International approach to reduce greenhouse gas emissions from sheep

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Abstract

Reduction of greenhouse gas (GHG) emissions, and particularly enteric methane (EM) emissions, from ruminant livestock is a global problem faced by all producers. In 2015 there were 1.18 billion sheep in the world emitting 158 Mt of carbon dioxide (CO_2) equivalents of EM, or 6% of the total EM emissions (FAO 2022). Animal breeding is a highly cost-effective strategy to achieve reductions in EM and has already been included in national plans and Nationally Determined Contributions (NDCS) to achieve mitigation targets in several countries (https://unfccc.int/NDCREG).

An international project ('Grass To Gas', 2019-2023) combined expertise and generated new knowledge towards the reduction of methane (CH_4), a potent GHG, from sheep. Proxy measurements were investigated including individual animal feed intake and N-alkane measures indoors and at pasture, rumen microbiome-generated data, nuclear magnetic resonance (NMR) spectroscopy with plasma and rumen samples, and rumen volume by Computer-Tomography (CT), amongst others, in native, improved and unimproved sheep breeds and with different feed and forage qualities. Direct measurements of CH_4, and CO_2 from ewes and lambs were compared using portable accumulation chambers (PACs), sheep and Greenfeed to enable animal ranking and first breeding values for GHG emissions for sheep. Heritabilities (with s.e.) for PAC raw CH_4 (g/day) were reported as being between 0.26(0.03) and 0.34(0.09) from 4 countries, with methane intensity CH_4 / (CH_4+CO_2) moles/day having lower values [0.21(0.03) to 0.29(0.06)]. Estimates for residual feed intake (RFI) were higher at between 0.37(0.08) to 0.45(0.08). As expected, genetic correlations between RFI and feed intake were moderate to strong (0.41(0.14) to 0.79(0.09), and low or close to zero with body weight or growth. Comparing low vs high RFI progeny of Romane sires showed that after 4 generations, the dry matter intake difference between RFI lines reached 123 g/day of concentrate and 80 g/day of forage in favour of the efficient line. A larger difference of 20% in feed intake was reported when comparing Australian Merino lambs contrasting in RFI.

The links between RFI, feed intake and CH_4 emissions are more complex and require further investigation. However, high CH_4 was associated with higher levels of feed intake with preliminary genetic correlations reported as being between 0.33(0.17) and 0.43(0.19) and those between CH_4 and metabolic body weight being 0.58(0.15) to 0.68(0.11). The use of routine CT scans enabled retrospective computation of rumen volume which has been shown to be moderately heritable. The genetic correlation between rumen volume and CH_4 is yet to be determined although larger reticulo-rumen volumes, as measured by CT scanning, were associated with increased methane emissions but not with RFI at the phenotypic level.

The rumen microbiota is a complex ecosystem, which include bacteria, archaea, protozoa, fungi and viruses, that provides ruminants with the ability of digesting fibres in plant cell walls into nutrients for the animals. The composition of the rumen microbiota did not enhance prediction accuracies over and above more conventional zootechnical predictors of feed efficiency for French sheep. In contrast in New Zealand, promising results were reported based on rumen microbial composition using high-throughput, restriction enzyme-reduced representation sequencing. The EBV accuracies for methane yield and RFI were comparable with those achieved only using the animals' genomic information.

Additional results emanating from the project demonstrated that incorporating methane emissions into national sheep breeding programmes is a cost-effective mitigation measure with significant abatement potential. Importantly, working internationally to share protocols, information, knowledge and experience benefits all countries, avoiding duplication and fostering shared responsibility to be part of the solution to a global problem. A new international project, 'Sustain Sheep' (2024-2027) is underway to advance and extend our understanding of some of the key issues highlighted in this paper.

