15. Strategies to mitigate greenhouse gas emissions from pasture-based sheep systems – an EU project consortium view

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Abstract

An international project ('Grass To Gas', 2019-2024) to combine expertise and generate new knowledge for the reduction of the potent methane (CH_4) greenhouse gas (GHG) from sheep is underway. Aims are to validate predictors of feed intake, methane emissions and feed efficiency, to investigate the association between feed efficiency and methane emissions measured indoors and outdoors, to explore the opportunity of using genetics and genomics (animal and microbiome) to reduce methane emissions in pasture-based sheep systems and quantify the economic and environmental benefits of more feed-efficient and lower GHG-emitting sheep linked to their microbiome. The potential impact is to deliver applied, sustainable solutions to reduce methane emissions for the international sheep breeding community, by bringing together the latest precision livestock monitoring and molecular technology to identify novel selection targets and potentially candidate genes.

Introduction

International commitments to reduce methane emissions to mitigate the impacts of climate change have been made by over 50 countries through the Paris agreement to limit global temperature rises to less than 1.5 degrees C; the recent Global Methane Pledge is to lower 2020 methane emissions level by 30% by 2030. Effective methane mitigation solutions are urgently needed to reduce CH_4 emissions for sustainable sheep production systems worldwide. In New Zealand, it has been shown that selective breeding can be used to achieve cumulative reductions in CH_4 per animal (Rowe *et al.* 2021), but there is no consensus about how best to implement direct and indirect measurements of CH_4 into breeding programmes internationally.

Materials & methods

Research objectives are designed to explore and validate results emanating from different countries undertaking replicated studies using native or economically important breeds. In the UK where new studies on individual animal feed intake indoors and outdoors are taking place, 250 Texel cross lambs sired by performance-recorded rams that had also been CT scanned as a lamb were monitored post-weaning for 6 weeks using CIEL-funded Biocontrol individual feed intake monitoring equipment in 2021 (and replicated in 2022). Their growth, body composition and rumen volumes (as determined by CT) at the start and finish of the trial (14 and 20 weeks of age on average) were also recorded. Paired lambs (related half- or full-sibs of those monitored indoors) were grazed out of doors on mixed perennial ryegrass swards and growth, body

composition and grazing offtake measured, which was also replicated in Turkey using 250 Akkaraman sheep. In Norway, the effects of roughage quality (grass silage and freshly cut grass) and sheep breed on enteric CH_4 were investigated. Twenty Norwegian White sheep (NWS) and 20 Old Norwegian spael (ONS) were housed in individual pens and fed *ad libitum* roughage. Feed intake was monitored using the BioControl system. In both experiments, repeated 50-minute CH_4 concentrations were captured in portable accumulation chambers (PAC). Each ewe in the grass silage experiment had 30 PAC measurements, while each ewe in the fresh grass experiment had 21 measurements. Individual animal CH_4 measurements were collected on 454 NWS adult females from 44 breeding flocks from 12 ram circles. Detailed results of these measurements are reported in a separate, related paper (Jakobsen *et al.* WCGALP, 2022).

A validation of the n-alkane technique in Ireland estimated dry matter intake (DMI) of mixed breed sheep grazing perennial ryegrass swards. A total of 242 *in vivo* DMI measurements were obtained. Lin's concordance correlations were used to examine the relationship between the ratio of C_{33} / C_{32} n-alkane when predicting DMI. A series of experiments were carried out using 60 nulliparous ewe lambs whereby to measure CH_4, O_2 and CO_2 levels were measured over 50 minutes using PAC. Data were recorded on multiple days pre- and post-respiration chamber measurements. PAC measurements were subsequently collected on 2,404 animals varying in life-stage, breed, sire line, production type and diet type to quantify genetic variation in methane output within the commercial Irish sheep population.

