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# BUILDING A REFERENCE POPULATION TO ENABLE GENOMIC SELECTION FOR METHANE RELATED TRAITS IN BEEF CATTLE

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## SUMMARY

Breeding for cattle that produce lower methane emissions, whilst maintaining or improving productivity has the potential to be a permanent and cumulative strategy for the minimisation of greenhouse gas emissions. Emissions traits in beef cattle are heritable and previous studies have shown that breeding for lower methane emissions is possible. However, a large number of animals measured for emissions traits, simultaneously with production traits, is required to underpin EBVs to allow for effective selection. This paper discusses key measurement technologies, concepts underpinning reference population design and summarises the current reference population for temperate beef cattle in Australia.

## INTRODUCTION

Enteric methane emissions from ruminants, such as cattle, contributes up to 80% of agricultural greenhouse gas (GHG) emissions. Hence, reducing methane production is essential in any GHG emissions reduction strategy. An added benefit of this is that selection for reduced methane also provides opportunities to improve feed efficiency as 6-10% of potential energy is lost from the animal as methane. Previous studies have shown that traits related to methane emissions in beef cattle are heritable; that breeding for lower methane emissions is possible and that average emissions may be reduced by ~0.5-2.5% pa (with an average across studies of ~1% pa) (Barwick *et al.* 2019; Fennessy *et al.* 2018). Some opportunities for the management of methane emissions are available by improving productivity, especially reproductive performance and days to slaughter. However, a large volume of animals measured for greenhouse gas emission traits, simultaneously with production traits, is required to underpin EBVs to allow for balanced selection. Previous studies have summarised the technologies available for methane measurements. This study will expand on these with a perspective on measurement technologies for individual animal recording for the construction of a reference population to underpin selection tools. Measurement tools, which animals to sample and ways to value methane to enable balanced selection will be discussed.

## METHODS FOR RECORDING METHANE

For animal breeding purposes the overall goal is to decrease overall lifetime methane production. This section will discuss alternative ways to record methane and their ability to estimate lifetime methane. Methane has been measured with a variety of tools for the past ~100 years, with tools ranging from closed circuit respiration chambers to sniffer-based sensors. Initial studies have focused on mean daily methane outputs for primarily animal science studies that have attempted to quantify the differences between treatments in controlled experiments.

**Closed circuit respiration chambers.** Often viewed as the gold standard for methane measurement, respiration chambers (RC) measure methane over a relatively short period of time (24-48 hrs) very accurately. The major challenge for this method is the short duration (24 hrs), ability to get large numbers of animals recorded and the cost of the system. Generally, chamber-based systems are unlikely to be the key measurement technologies for large scale reference data generation. The largest study of beef cattle recorded in chambers is Donaghue *et al.* (2016) where 1,046 Angus animals were cycled through RC and heritability and GEBV estimated. Heritabilities were generally moderate (~0.3) and GEBV accuracies of 0.5-0.6 achieved within the population (Hayes *et al*. 2016).

**Modified SF6.** An alternative to respiratory chambers is the modified sulphur hexafluoride (SF6) tracer method. It also gives an accurate measure of total methane released over a short period of time. Previous studies have shown that SF6 has a high correlation with chamber-based measures (0.83, Manzanilla-Pech *et al*. 2021). The challenges for SF6 are that the method is costly and labour intensive per animal recorded and is often not used for large scale animal recording (100's not 1000's of animals recorded). The SF6 tracer is also a potent greenhouse gas and collection of permeation tubes post recording is challenging for processors. One of the largest examples of using the SF6 method for reference population data recording is 463 cows measured repeatedly using this system at the Department of Economics Development, Jobs, Precincts and Regions, Ellinbank (National Centre for Dairy Research and Development) from 2013 to 2017 (Richardson *et al.* 2021). Heritability from this data was reported to be 0.2 (SE 0.11) (Manzanilla-Pech *et al*. 2021). Whilst this is a start, much larger numbers of animals are required, it is unlikely that the use of SF6 will allow for reference populations with thousands of animals to be recorded in beef cattle.

**GreenFeed.** In the past decade one of the most common methods for recording methane on beef cattle is using GreenFeed Emissions Monitors. GreenFeed is an automated feeder system that monitors $CH_4$ and $CO_2$ fluxes from breath and eructation gas from cattle that was created as an alternative to SF6 (Hammond *et al*. 2016). These machines use multiple short methane samples (2-3mins) captured over a testing period. A major challenge for this type of system is that animal access is voluntary so careful management is required to achieve high rates of animal visitation over a trial period. In intensive situations units can be placed at a ratio of 1 unit to 40 animals and in more extensive situations 1:20 is more suitable. This equates to approximately 240 animals per unit annually if recording is for 28 days. Whilst this is considerably more than SF6, GreenFeed units are expensive and still require some labour to manage the units over a trial period. Recording of methane with GreenFeed is still expensive with more cost-effective alternatives required.

**Methane sensors (sniffers).** Methane concentration (ppm) can be measured with standalone methane sensors. Examples of these have been described previously with two common sniffers being: the nondispersive infrared $CH_4$ sensor (Guardian NG, Edinburgh Instruments Ltd.) and the portable Fourier transform infrared Gasmet DX-4000 (Gasmet, Gasmet Technologies Oy). The correlation between respiration chamber $CH_4$ emissions and sniffers has been reported to be high under controlled conditions (0.77, Manzanilla-Pech *et al.* 2021). However, a challenge for sniffer systems is to validate their accuracy in extensive, outdoor conditions. Another limitation for sniffer systems is that methane concentration is measured (ppm) and a conversion is required to understand the volume of methane the animal has emitted. Sniffer-based recording protocols have been designed for recording dairy cattle; however, the extensive nature of beef cattle production has limited their use in recording programs.