Keywords: enteric methane, greenhouse gas, genetic parameters, genetic improvement, sheep.
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Introduction

Reduction of greenhouse gas (GHG) emissions, and particularly enteric methane (EM) emissions, from ruminant livestock is a global problem faced by all producers. Although large ruminants make up the majority of the enteric methane emissions from livestock, in 2015 there were 1.18 billion sheep in the world emitting 158 Mt of carbon dioxide (CO_2) equivalents of EM, or 6% of the total EM emissions (FAO 2022). Animal breeding is a highly cost-effective strategy to achieve reductions in EM and has already been included in national plans and Nationally Determined Contributions (NDCS) to achieve mitigation targets in several countries (https://unfccc.int/NDCREG). Animal breeding is a long-term strategy to make cumulative reductions in methane and GHG emissions, which are permanent and highly cost-effective, for meat (Lambe, 2022), wool (Navajas *et al*., 2022a) and potentially for dairy sheep.

An international project ('Grass To Gas', 2019-2023) combined expertise and generated new knowledge towards the reduction of methane (CH_4), a potent GHG, from sheep. The countries involved in this project all have established national sheep breeding programmes and prior to the onset of the project, were independently measuring some or all of the following: individual animal feed intake, RFI, GHG emissions (CO_2 and CH_4), N-alkane measures at pasture, rumen microbiome-generated data, NMR spectra and rumen volume by Computer-Tomography (CT), amongst others, in native, improved and unimproved sheep breeds and with different feed and forage qualities. The overall aim of the project was to bring together the collective expertise to validate existing protocols, benchmark, and enhance the measurement and analyses of these new phenotypes for their development and use in sheep breeding programmes. The project also aimed to determine the use of selective animal breeding as a tool for GHG abatement, considered in relation to other measures available with modelling applicable to one country (UK). Specifically, the objectives were,

1. To validate predictors of feed intake (FI), residual feed intake (RFI) and CH_4|emissions.
2. To compare indoor and outdoor FI and RFI.
3. To investigate the opportunity to use genetics and genomics to reduce CH_4 emissions by determining the genetic basis of new phenotypes including rumen microbiome.
4. To quantity the economic and environmental benefits of more feed-efficient and lower GHG-emitting sheep, and ensure that the relevance of the project from farm to international impact scale.

Material and methods

Feed intake, Feed efficiency

Residual feed intake (RFI) is a measure of feed efficiency defined by Koch *et al*. (1963) as the difference between actual and predicted dry matter feed intake, based on average daily gain and metabolic body weight. In France, the methodology used to collect feed intake and feed efficiency data in 951 male Romane lambs is documented in Tortereau *et al*., 2020, using an 8 week testing period per batch of between 92-149 lambs at around 70d of age and weighed at the start and end of the trail period. In Uruguayan Merino sheep, phenotypic records of 1138 animals with feed intake, and 1120 with RFI were recorded using Intergado® automated feeders as described by Amarilho-Silveira *et al*., 2022 and genetic parameters analysed according to the methodology by Marques *et al* 2024. In New Zealand maternal sheep, individual measures of feed intake, feeding behaviour (length and duration of eating events) CH_4 and CO_2 were generated on 986 growing maternal ewe lambs sourced from three pedigree recorded flocks, the methodology for which was reported by Johnson *et al*., 2022. Norway compared 40 Old Norwegian with Norwegian White dry adult ewes fed different silage qualities as described by Aby *et al*., 2023a. In Ireland, 242 individual feed intake measurements taken at grass from 242 Texel-cross lambs at grass was measured using the N-alkane technique as described by McGovern *et al*., 2021 and in the UK, 250 indoor- and outdoor- reared Texel-cross lambs were used to compare sire ranking of full-sib offspring reared in the 2 systems as described by Conington *et al*., 2022. More detail on the predictions of feed intake and methane emissions in sheep using different proxies has been provided in Le Graverand *et al*., 2024.

