

Genetically more efficient Australian dairy cows and sheep are higher emitters of methane per unit of food

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Abstract

Both the dairy cattle and sheep industries face the simultaneous challenge of improving feed efficiency and reducing methane emissions. Genomic selection is a valuable tool to reduce residual feed intake (RFI) and reduce methane yield (MeY), which are widely used traits for estimating efficiency and emissions. However, it is important to know how selecting one of these traits would affect the other and this relationship has been contentious in the literature. Here we estimated the genetic correlations between RFI and MeY in 584 Holstein dairy cattle and 445 Australian Maternal Composite ewes using bi-variate genomic best linear unbiased prediction models. In both datasets, negative genetic correlations between RFI and MeY were found, which means that selecting more feed efficient animals would increase the amount of methane emitted per kg of dry matter intake. Diet could play a role in this relationship.

Introduction

Feed is the main production cost for both dairy cattle and sheep production and improving feed efficiency is of economic importance to livestock producers. Residual feed intake (RFI) is an accepted measurement of feed efficiency obtained as the difference between actual and predicted dry matter intake (DMI) required for maintenance, growth, and production. Methane is a greenhouse gas emitted as a by-product of enteric fermentation in ruminants. Reducing methane emissions from dairy cattle and sheep is an important step to achieving the Australian goal of reducing greenhouse gas emissions by 26 to 28% from 2005 levels by 2030. On top of the environmental benefits, reducing methane emissions could increase productivity because methane represents a waste of energy that could be used for production. Methane yield (MeY) is a methane emission trait calculated as the ratio of methane production (MeP) and dry matter intake (DMI). Both RFI and MeY are time-consuming or expensive to measure traits, making routine recording on commercial farms unviable. For these reasons, and given their socio-economic importance, RFI and MeY are ideal traits to be improved with genomic selection. However, it is necessary to know how selecting one of these traits would affect the other. Our hypothesis was that as methane emissions represent a waste of energy, more efficient sheep and cows were expected to be lower emitters of methane per kg of DMI (MeY).

Materials & Methods

Animals. Feed intake and methane emissions were collected on 584 Holstein dairy cattle from 12 herd-year-season (HYS) cohorts between 2013 and 2017 and 445 Australian Maternal Composite ewes between 2013 and 2014. Cows were located at the Ellinbank SmartFarm (Ellinbank, Victoria, Australia), milked twice per day, and were between 55 and 127 (71 ± 26) days in milk (DIM) and across parities 1 to 9. Daily milk production was recorded in cows, and milk protein and fat were calculated using midinfrared spectroscopy. Energy corrected milk (ECM; kg/d) was calculated with the methodology of Visscher et al. (1994) as follows:

$$\text{ECM} = 0.1 * \text{milk yield (kg)} + 5.2 * \text{fat (\%)} + 2.6 * \text{protein (\%)} \quad (1)$$

Ewes were located at the Hamilton SmartFarm (Hamilton, Victoria, Australia), were not in late pregnancy or lactating, and their phenotypes were measured at post-weaning, hogget, and adult age stages.

Phenotypes. Dairy cows were fed *ad libitum* for 3 hours after morning and afternoon milking with alfalfa hay or cubes, after which they were moved to an area without food but with water available *ad libitum*. One feeding bin was allocated per one or two cows. Additionally, cows consumed 6.2 ± 0.25 kg of dry matter per day of crushed wheat in 2 portions at milking. The alfalfa hay and cubes provided to cows contained 8.7 and 10.2 MJ of ME/kg of dry matter (DM), 18.2% and 22.7% crude protein (CP), and 46.3% and 37.3% Neutral detergent fibre (NDF), respectively. The crushed wheat fed to cows had 13.8 and 13.8 MJ of ME/kg of DM, 13.1 and 13.9% CP, and 10.2 and 14.3% NDF, respectively. Ewes were fed *ad libitum* (24 hours per day) with a cereal straw-based pelleted diet for 10-14 days before recording feed intake. The pellets provided to ewes had 9.6 ± 0.58 MJ of ME/kg of DM, 9.8 ± 1.6 % CP, 48 ± 3.18 % NDF. Ewes were spread across 10 pens, with less than 24 ewes per pen and two automated feeders. Pen number was constant within all experiments, but ewes could be assigned to different pens at different ages. Feed intake was measured between 32 and 37 days in cows and for a total of 53, 42, and 32 days for post-weaning, hogget, and adult ages in ewes, respectively. Ewes were weighed three times a week to calculate the average daily gain (ADG). RFI of cows was estimated by Bolormaa et al. (2021) as