Feed efficiency and CH_4 emissions have also been measured in Uruguay in 940 Australian Merino, 223 Corriedale, 360 Dohne Merino and 77 Texel lambs, which are part of to the national genetic evaluations, with the objective of investigating phenotypic and genetic associations among these traits, as well as with others relevant for sustainable sheep farming. Individual feed intake is recorded by Intergado® automated feeders during 56-day tests in which lambs are fed *ad libitum* with lucerne haylage. At the end of each test, CH_4 and CO_2 emissions are measured twice using PACs.

In France, male lambs of the Romane breed were measured at 3 months of age for performance, feed intake and feeding behaviour. These lambs emanated from two divergent lines for Residual Feed Intake (RFI) under a concentrate diet. The same measurements were also taken using a mixed diet. A protocol for GHG emissions phenotyping with GreenFeed (C-Lock) was defined. After each feeding period, blood, rumen fluid and faeces were sampled. Metabolomics analyses were undertaken on plasma and rumen fluid, and ruminal microbiota was sequenced for the 16S and 18s rRNA genes with the objective of identifying proxies of feed intake and GHG emissions, by using multivariate analyses (PLS, PLS-DA, Random Forest, SVR). In 2020, rumen volumes of 24 extreme Romane male lambs were CT-scanned to determine body composition and rumen size.

In New Zealand, animals selected divergently for methane emissions have also been measured for rumen microbial composition, milk, meat and wool composition, growth and slaughter traits, faecal egg counts and physiology of the alimentary tract. Part of this program has involved over 1000 female lambs measured for feed efficiency and feed intake traits to estimate relationships between methane emissions, microbial composition and gut related physiology. During this project, the role of New Zealand is to share protocols and data and to supply newly developed rapid sequencing technology of rumen microbes (Hess *et al.*,2020). Details of the feed efficiency programme are described in Johnson *et al.* (2018) and associated PAC measurements in Jonker *et al.* (2018).

Results

For feed intake in the UK after only 1 year of trial work, the Texel-X lambs showed significant between-sire variation in both average daily gain (ADG) and dry matter intake (DMI). At the sire level, initial results did not show a strong relationship between ADG and DMI. Live weight explained 41% of the variation in rumen volume (a potential methane predictor), with considerable variation in live weight adjusted rumen volume (CV 22 and 29% at each scan age), which differed significantly between sires, but was not significantly related to DMI in initial analyses. In the Norwegian grass silage experiment, DMI of early and average cut silage was 2.479 kg and 1.835 kg for NWS, and 1.337 kg and 1.143 kg for ONS, respectively. The difference in DMI was also reflected in enteric CH_4, being 1.65 vs 0.95 grams CH_4/hour for NWS and ONS respectively. When corrected to a mean DMI, ONS still had lower enteric CH_4 compared to NWS, (1.09 vs 1.52 grams CH_4/hr). Similarly, the early cut grass silage resulted in lower CH_4 emissions compared to the average, being 1.28 vs 1.32 grams CH_4/hour. Results from the 4,534 gas measurements of breeding females from 44 NWS flocks revealed mean CH_4 g/hr of 1.37 (s.d 0.28) and heritability CH_4/hr of 0.17 (se 0.04).

When estimating feed intake from grass, using the n-alkane technique in Ireland, the Lin's concordance correlation coefficient and the r-squared calculated between n-alkane estimated DMI using the ratio of C_{33} / C_{32} and measured DMI was 0.59 and 0.79, respectively. Offering animals low and medium herbage mass provided a more accurate estimate of DMI using the n-alkane technique. When investigating the measurement of CH_4 output, the time of day that animals were measured had no effect. For all three gases (CH_4, oxygen and carbon dioxide), the between-day variance (39 to 40%) accounted for a larger proportion of total variance compared to between-animal variance. The correlation between CH_4 production measured in PAC and the respiration chambers was 0.55, similar results were reported in New Zealand (Jonker *et al.*, 2018). When the data edits were applied to the dataset based on the rate of gaseous emissions 44 animals were retained, a higher correlation of 0.66 was estimated.