**Portable (or semi portable) Accumulation Chambers (PAC).** These have been used to spot sample animals and larger, less portable, versions have also been proposed and tested for use in beef cattle in New Zealand. Animals are confined to a sealed chamber for a given time and the amount of methane is recorded. The volume of methane produced is then a function of initial methane, final methane and the volume of the chamber (Goopy *et al.* 2016). One of the challenges for the PAC system, and other spot sampling methods, is the adjustment for the time since the animal had eaten. Methane follows a diurnal pattern as the animal grazes and ruminates and this needs to be considered to improve precision of methane measurements from spot sampling methods. The advantages for sniffer and PAC methods are that they enable high volume recording of large cohorts of animals, whilst they may not be suitable for absolute methane (in g/day) they may provide useful tools to measure animals as part of the reference population for emissions traits.

## REFERENCE POPULATION DESIGN

A reference population is simply animals recorded for a trait, that have known relationships to selection candidates (either derived by pedigree or genomics) and known management information. The creation of a reference population for methane has 3 key principles: 1) build accuracy of selection, 2) understand correlations between methane and other important traits and 3) provide tools to breeders to select for improved methane in a large part of the population (diversity).

Previous studies have shown that genomic prediction accuracy is impacted by the relationship within and to the reference population (Pszczola *et al*. 2012). When the reference population is small the key is to record animals closely related to selection candidates, as the reference population grows the baseline accuracy of prediction increases and relationship to the reference becomes less important (Kamprasert *et al*. 2024). In beef cattle, the diversity of breeds used for production make it challenging to create reference datasets for all breeds.

To ensure balanced selection occurs, animals with key traits recorded on them should be prioritised for methane recording so that correlations between existing traits and new traits like methane can be estimated. This also enables the benefits of multi-trait selection to be realised, especially allowing for the impact of indirect selection for other traits like reproduction, productivity, welfare and feed intake on methane outputs of the breeding program to be estimated. If multiple methods are used to measure methane careful consideration is required as to whether alternative ways to measure methane require separation as different traits. Other traits like microbiome profiles and volatile fatty acid content from rumen fluid or saliva have also been suggested as proxies for methane outputs. A further complication for methane is that animals can be recorded in the feedlot or at pasture and this may further require additional trait definitions.

## CURRENT REFERENCE POPULATION FOR TEMPERATE BEEF CATTLE

In Australia, a reference population for methane traits is currently being generated using GreenFeed emissions monitoring units to record animals. In the construction of this reference population there has been a focus on recording close relatives of key sires by utilising two key populations in temperate Australia, the Southern Multi-Breed project (6,000 steers and heifers) (Walmsley *et al*. 2021) and the Angus Sire Benchmarking project (2,000 steers) (Parnell *et al*. 2019). Overlaying on existing projects has meant that other key traits like birth, growth, carcase and reproductive performance have also been recorded on the same animals. Table 1 illustrates the animals that have been recorded to date. These animals were from breeds that were chosen based on their importance to the beef herd in temperate Australia (Walmsley *et al*. 2021).

**Table 1. Number of records per individual and per breed in the reference population**

| **Breed** | **Feedlot (steers)** | | **Pasture (heifers)** | |
|---|---|---|---|---|
| | Individuals | Records | Individuals | Records |
| Angus | 1,048 | 65,591 | 509 | 33,751 |
| Charolais | 155 | 8,333 | 129 | 7,595 |
| Hereford | 329 | 20,754 | 281 | 15,022 |
| Shorthorn | 181 | 7,360 | 133 | 9,239 |
| Wagyu | 166 | 6,976 | 113 | 5,882 |
| Brahman | 25 | 291 | 43 | 698 |
| BrahmanX | 87 | 1,958 | 141 | 4,636 |
| Total | 1,991 | 111,263 | 1,349 | 76,823 |

The mean performance for animals at pasture and in the feedlot has been similar, as shown by Table 2. Slightly higher means and standard errors observed in the feedlot, which is expected given the steers are generally heavier than heifers at point of recording.

**Table 2. Means for methane and carbon dioxide for animals in the reference population (SE)**

| Gas (g/day) | Feedlot | Pasture | Both |
|---|---|---|---|
| CH4 | 168 (77) | 166 (57) | 167 (69) |
| $CO^2$ | 9711 (2479) | 7038 (1737) | 8617 (2567) |

## CONCLUSION- TRAIT DEFINITIONS AND VALUING METHANE

The final piece of the puzzle for including methane in the breeding program is including it in a selection index with other key traits. Methane can be minimised at the breeding program level by either per head or per kg product (van der Werf 2023). Each is essentially a breeding objective; a further question is often asked when considering methane; should methane output be optimised per unit of feed intake (methane yield) or per unit of production (methane intensity)? The main impact this has on the development of the reference population for methane is regarding the other traits that are to be recorded. If methane output per kg of feed is used, then it is essential for daily feed intake to be recorded on animals which is an added challenge for recording methane yield on pasture.

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