

TOWARDS SELECTING FOR LOWER METHANE SHEEP

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SUMMARY

The aim of this project is to enable Australian sheep breeders to select for reduced enteric methane emission, allowing industry to achieve a permanent and cumulative 4.2% reduction (0.8 MtCO₂e) in methane emissions from sheep by 2030 and 15% reduction (2.6 MtCO₂e) by 2040. A mobile field test using portable accumulation chambers for measuring methane emissions on 10,000 sheep across research and breeder flocks is being rolled out. Five thousand sheep will have feed intake, rumen microbiome and volatile fatty acids profiles recorded to better understand and improve CH₄ emission predictions. Combined with their genotype information, this data will allow genomic prediction of breeding values on selection candidates. Work to date has demonstrated that the protocol for methane measurement is robust and the preliminary data gathered has shown that there is sufficient variation in methane production among animals to enable selection for reduced methane production. Different technologies used to measure emissions data are highly correlated.

INTRODUCTION

Meat & Livestock Australia (MLA) has set a Carbon Neutral Target for 2030 and has initiated a number of programs to reduce the carbon footprint from the Australian livestock industry, including an Emissions Avoidance Program (EAP). The EAP aims to provide various strategies to mitigate methane, including feed additives, new forages and selection for low methane livestock. A program for selecting for more methane efficient sheep was initiated in collaboration between the University of New England, the NSW Department of Primary Industries and MLA. The aim of the project is to collect a large number of phenotypes for methane production and feed intake and to use that data to 1) estimate genetic parameters, including genetic correlations of these traits with production and reproduction traits, and 2) to predict genomically informed breeding values of young rams and ewes in order to select for these traits in studs and commercial enterprises.

Previous work in New Zealand and Australia has shown that there is variation between sheep in how much methane they produce, and this variation is heritable (Pickering *et al.* 2015), i.e. it is possible to change the average methane output via selection. Further modelling work shows that selection for methane alongside selection for other traits can simultaneously improve methane and production efficiency (Robinson and Oddy 2016; Rowe *et al.* 2019). Methane efficiency in dairy cattle has been shown to be positively correlated with feed efficiency, measured as residual feed intake (Manzanilla-Pech *et al.* 2022), but results in sheep have showed a less clear relationship (Muir *et al.* 2020). Furthermore, recent studies have shown that additional information about the methane phenotype of sheep can be obtained from VFA and rumen microbiota (Rowe *et al.* 2019; Ross *et al.* 2020). Based on reliable genetic parameters selection index theory can help clarify the best strategy for selection on productivity as well as feed- and methane efficiency.

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Various trait definitions have been proposed for selection and for assessing the objective of a breeding program for methane (Johnson *et al.* 2022). We propose to simply measure methane output per head, and feed intake, and optimise a breeding objective via bioeconomic modelling, where minimizing the methane production per unit of product is likely to be an overall objective.

Sustainable genetic improvement in methane efficiency of the national sheep flock will require ongoing trait measurement and a data pipeline to turn this information into selection criteria for breeders. Developing a reference population with methane measured animals will allow genomic prediction of breeding value as well as provide more accurate estimation of genetic parameters, including correlations with other traits needed to accurately optimize selection across all economically relevant traits. This requires the development and validation of estimated breeding values and routine measurement of the trait phenotype in a model akin to the MLA resource flock. Thus, the project aims to collect methane and feed intake data on 10,000 and 5,000 animals over 5 years. Lambs from the MLA Resource Flock will be measured as well as ewes in breeders' flocks. The study commenced in 2022. This paper reports on the measurement protocols and some early results of lambs and ewe measurements.

MATERIALS AND METHODS

Methane measurement. Methods and protocols for practical use of portable chambers to measure methane production by sheep are well established and have been validated (Robinson *et al.* 2020). The animals to be measured are held on feed and water until a known time prior to measurement, generally 1 hour. Measurement of gases takes place over a period of 40-60 minutes per animal with methane measurement, oxygen consumption and carbon dioxide output all recorded.

Animals are weighed as close to measurement as possible and a sample of rumen fluid is obtained by stomach tube, after measurement of gas exchange. Rumen fluid is stored for subsequent analysis of volatile fatty acid (VFA) composition and rumen microbial composition.

For this preliminary study, 500 lambs from the MLA Resource Flock cohort were measured at the Kirby Research Station in Armidale (NSW). The Resource Flock design and the proportion of animals from the various sheep breed types is described by van der Werf *et al.* (2010). Animals were born in October 2021 and measured in early April 2022 at approximately 6 months of age. Their average body weights were 29.2 (SD = 6.3) kg. Sheep were removed from the paddock at the beginning of each day and kept in a holding yard with access to feed and water. There were up to four runs each day with 12 animals in each run housed in individual methane accumulation chambers (48 animals in total per day). Lambs were taken off feed 1 hr prior to entering the chambers and gas measurements were taken at 25 and 50 minutes after entering the chamber.

Approximately 500 adult (2016 and 2017 drop) ewes from the Merino Lifetime Productivity (MLP) project were measured at "The Vale", Temora from 31 October -5 November 2022. Their average body weights were 67.6 (SD = 10.3) kg. A total of 84 sheep were removed from the paddock at the beginning of each day and kept in a holding yard with access to feed and water. Each day there were seven runs of twelve animals (84 animals in total each day). Ewes were taken off feed 1 hr prior to entering the chambers and gas measurement on ewes were taken at 20 and 40 minutes after entering the chamber.

For both groups of animals, measurements taken included methane (CH₄, using a micro-Flame Ionisation Detector), CO₂ and O₂ (both using a FoxBox-Pro). Data obtained from these validated instruments were compared with data collected using an Eagle2 Gas Monitor (RKI Instruments).