Methane measurements

Direct methane measurements were undertaken using different technologies. Portable accumulation chambers (PACs) were used in Norway, New Zealand and Ireland, using the protocols first developed in Australia (Goopy *et al*., 2011, applied in New Zealand by Jonker *et al*., 2018, and validated by O' Connor *et al*., 2021. A PAC is an airtight chamber in which an animal is kept for less than one hour. Emissions of CO_2 and methane are estimated based on the difference in the concentration of gases when the animal is entering and leaving the PAC. Temperature and pressure are also measured, and with the body weight of the animal, are used to calculate the emission of these two gases in grams per day. In Uruguay, PACs to measure GHG emissions were used to measure 930 Merino as described by Marques *et al*., 2022; RFI and CH_4 have been measured at post-weaning in Corriedale, Dohne Merino, Merelin and Texel lambs since 2018. Animals evaluated in Uruguay belong to the Selection Nucleus (Australian Merino), Information Nucleus (Corriedale, Dohne Merino, Texel) and commercial stud-flocks (all breeds) and are strongly connected with populations in the genetic evaluation (performance recorded) (Navajas *et al*., 2022)In France, the C-lock® GreenFeed individual small animal measurement system was used to quantify daily CH_4 and CO_2 emissions from individual animals as described by Tortereau *et al*., 2023.

CT-derived rumen volume and other proxy traits

In the UK, and New Zealand, Computer Tomography (CT) is used routinely as part of national meat sheep breeding programmes to quantify, with almost perfect precision, the body composition of elite rams. Using additional data generated as part of that process, the rumen size was determined to estimate the correlations between reticulo-rumen size and CH_4 emissions, as documented by Lambe *et al*., 2022 and Hitchman *et al*., 2023. CT-measured rumen volumes were measured within the Grass to Gas project in the UK (from unselected Texel-crossbred lambs) and France (from Romane lambs divergently selected for RFI) and related to feed intake and efficiency measures. In Norway, rumen tissue volume was undertaken on samples collected post-mortem as described by Åby *et al*., 2023b.

Different traits were considered as proxy measurements for feed intake, feed efficiency or methane emissions depending on the study. Most of the proxy traits were recorded during feed intake trials, such as bodyweight or body composition. In Scotland, New Zealand and Norway, the number of feeding events per day were collected and tested as a possible proxy. In France and New Zealand, blood and rumen fluid samples were collected during (New Zealand) or at the end of the intake trial (France). DNA was extracted from blood samples, and genotyping was performed using the Illumina 50K SNP chip. Ruminal microbiota was assessed through meta-barcoding or meta-genomics in France (Le Graverand *et al*., 2023) and New Zealand (Hess *et al*., 2023), respectively. For meta-barcoding to determine the rumen microbiota composition, to predict feed intake, the details are provided in Le Graverand *et al*., 2023a. In New Zealand, metagenome profiles were generated using restriction enzyme-reduced representation sequencing (RE-RRS) (Hess *et al*., 2020).

Impact of breeding for reduced methane emissions

The use of animal breeding as a tool for GHG reduction in small ruminants has not received serious attention by some Governments as a tool for the estimation of the abatement potential, despite the unequivocal evidence of the contribution of animal breeding to improve animal efficiency. The contribution to the reduction in GHG emissions in 5 Irish sheep breeds reared in 4 flocks was summarised by McHugh *et al*., 2022 and the difference in high- vs low-genetic merit Irish flocks was reported by Farrell *et al*., (2022). Bioeconomic modelling to compare profitability of high- vs low-emitting sheep flocks was used in the study.

In the UK, animal breeding for efficiency and directly breeding to reduce CH_4 emissions were included in new marginal abatement cost curves (MACC) for sheep which was estimated in terms of the cost-effectiveness and abatement potential.

Results

McGovern *et al*., (2024) provides full details of summary statistics and the models used to analyse the phenotypic results from each participating country. Table 1 summarises the heritability estimates for traits used in the estimation of feed efficiency and GHG emissions. Full details of the genetic models to estimate these parameters can be seen in the respective publications.

The genetic correlations of RFI with other traits are summarised in Table 2. They confirm a strong association of RFI with feed intake, and low, or close to zero correlation with body weight and growth during the feed efficiency tests.

