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THE GREEN COW PROJECT: DEVELOPING A METHANE EFFICIENCY GENOMIC BREEDING VALUE FOR THE AUSTRALIAN DAIRY INDUSTRY

C.J. Vander Jagt[1], F. Almasi[1], B.J. Sepulveda[1], L.C. Marett[1,5], S.R.O. Williams[1], R. Xiang[1], S. Bolormaa[1], M. Shaffer[2], J. Penry[3], C. Hill[3], D. Gibson[4], J.L. Jacobs[1,5] and J.E. Pryce[1,5]

[1] Agriculture Victoria Research, Victoria, Australia
[2] DataGene Ltd, Bundoora Victoria, Australia
[3] Dairy Australia, South Bank Victoria, Australia
[4] Gardiner Foundation, South Bank Victoria, Australia
[5] La Trobe University, Bundoora Victoria, Australia

SUMMARY

Methane (CH_4) represents a critical target for near-term climate mitigation and the Australian dairy industry is under increasing pressure to further reduce its emissions footprint, particularly enteric methane emissions (EME). While management practices and feed additives show promise in reducing EME, the temporary nature of additives and public concerns surrounding their use highlight the need for more sustainable and permanent solutions. The Green Cow project, led by Agriculture Victoria Research (AVR), aims to address this challenge by developing a multi-trait Methane Efficiency Australian Breeding Value (ME-ABV) for the dairy industry. By leveraging multiple selection criteria (proxy traits) in a multi-trait model, the ME-ABV will initially be integrated into the Australian dairy Sustainability Index (SI) before being released as a standalone breeding value. This approach provides a practical and publicly acceptable solution to reducing EME in the dairy sector while simultaneously improving feed efficiency and maintaining productivity.

INTRODUCTION

Methane (CH_4) is a potent greenhouse gas with a warming potential 80 times greater than CO_2 over 20 years, making its reduction crucial for near-term climate mitigation (Black *et al*. 2021). The Australian dairy industry has one of the world's best emissions footprints, averaging 0.93 kg CO_2e/kg Fat and Protein Corrected Milk (Dairy Australia 2023), it faces growing pressure to further cut gross EME (g/day) and EME-intensity (g/kg milk) for environmental and social reasons.

Various strategies can reduce gross EME and EME-intensity, including improved management practices and feed additives. While feed additives have been shown to reduce EME, their effect is temporary (Black *et al.* 2021). Recent controversy surrounding the use of 3-NOP in the dairy and beef livestock industries (Dalton 2024) highlight a significant challenge: public perception and acceptance of feed additives. The most effective and broadly supported long-term solution is likely through genetics, as genetic gains are permanent and cumulative, with EME reductions improving progressively with each generation. Moreover, genetic approaches could complement other strategies, offering the potential for a stacked approach to maximise impact. Genomic selection is a cost-effective solution to address this challenge, requiring only EME measurements (or proxy measures) from genotyped cows to develop a genomic breeding value.

In 2022 DataGene, the organisation responsible for providing breeding values to the Australian dairy industry, introduced the SI (Nguyen *et al.* 2023, Richardson *et al.* 2021), which places additional emphasis on green traits such as feed efficiency, longevity, and milk production. This index is projected to reduce EME-intensity by 5 to 10% by 2050 (Richardson *et al.* 2022). However, incorporating EME into the breeding objective could achieve even greater reductions, with an estimated up to 30% reduction in EME-intensity by 2050 (Richardson *et al.* 2022). The Green Cow project, led by AVR, aims to address this challenge by developing a Methane Efficiency Australian Breeding Value (ME-ABV) for the Australian dairy industry. The Green Cow project is a 4-year

project that commenced in December 2023. Co-developed with the Victorian Dairy Innovation Agreement (VDIA, a collaboration between Gardiner Foundation, Dairy Australia and AVR) and DataGene, the project will provide farmers with a powerful tool to reduce emissions through genomic selection. Central to this work are key national and international collaborations including VDIA's DairyBio and DairyFeedbase programs, the ZNE-Ag CRC and the GMH, an international initiative dedicated to reducing methane emissions. This paper details these efforts and the project plan, and highlights progress made so far.

