy and lactation, on enteric methane emissions. Although previous research have identified seasonal variations for methane emissions from breeding cows, the physiological effects, such as pregnancy, remain unaccounted for (Martinez-Fernandez *et al.* 2020). Pregnancy is known to affect some of the key parameters, such as feed intake and weight gain, which are also known to be associated with methane emissions (Congio *et al.* 2023). Thus, it is logical to hypothesise that methane emissions will vary during the annual reproductive cycle of breeding herds. The ongoing research for the LESTR project will investigate the effect of heifer pregnancy on methane emissions.

The future for this methodology is very promising; it will be an important option in the toolbox of the environmentally aware cattle researcher. New prediction equations, based on the best available information, are key to creating sustainable livestock industries (Congio *et al.* 2023). It is noteworthy that methane has a short half-life in the atmosphere. Reducing methane emissions can have a relatively quick and positive impact on global warming (Saunois *et al.* 2020). Therefore, measuring methane accurately has important consequences.

## Supplementary material

Supplementary material is available online.

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**Data availability.** The data generated for the LESTR project are jointly owned by UQ and MLA, and may be available for research purposes upon request.

**Conflicts of interest.** Dr Kieren McCosker is an Associate Editor for Animal Production Science but was not involved in the peer review or decision-making process for this paper. The authors have no further conflicts of interest to declare.

**Declaration of funding.** This project was funded by Meat and Livestock Australia (MLA) in partnership with the University of Queensland, as a donor company (MDC funding, grant number *P.PSH.2010*).

**Acknowledgements.** The LESTR project is a large and multidisciplinary project funded by Meat and Livestock Australia (MLA). The authors acknowledge all collaborators involved in the LESTR project. We thank all the Queensland Animal Science Precinct (QASP) staff for their work on this project. We acknowledge the essential contributions of Christian Forster and Dan-Nar-Leigh Crouch from the Science Faculty workshop in manufacturing the permeation tubes and all the required bespoke equipment for the LESTR pregnancy trial, including fixing the halters and measuring the airflow to standardise the sampling.

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