The comparison of progeny of high feed efficiency (low RFI) and low efficiency (high RFI) Romane sires, after one generation of selection showed that improving RFI represents 3% less concentrate per day at the same level of performance (Tortereau *et al*., 2020). After 4 generations of selection, these differences between the 2 RFI lines reach 123 g of concentrate and 80 g of forage in favour of the efficient line (Marie-Etancelin *et al*., 2023). A larger difference of 20% in feed intake was reported by De Barbieri *et al*. (2020), when comparing Australian Merino lambs of contrasting RFI. In Norway, Åby *et al*. (2023a) found differences in methane emissions between two Norwegian breeds which were strongly linked to feed intake. The breed differences were still present after adjusting for feed intake, which suggest that other factors such as rumen size and anatomy may influence methane emissions. In France, comparisons of RFI lines in term of GHG showed that the most efficient animals (with lower feed intake) produce significantly more CH_4 than the least efficient animals, whether in males fed mixed

Table 1. Heritability estimates (standard error) for residual feed intake (RFI), traits used for the estimation of RFI and individual methane (CH_4) and carbon dioxide (CO2) emissions.

Study	Johnson et al. (2022)	Hickey et al. (2022)^{1}	Tortereau et al. (2020)	Marques et al. (2022)	Jakobsen et al. (2022)
Breed	NZ maternal	Several	Romane	Australian Merino	Norwegian White
RFI	0.42 (0.09)	--	0.45 (0.08)	0.37 (0.08)	--
Feed intake	0.35 (0.10)	--	0.28 (0.08)	0.41 (0.08)	--
Average daily gain	0.42 (0.10)	--	0.22 (0.07)	--	0.19(0.01)^{5} 0.20(0.01)^{6}
Metabolic body weight	0.44 (0.11)	--	--	--	--
Body weight	--	--	0.21 (0.07)^{2}	0.41 (0.01)^{3}	--
Backfat thickness by US	0.57 (0.09)	--	0.39 (0.08)	0.32 (0.04)	--
Muscle depth by US	--		0.41 (0.08)	0.39 (0.04)^{4}	--
CH4 (g/day)	0.32 (0.08)	0.26 (0.03)	--	0.34 (0.09)	0.17(0.04)
CO2 (g/day)	0.32 (0.08)	--	--	--	--
CH4/(CH4 + CO2)	0.29 (0.06)	0.21 (0.03)	--	--	--

[1] Gas measurement by Respiration Chambers, the other studies used PACs.
[2] Includes body weight at the end of feed efficiency test;
[3] body weight at shearing;
[4] muscle area.
[5] Direct & maternal h^2 summed for 42d weight
[6] Direct & maternal h^2 summed for 140d weight.
[7] CH_4 g/hr

Table 2. Genetic correlation (S.E.) between RFI and performance traits recorded in feed efficiency tests.

Study/trait	Johnson et al. (2022)	Tortereau et al. (2020)	Marques et al (2022)
Feed intake	0.41 (0.14)	0.78 (0.08)	0.79 (0.09)
Metabolic body weight	-0.23 (0.17)	--	--
Body weight	--	-0.03 (0.19)[1]	-0.22 (0.14)[2]
Average daily gain	-0.09 (0.17)	-0.03 (0.19)	--
Ultrasonic Backfat thickness	-0.14 (0.15)	0.00 (0.16)	-0.17 (0.16)
Ultrasonic Muscle depth	--	-0.30 (0.16)	-0.15 (0.20)[3]

[1] Body weight at the end of feed efficiency test;
[2] Body weight at shearing;
[3] Muscle area.

Table 3. Genetic correlations (standard errors) of methane emissions (CH4), carbon dioxide (CO2), total greenhouse gas (CH_4+$C0_2$) and methane intensity CH_4/(CH_4 + CO_2) with RFI and traits recorded in feed efficiency test