MATERIALS AND METHODS

The Green Cow project will take a multi-trait approach to develop the ME-ABV, using EME proxy-data from microbiome profiling (ruminal and faecal), milk mid-infrared spectrometry (MIR) and CH_4 sniffers. Validation of these EME proxies against the at-pasture baseline EME measuring approach, SF_6 tracer technique, is ongoing. A multi-trait approach will provide a more accurate and robust prediction by leveraging trait correlations and capturing different aspects of EME biology. Additionally, using multiple phenotyping methods reduces the risk of systematic errors that might arise from relying solely on a single approach. Since genotype-by-environment interactions are expected to significantly influence trait expression, utilising a diverse range of phenotyping techniques allows us to better capture these interactions, leading to more robust genomic predictions of EME across varying environmental conditions. This approach also ensures that breeding programs remain resilient to potential failures or inaccuracies from any single data source.

The current prioritised order for phenotyping CH_4 is as follows:

1) **Rumen microbiome:** recent studies suggest that the ruminal microbiome is heritable and strongly correlated with EME (González-Recio *et al.* 2023). Additionally, it offers the potential to link EME data from other countries for improved prediction (Sepulveda 2024), as all EME traits primarily originate from the rumen microbiota. However, there are challenges such as recruiting farmers to allow ruminal sampling, ensuring farms have appropriate cattle restraints, and securing enough trained personnel to carry out the sampling. Furthermore, the project may benefit from potential access to collaborative international microbiome data through the GMH.
2) **MIR:** milk composition measured by MIR light absorption can be used to predict traits such as EME (Rojas de Oliveira *et al*. 2024). It is cost-effective, non-invasive and suitable for large-scale phenotyping and already used for reproductive outcomes (Ho and Pryce 2020, DataGene 2022) and the Canadian Methane Efficiency breeding value (Rojas de Oliveira *et al.* 2024). If validated in Australia, it could maintain the ME-ABV cost-effectively. As MIR analysis is already routinely conducted at herd test centres, integrating it for methane phenotyping would minimise additional costs and logistical challenges while enabling large-scale, ongoing data collection to support the ME-ABV's long-term sustainability. However, reliability may be affected by the diversity of Australian dairy systems due to genotype-by-environment interactions.
3) **CH_4 sniffers:** these sensors measure CH_4 concentrations in the air near cows, offering a non-invasive, cost-effective method for large-scale EME phenotyping. ArcoFlex CH_4 sniffers have been installed in feed bins at AVR's Ellinbank SmartFarm, measuring CH_4 ppm every 10 seconds while cows are in the milking stall. If validated, these sensors could enable scalable EME phenotyping, leveraging the existing network of farms where these devices are already installed and operational. Other CH_4 sniffers in Australia, including La Trobe University's devices, may be used in the Green Cow project. Their approach estimates CH_4 from ruminal gas by calculating oxygen absence as a dilution factor.
4) **Faecal microbiome:** the faecal microbiome has shown potential as a useful proxy for predicting daily methane emissions in beef cattle (Manafiazar *et al*. 2021). If the faecal

microbiome proves to be predictive of EME in dairy cows, it might not be as accurate as the ruminal microbiome but could allow for easier phenotyping, albeit with the need for a much larger sample size.

The Green Cow project will target specific types of farms to optimise participation and outcomes. The priority is research farms, as most herd members are already genotyped, reducing costs and saving time. These farms are more likely to have staff available for animal handling and can better tolerate production disruptions. Additionally, they typically possess appropriate cattle restraints, which are essential for ruminal sampling. The second focus is Ginfo farms (Ginfo is Australia's national reference herd for genetic information), where most animals are genotyped and detailed animal records are maintained. These farms have a strong history of research participation and established relationships with AVR, ensuring smooth collaboration. A third target is farms that participated in the VDIA's DairyBio 10K bloods project (VDIA 2025). These herds are genotyped, have comprehensive records, and farmers are willing to engage in research. If the ZNE-Ag CRC project, which aims to determine whether blood volatile fatty acid profiles can predict CH_4 emissions, proves successful, these farms could contribute an additional 10,000 phenotypes. Lastly, if the ArcoFlex CH_4 sniffers validate against SF_6, the network of ArcoFlex farms could provide ~6,000 EME phenotypes from diverse environments, with the potential for more over time. If validated, ArcoFlex farms will become the top priority for phenotyping.