In determining the most appropriate model for the estimation of residual feed intake (RFI) using Uruguayan data, important fixed effects include pen-trial, average daily gain and metabolic body weight, whilst body composition and fleece growth traits were not significant ($P>0.05$) (Marques *et al.*, 2021). Amarillo *et al.* (unpublished data) also found the potential of reducing by 7 days the length of the feed efficiency test, without compromising the accuracy of RFI estimates, with R^2 being around 70%. The comparison of high, medium, and low RFI groups of lambs confirmed that more efficient animals are those with lower feed intake at similar performance levels in all breeds. Phenotypic Correlation coefficients in Corriedale, Dohne and Merino were moderate to high between CH_4 emissions and feed intake (0.8 to 64) but weaker with RFI (0.01 to 0.20). No differences in RFI were found between resistant (R) and susceptible (S) Corriedale sheep to internal parasites selected from divergent selection lines (Ferreira *et al.*, 2021). A Principal Component Analysis including body weight, average daily gain and gas emissions measured by PACs indicated the possibility of estimating feed intake, with a precision above 72% in allocating animals to high, medium, and low observed feed intake groups. More detailed results are in Le Graverand *et al.* WCGALP 2022.

In New Zealand, Johnson *et al.* (2018) reported that the most appropriate models to estimate RFI included fitting cohort, average daily gain and metabolic mid-weight ($P<0.05$) reaching an accuracy of 0.88. It also showed that whilst reduced periods of feed intake data could be used to estimate RFI, to obtain the most accurate average daily gain estimates the full 42d test period was most appropriate. The heritability estimates for CH_4 measured as absolute emissions and CH_4 yield ($CH_4/CH_4 + CO_2$) were 0.26±0.04 and 0.24±0.03, with the heritability estimate for RFI 0.46±0.13. The phenotypic (r_p) and genetic (r_g) correlation between CH_4 and the weight of the lambs were 0.62±0.03 and 0.84±0.05 respectively for absolute CH_4 emissions and 0.42±0.10 and 0.07±0.05 for methane yield. The phenotypic and genetic correlation between CH_4 and RFI were -0.18±0.12 and 0.08±0.05 respectively for absolute CH_4 emissions and -0.45±0.13 and -0.25±0.04 for

methane yield. There was a negative r_p between RFI and ultrasound fatness at the start of the measurement period (-0.23±0.04), and a positive r_p for RFI and change in fatness across the trial (0.21±0.04).

After three generations of divergent selection (2 s.d.) for RFI in France, more efficient animals (low RFI) ate 10% less concentrate, had shorter daily feeding duration with longer time intervals between feeder visits than less efficient (high RFI) animals. Ruminal microbiota and metabolomics do not yet show any significant difference between efficient and less efficient animals, regardless of the diet, however, more efficient animals have higher relative concentrations of citrate and malate and lower relative concentrations of amino acids in their blood than less efficient animals. Efficient animals also tended to have larger rumens, when expressed in relation to body weight (P-value=0.09). The correlation between indoor concentrate intake and indoor forage intake is positive and that with RFI are positive, being 0.22 and 0.25 respectively. In New Zealand, it has been shown that divergent selection for methane is associated with rumen size with high emitters of methane having larger rumens.

Discussion

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. Although a full meta-analysis of data is yet to take place, sharing of methods, protocols and equipment has ensured that studies are comparable. Very similar heritabilities and repeatabilities from the analysis of methane emissions from PACs across different countries and sheep breeds indicate that the same trait is being evaluated and that global comparisons are possible using co-developed or co-validated proxy methods. The roles of feed intake, feed quality, liveweight, body composition and health status in accounting for variations between animals' performance and CH_4 emissions has been central to the investigations so far in this project. The use of PACs in experimental as well as commercial settings has generated data to optimise breeding programmes of the future. Participating countries should consolidate their reference populations locally and internationally to augment the role that genomic selection will have in tackling the problem of GHG emissions from livestock.

Acknowledgements

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