	CH_4	CO_2	CH4 + CO2	CH4/(CH4 + CO2)
	Johnson et al. (2022)			
RFI	-0.28 (0.16)	0.05 (0.17)	0.04 (0.17)	-0.41 (0.15)
Feed intake	0.33 (0.17)	0.59 (0.14)	0.60 (0.14)	-0.24 (0.09)
Metabolic body weight	0.68 (0.11)	0.62 (0.13)	0.73 (0.08)	-0.06 (0.08)
Average daily gain	0.34 (0.10)	0.06 (0.19)	0.07 (0.19)	0.10 (0.16)
Backfat thickness by US	-0.04 (0.15)	-0.33 (0.15)	-0.32 (0.15)	0.06 (0.08)
	Marques et al., (2022)			
RFI	0.43 (0.19)	--	--	--
Feed intake	0.79 (0.09)	--	--	--
Body weight	0.58 (0.15)	--	--	--
ADG	--	--	--	--
Ultrasonic backfat	0.37 (0.17)			
Ultrasonic Muscle	0.36 (0.15)			

diet or in females on pasture (Tortereau *et al*., 2023). Table 3 confirms that methane production is closely associated with feed intake. Higher feed efficiency measured by RFI (i.e. low RFI) is associated with lower feed intake, which lead to the hypothesis that increased efficiency also means less methane production, although these results are not conclusive from our studies.

Proxy traits

Microbiome

Predictions of host feed efficiency from 16S (assessed as the average correlation between actual RFI and its prediction) ranged from 0.11 under a concentrate diet to 0.35 under a mixed diet, while predictions from fixed effects varied from 0.31 to 0.55, respectively (Le Graverand *et al*., 2023a). When young rams fed concentrate only, SNPs were the best predictors of RFI (accuracy of 0.44), but blood NMR spectra were also promising with an accuracy of 0.33 (Le Graverand *et al*., 2023b). In New Zealand, accuracy of methane and feed intake prediction was significantly improved by including a microbial relationship matrix in the linear mixed model alongside the genomic relationship matrix (Hess *et al*., 2023). Bilton *et al*., 2022 further showed that the genetic correlation between direct measures from PAC chambers and methane predicted from rumen microbial profiles was 0.76 (s.e. 0.12) and 0.64 (s.e. 0.11) for feed intake.

Computer Tomography

Rumen volume measurements, taken from CT scans in the UK and France, were significantly correlated with body weight, growth and feed intake, and in the UK also with age and muscle depth. However, no significant link between RFI and rumen volume was identified. Selecting lambs on RFI under a concentrate diet in the growing period did not seem to impact rumen volume later in life. These results suggest that rumen volume cannot be proposed as a proxy of feed efficiency. However, it might be tested in further analyses as a proxy of feed intake.

Predictions from other phenotypes

In general across all available data sets from the project, when investigating various proxy measurements (Le Graverand *et al*., 2024), validation prediction accuracies were higher for feed intake than for feed efficiency criteria (residual feed intake and feed conversion ratio). The best predictions for feed intake were obtained when body weight and the average number of feeding events per day were included in the models (R^2=0.78). Methane emissions were predicted with the highest accuracy when feed intake was considered among the proxies, alongside body weight, average daily live-weight gain and ultrasound measured body composition (R^2=0.34). Prediction accuracies for methane emissions obtained with metagenome were higher than with the sheep genome, although this accuracy remains quite low (r=0.32).

Economics and abatement potential

The results from the Irish study comparing high-vs low- genetic merit flocks for differences in profitability and predicted GHG emissions, indicated that the flock of high genetic merit was more profitable with a higher net profit of €18/ewe than the flock of low genetic merit (Farrell *et al*., 2022). Although the GHG emissions, assessed by life-cycle analysis (LCA) showed an increase of 2.9% in total emission (expressed as CO2eq), the emissions intensity (kg CO2eq/ kg carcass weight sold) was 6.9% lower for the flock with high genetic merit. In the UK,

the results of the marginal abatement cost curve (MACC) in terms of cost effectiveness (CE) and abatement potential (AP) are presented in Table 4. The maximum AP estimated for UK sheep was of 2.7Mt, which is equivalent to 27% reduction in emissions. Sheep breeding strategies explain almost 30% of this reduction.