As more phenotypes are collected, they will be incorporated into EME genomic prediction models to refine and develop iterations of the ME-ABV. This ABV will be introduced in phases, starting with inclusion in the SI. Once higher reliability is achieved, it will be released as a standalone ME-ABV. By 2030, the goal is to integrate the ME-ABV into the main industry index, the Balanced Performance Index. Reliability will improve as more animals are phenotyped, either through direct EME measurements or proxies. Achieving the typical reliability of a standalone ABV (≥30%) requires data from 10,000 to 15,000 animals. However taking a multi-trait model approach, which combines multiple interconnected measurements, may reduce the number of phenotypes needed to reach this level of reliability (Song *et al.* 2020). Given the high cost of phenotyping, a stepwise approach is essential. Introducing the ME-ABV through the SI first, combined with the use of multi-trait modelling, ensures the fastest and most impactful route for delivering benefits to the industry.

RESULTS AND DISCUSSION

Progress to date. Since commencing December 2023, the Green Cow project has progressed significantly. Focus has been on analysing existing EME-related data from experiments conducted at the Ellinbank SmartFarm, integrating it with ongoing data collection. This has enhanced the reliability of the EME genomic breeding value (based on SF_6) developed through the VDIA's DairyBio program, raising it from below 0.10 to around 0.15. Recent incorporation of MIR and microbiome phenotypes has further improved the model's reliability to ~0.24, potentially increasing its resilience to genotype-by-environment interactions. Despite these improvements, results remain inconsistent across different analysis scenarios, highlighting significant data noise and underscoring the need for more phenotypic data to reduce variability and stabilise the genomic prediction model. The model requires testing in diverse environments and farming systems beyond Ellinbank.

Efforts to validate the ArcoFlex CH_4 sniffers at the Ellinbank SmartFarm have advanced, with 50 devices operational since February 2024. The team has matched sniffer measurements to individual cows and estimated the heritability and repeatability of the data. Work to determine the correlation of the sniffer data to SF_6 data is still underway. While initial results highlight the sensors' potential for predicting EME, further validation using additional SF_6 data is required.

Early analysis of 25 animals revealed that faecal metagenome features had phenotypic correlations of up to 0.40 ± 0.18 with EME production (g/d) and up to 0.62 ± 0.12 with features from the ruminal metagenome (Sepulveda *et al.* 2025). While not as strong as the associations

observed with ruminal features (genetic correlations between 0.59-0.93, González-Recio *et al.* 2023), it remains highly promising. Notably, DNA extraction and sequencing from ~2,000 additional ruminal and faecal samples, is underway (to conclude in 2025). This expanded dataset will enhance the reliability and stability of the genomic prediction model(s). The team has also developed scalable protocols for faecal microbiome sampling on commercial farms, offering a non-invasive and efficient method for phenotyping. While activities beyond Ellinbank are yet to occur, these preparations position the project for rapid expansion in the near future.

CONCLUSION

Designing a reference population for EME is challenging. The Australian dairy industry is addressing this through a multi-trait approach to developing the ME-ABV, aiming to deliver more accurate and robust predictions by capturing various aspects of EME biology. Close to 1,000 SF_6 CH_4 records from AVR's Ellinbank SmartFarm support validation of other selection criteria. Higher-throughput phenotypes, such as MIR, show promise as selection criteria but require external validation beyond Ellinbank. The ruminal microbiome is a validated proxy trait and may capture genotype-by-environment interactions, a key challenge in developing EME breeding values. Scaling ruminal microbiome data for reliable breeding values is difficult. Early research into the faecal microbiome and CH_4 sniffers is also encouraging, and, if validated, these methods could provide a substantial number of phenotypes. Initial development of the ME-ABV has shown that reliability levels fluctuate depending on the analysis method used, highlighting significant data noise and emphasising the need for more phenotypic data. Plans are underway to expand phenotyping efforts beyond Ellinbank in 2025, which should strengthen these models and reduce data variability. In addition, global and domestic collaborations through initiatives such as the GMH and ZNE-ag CRC will improve access to additional data and support the development of EME breeding values.

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