Feed Intake. Information on methane phenotypes as well as feed intake on about 1,000 animals per year will be collected. The "Masteryard" feed intake system designed and implemented by Crown Agriculture will be installed at Kirby Research Station. The system consists of 50 feed intake units with Calan gates and EID readers, each capable of measuring approximately 6 animals at a time. Animals will be tested for a defined test period (likely 7 weeks total) and all feeding events as

well as body weight will be recorded, allowing estimation of daily feed intake as well as feeding behaviour. The diet will be as close as possible to a pasture, i.e. with a high proportion of roughage, while lambs will have a higher energy diet to allow maximum growth.

Implementation. All phenotypic and genotypic data collected in this project will be submitted to the Sheep Genetics database. We are also collaborating with other groups in Australia that collect such data to ensure consistency in measurement protocols and experimental designs that include links sires between data sets.

Collaboration. We are collaborating with research partners in Western Australia at the Department of Primary Industries and Regional Development DPIRD and Murdoch University, with the aim of creating a larger genomic reference population. We are also collaborating internationally (with partners in New Zealand, Uruguay and Ireland) in order to develop more accurate estimates of genetic parameters.

Quantitative genetic and genomic analysis of methane and feed intake phenotypes will be undertaken, initially to understand sources of variation, to determine the best model for analysis and to estimate variance components and genetic parameters. Microbiome data will be analysed and association studies with methane phenotypes undertaken.

After preliminary data analysis in the first year, trait definitions and a model for genetic evaluation will be proposed. This will then be tested through the OVIS software for single step analysis and predictions will be validated and further developed. This includes data to generate estimated breeding values for methane emission as well as feed intake.

The pipeline also involves development of best methods for genomic prediction, including research on genetic markers that might have a large effect on the traits for which genomic prediction is tested. Such marker identities will be made available to genotyping service providers such that they can add them to their genotyping arrays.

Existing data on genotypes and full genome sequence will be used to impute genotypes on animals with new phenotypes. Collaboration with AGBU and Sheep Genetics will develop selection indices and help deliver this information to breeders and let them achieve the desired genetic change. The modelling work will result in clear messages to breeders on what can be achieved with selection on methane and/or feed efficiency ASBVs and how they can best implement that information in their breeding strategies.

RESULTS AND DISCUSSION

Means and standard deviations for body weight and emission traits for Kirby lambs and Temora ewes as measured by the FoxBox-Pro and EAGLE-2 are shown in Table 1 and 2 respectively.

Table 1. Means and standard deviation of lamb and ewe emission traits at Kirby and Temora

Lambs Time	Measure (mL/min)	FID/FoxBox		Ewes Time	Measure (mL/min)	FID/FoxBox	
		Mean	SD			Mean	SD
25 mins	CH ₄	8.45	4.88	20 mins	CH ₄	6.24	7.00
	CO ₂	241.4	71.4		CO ₂	498.1	153.9
	O ₂	-513.5	103.3		O ₂	-1152.0	180.6
50 mins	CH ₄	10.56	4.51	40 mins	CH ₄	8.96	7.88
	CO ₂	225.0	52.3		CO ₂	409.9	108.6
	O ₂	-358.7	68.1		O ₂	-787.0	122.6

Methane output (mL/min) was significantly positively correlated with body weight for Kirby lambs (0.575, Figure 1a) but not ewes at Temora (0.11, Figure 1b). The same measurements on different machines were generally well correlated. Correlations between the FID/FoxBox-Pro and

the Eagle for CH₄, CO₂ and O₂ were 0.951, 0.956 and 0.948, respectively for lambs at Kirby and 0.977, 0.970 and 0.905, respectively for ewes at Temora. Although the correlations were high, the slope of the regression was not one, which was more pronounced for CH₄, hence there is still a small scaling difference between measurements with the instruments.

Table 2. The correlation (unadjusted) between CH₄ and O₂ and CO₂ in Kirby lambs

Lambs	CH ₄	O ₂	CO ₂
CH ₄	-		
O ₂	-0.682	-	
CO ₂	0.749	-0.922	-

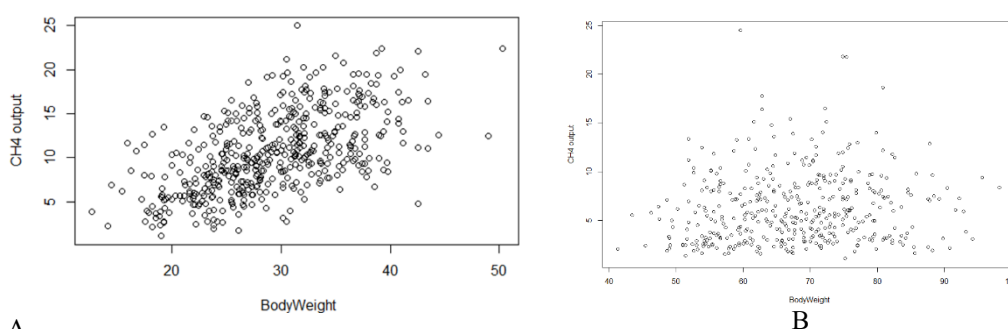


Figure 1a and 1b the relationship between Methane output (ml/min) and body weight in a) lambs at Kirby; and b) mature ewes at Temora

CONCLUSIONS

The protocol for methane collection appears robust and the preliminary data gathered to date has shown that there is enough variation in methane production among animals which should enable the sheep industry to select for lower methane production without negatively impacting other traits.

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