Another example is the integration of sheep genetics for reducing GHG emissions in the context of a Regenerative Livestock Farming programme in Uruguay. Research and development for this initiative have been supported by national and international funding. As the industry captured the demands of high-value markets for high intrinsic quality wool produced in regenerative livestock farming systems, nowadays there are 19 farms included in this initiative (Blumetto *et al*., 2023), and the approach is expanding

Table 4. Cost effectiveness (CE) and abatement potential (AP) of mitigation measures applied to UK sheep systems, with interactions.

Name	Applied to	CE (£/tCO2e)	AP (ktCO2e)	AP as %
Bio N fixation in grasslands	Managed grass	-1034	250	2.5%
Optimising pH for grass growth	Managed grass	-31	278	2.8%
Breeding for improved productivity	All sheep	-10	504	5.0%
Breeding for lower CH4	All sheep	20	252	2.5%
Better health planning for sheep	All sheep	38	391	3.9%
3NOP	Non-grazing sheep	119	99	1.0%
3NOP	All sheep	158	925	9.2%
Total			2699	27%

to other industries. Modelling work carried out based on Life Cycle Analysis indicates a potential abatement of emission intensity associated with wool products, ranging from 6 to 20%, assuming the flock has a CH4 emission equivalent to the top 25% genetically superior (animals with lower emission) (Blumetto *et al*., 2023).

In 2023, GEPDs for RFI and CH4 emission were made available for an Australian Merino breed in Uruguay as research breeding values, being a first step before incorporating routinely these traits into the breeding program. These estimates were based on 1.200 lambs phenotyped for RFI and CH4 and 3000 genotyped animals.

In New Zealand, the cost of mitigating a tonne of CO_2 equivalent through breeding for reduced methane in the national scheme was estimated at less than NZ$2. Empirical evidence showed that a 0.5-1% per year reduction is possible for a moderate economic weighting on carbon demonstrating that breeding has major potential as a national mitigation strategy (Rowe *et al*., 2021). The authors also demonstrated use of methane emissions in a selection index for a 750 ewe research flock where methane emissions were being reduced by greater than 1% per year whilst maintaining genetic gain in the production index.

Discussion

Some viable proxy traits for predicting feed efficiency and methane emissions are emerging. Work continues to progress on rumen microbial profiling to predict methane emissions (Rowe *et al.* 2019; Hess *et al.* 2020), which has the potential to provide an accurate and relatively quick method to determine genetic merit for use in breeding programmes. Likewise, CT-measured rumen volumes are now being measured in greater numbers within research flocks and breeding programmes in various countries to relate to methane emissions at the genetic level. If this proxy trait proves to accurately predict methane at the genetic level, then a mechanism is already in place in some countries (e.g. UK, NZ, Ireland) to incorporate these alongside other CT traits in breeding programmes. However, direct measurement of methane emissions, using PAC technology, is emerging as the most promising route to large-scale measurement of emissions from sheep across different production systems. PACs are being used within research flocks (e.g. in Australia, NZ, Uruguay, Ireland, Norway), often with strong genetic links to national breeding programmes. In some cases (e.g. NZ, Ireland, Norway, UK), PACs are being taken to breeders' farms to measure methane from industry flocks participating in progeny testing or national breeding programmes. The GrassToGas team has produced standardised protocols for both PAC and feed efficiency measurements, which could help in collation of methane records from different countries via international collaborations. Measurement of more animals can help accelerate the contribution of sheep breeding to methane mitigation.

The next steps are to develop environmental selection indices, including methane emissions, for breeding programmes, once sufficient data are available. Mitigation targets can be expressed as absolute methane emissions or "methane intensity" (methane per kg product). Improving productivity and reducing production inefficiencies favourably impacts methane intensity, but may increase absolute emissions. However, direct genetic selection for low emitting animals reduces total methane emissions. One main project conclusion is that selection for lower methane emissions should be considered in the framework of a multi-trait selection index, allowing optimised genetic improvement across several important traits, even when some genetic associations are unfavourable.

One of the most successful outcomes for the project is truly independent validation of methods and protocols to monitor complex phenotypes associated with environmental impact. The consortium will continue working together under the newly-funded Green-Era-Hub 'Sustain Sheep' project, and hopefully into the future via the Global Methane Hub.

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