$$\text{RFI}_{\text{cows}} = \text{DMI} - (\text{mean} + b_1 \text{ parity} + b_2 \text{ DIM} + b_3 \text{ HYS} + b_4 \text{ poly}(\text{age}, -2) + b_5 \text{ diet} + b_6 \text{ ECM} + b_7 \text{ BWT} + b_8 \Delta \text{BWT}), \quad (2)$$

where $b_1 - b_8$ are partial regression coefficients; BWT is mean body weight in the test; Δ BWT daily BWT change. RFI of ewes was calculated as

$$\text{RFI}_{\text{ewes}} = \text{DMI} - (\text{mean} + b_1 \text{ MMWT} + b_2 \text{ ADG} + b_3 \text{ EXP} + b_4 \text{ PEN:EXP} + b_5 \text{ AGE}), \quad (3)$$

where $b_1 - b_5$ are partial regression coefficients; MMWT is the metabolic mid-test weight obtained as the average weight of the animal during the feeding test to the power of 0.75; EXP is the feed intake experiment ($n = 5$); PEN:EXP is the pen nested into the experiment (45 levels), and AGE is the age in days at the start of the experiment. RFI was the residual error of the expression, i.e. additional consumption after accounting for energy sinks, which were maintenance (MMWT) and growth (ADG).

Methane production (MeP; L/d) in cows was measured over 5 days with the sulphur hexafluoride (SF_6) tracer method described by Deighton et al. (2014) and averaged to obtain one observation per animal. Methane emissions were measured twice in ewes during each feed intake experiment with the portable accumulation chamber (PAC) technique described by Goopy et al. (2011). In the sheep population, the first methane measurement was obtained approximately four weeks after the start of the experiment and the second one approximately ten days later after the first measurement, and MeP (L/d) was estimated. MeY was calculated as MeP divided into DMI for both cattle and sheep.

Genotypes. The dairy cattle animals were genotyped for 54,609 single nucleotide polymorphism (SNP) from the BovineSNP50k v2 BeadChip (Illumina Inc., San Diego, CA).

These SNP were mapped to the ARS-UDC1.2 bovine genome sequence assembly. SNP with call rate smaller than 0.9, minor allele frequency smaller than 0.025, and duplicate map positions were removed. After this filter, 36,951 SNP were retained for cattle. Ewes were genotyped with one of three chips — with 12,785, 15,000 or 54,241 SNP — and imputed with FImpute to 46,091 SNPs. Genomic relationship matrices (G) were created with the function Gmatrix of the R package AGHmatrix (Amadeu et al. 2016) following the method of Yang et al. (2010), and principal component analyses on G were completed. All animals had a genotype call rate greater than 0.9.

Genetic correlations. Genetic correlations between RFI and MeY were obtained with the R package ASReml-R (Butler et al. 2009) with bi-variate GBLUP models of the form

$$\mathbf{y} = 1\mu + \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{g} + \mathbf{e} \quad (4)$$

where \mathbf{y} is a vector of phenotypes; μ is the overall mean; $\boldsymbol{\beta}$ is a vector of fixed effects; \mathbf{g} is a vector of random additive genetic effects; \mathbf{X} and \mathbf{Z} are the incidence matrices for $\boldsymbol{\beta}$ and \mathbf{g} , respectively; and \mathbf{e} is a vector of random residuals distributed as $N(0, \mathbf{I}\sigma_e^2)$. The distribution of \mathbf{g} is $N(0, \mathbf{G}\sigma_g^2)$, where \mathbf{G} is the genomic relationship matrix calculated from SNP genotypes following Yang et al. (2010). The fixed effects in cows were HYS, DIM, age and parity, and in sheep were EXP, PEN:EXP, and AGE.

Results

RFI had means of zero and standard deviations of 1.01 and 0.27 in dairy cows and sheep, respectively (Table 1). MeY means were 19.96 ± 3.61 and 25.08 ± 7.14 for cattle and sheep, respectively. In MeY, the range was wider, and the coefficients of variation and maximum values were lower in dairy cattle than in sheep. The coefficients of variation were 18.09 and 28.48, and maximum values were 32.89 and 54.19 for cattle and sheep, respectively. The coefficient of determination (R^2) of the observed DMI regressed on the predicted DMI in sheep was 0.79. The first two principal components of the \mathbf{G} matrices together explained 11% and 12% of the variation in cattle and sheep; 14 and 3 distant animals in cattle and sheep were removed, respectively. The genetic correlations between RFI and MeY were strong and reasonably consistent and were -0.77 ± 0.15 and -0.86 ± 0.13 for dairy cattle and sheep, respectively.

Table 1. Descriptive statistics of residual feed intake (RFI) and methane yield (MeY) in dairy cattle and sheep.

Species	Trait	Animals	Mean	SD	Min	Max	CV
Dairy	RFI	584	0.00	1.01	-3.49	4.69	NA
Cattle	MeY	424	19.96	3.61	11.11	32.89	18.09
Sheep	RFI	443	0.00	0.27	-1.08	1.05	NA
	MeY	458	25.08	7.14	6.72	54.19	28.47

Discussion

The range of phenotypic values of RFI and MeY and the coefficient of variation of MeY show variability in the dairy cattle and sheep populations, which can be used for the genetic improvement of these traits. The percentages of the \mathbf{G} matrices explained by the first two principal components showed that population structures in the populations were minor.

The results from this study show that the selection of ruminants for improved feed efficiency (i.e. lower RFI), would lead to higher methane emissions per kg of DMI. This is somewhat

unexpected, as it was hypothesised that lower RFI (more efficient animals) would have decreased MeY, as methane represents energy that could otherwise be used for production. In accordance with this approach, studies in dairy cattle and sheep fed with lower fibre and higher protein diets than our research found that selecting for low RFI would decrease MeY (Negussie et al. 2014; Paganoni et al. 2017). In contrast with these studies and with diets with lower protein and higher fibre, we found that MeY was higher for low RFI in dairy cattle and sheep. Similar to our results, heifers with low RFI had higher MeY values under a high-forage diet (Flay et al. 2019). Additionally, an inverse relationship between DMI and MeY has been reported in ruminants fed with forage diets (Hammond et al. 2013) and RFI as a portion of DMI could be expected as negatively correlated with MeY. The underlying biology of the genetic correlations between RFI and MeY is not clear yet and requires further research to elucidate.

References

- Amadeu R.R., Cellon C., Olmstead J.W., Garcia A.A., Resende M.F. *et al.* (2016) Plant Genome 9(3):1-10. <https://doi.org/10.3835/plantgenome2016.01.0009>
- Bolormaa S., Macleod I.M., Khansefid M., Marett L., Wales B. *et al.* (2021) Proc. of the 24th AAABG, Adelaide, Australia.
- Butler D., Cullis B.R., Gilmour A. and Gogel B. (2009) ASReml-R Reference Manual. The State of Queensland, Department of Primary Industries and Fisheries.
- Deighton M.H., Williams S.R.O., Hannah M.C., Eckard R.J., Boland T.M. *et al.* (2014) Anim. Feed Sci. Technol. 197:47-63. <https://doi.org/https://doi.org/10.1016/j.anifeedsci.2014.08.003>
- Flay H.E., Kuhn-Sherlock B., Macdonald K.A., Camara M., Lopez-Villalobos N. *et al.* (2019) J. Dairy Sci. 102(3):2708-2713. <https://doi.org/10.3168/jds.2018-15234>
- Goopy J.P., Woodgate R., Donaldson A., Robinson D.L. and Hegarty R. (2011) Anim. Feed Sci. Technol. 166-167:219-226. <https://doi.org/10.1016/j.anifeedsci.2011.04.012>
- Hammond K.J., Burke J.L., Koolaard J.P., Muetzel S., Pinares-Patiño C.S. *et al.* (2013) Anim. Feed Sci. Technol. 179(1):121-132. <https://doi.org/https://doi.org/10.1016/j.anifeedsci.2012.11.004>
- Negussie E., Mäntysaari P., Mäntysaari E.A. and Lidauer M.H. (2014) Proc. of the 10th WCGALP, Vancouver, Canada.
- Paganoni B., Rose G., Macleay C., Jones C., Brown D. *et al.* (2017) J. Anim. Sci. 95(9):3839-3850. <https://doi.org/doi:10.2527/jas2017.1499>
- Visscher P.M., Bowman P.J. and Goddard M.E. (1994) Livestock Production Science 40(2):123-137. [https://doi.org/10.1016/0301-6226\(94\)90042-6](https://doi.org/10.1016/0301-6226(94)90042-6)
- Yang J., Benyamin B., Mcevoy B.P., Gordon S., Henders A.K. *et al.* (2010) Nat. Genet. 42(7):565-569. <https://doi.org/10.1038/ng.608>