An Roinn Talmhaíochta,
Bia agus Mara
Department of Agriculture,
Food and the Marine

Project Ref - 16/RD/ERAGAS/1 Predicting appropriate GHG mitigation strategies based on modelling variables that contribute to ruminant environmental impact

Final Report

This project was funded under the Department of Agriculture, Food and the Marine Competitive Funding Programme.

SUMMARY

The main objectives of this project was to better understand the relationship between the rumen microbiome and methane emissions in beef cattle. To achieve these objectives, a 3 tired approach was implemented including 1) Identifying cattle divergent in their level of feed efficiency and environmental output; 2) Identify key rumen microbes associated with methane emissions and 3) to discover novel DNA based biomarkers associated with enhanced feed efficiency, lower environmental load and efficient rumen microbiome for integration into the genomic selection breeding programme. Furthermore this study aimed to standerdise molecular methods utilised in the study of the rumen microbiome. Through this project, the first large scale measurement of methane emissions in Irish cattle was conducted. Findings from this project have identified the residual methane emissions concept as the optimal phenotype for assessing the methanogenic output of an animal, as it is the only phenotype observed to be independent of all measures of animal productivity but strongly correlated with enteric methane output. Furthermore, when animals were ranked on the basis of residual methane emissions, low ranked animals produced 30% less methane, but had the same level of growth performance and carcass output has high ranked animals. A cohort of lactic acid producing bacteria were observed to be associated with a low residual methane emissions phenotype and therefore may act as potential ruminal biomarkers for low methane output. Findings from this research project will be utilised to identify host DNA based biomarkers associated with RME, as part of the EU Horizon 2020 funded MASTER project, whereby similar data has been amassed on near 1000 beef cattle.

KEYWORDS

Methane, cattle, rumen

ACRONYM	RumenPredict
PROJECT COORDINATOR, INSTITUTION	Professor Sinead Waters, Teagasc, Animal & Grassland Research and Innovation Centre
EMAIL	sinead.waters@teagasc.ie
COLLABORATORS, INSTITUTION	Queen's University, Northern Ireland. Natural Resources Institute Finland, Finland. Agresearch, New Zealand. Swedish University of Agricultural Sciences, Sweden. Teagasc, Ireland. Irish Breeding Federation, Ireland. University College Dublin, Ireland. Wageningen University, The Netherlands. Institut National de Recherche en Agronomie (INRA), France.
PUBLICATION DATE	April 2022

Section 1 - Research Approach & Results

Start Date

01 September 2017

End Date

30 September 2021

Select Research Programme

Research Stimulus Fund

Please select the appropriate TRL Scale

TRL 1: Basic Principles Observed

NRPE Priority area

Sustainable Food Production and Processing

Total DAFM Award

€244,418.07

Total Project Expenditure

€209,959.00

Rationale for undertaking the Research

Agriculture is Ireland's oldest indigenous industry and employs 7.1% of the Irish work force (DAFM, 2021a). In 2019, Irish agri-food exports were valued at €14.5 billion (DAFM, 2021a). Beef dominates as Ireland's primary farming enterprise (Dillon et al., 2019) with over 100,000 Irish farms contributing to beef production with 528,231 tonnes of beef exported in 2019 to value of €2.3 billion (DAFM, 2021a). In spite of the key role cattle production has in Irish society, enteric methane emissions originating from cattle, produced as a byproduct of rumen microbial fermentation, is accountable for ~19% of Ireland's greenhouse gas (GHG) emissions. Indeed, a target of a 10% reduction in enteric methane emissions has recently been established as part of the FoodVision 2030 strategy for the Irish agrifood sector. Furthermore, to achieve both the agrifood sector and state's ambition to be carbon neutral by 2050, a 24-47% reduction in enteric methane has been deemed a necessity. The genetic selection of low methane emitting animals has long been advocated as mitigation strategy for the ruminant livestock sector. However, prior to the commencement of this project, no large scale measurements of methane output had been conducted in Irish beef cattle. Furthermore, due to the relationship of feed intake with methane output, there was little consensus about how best to identify low methane emitting cattle or the key rumen microbes associated with the methanogenic output of an

animal. In addition, there was little consensus of the optimal way to perform rumen microbial 16S analysis.

Methodology

To perform the first large scale measurement of enteric methane output in Irish beef cattle, a GreenFeed System was purchased through the RumenPredict project. Subsequently, the expertise gained in the workings of the GreenFeed, from this project, then provided the platform for an additional 5 GreenFeeds to be purchased through both EU and national funded projects. In total, over the duration of the RumenPredict project, 6 GreenFeeds were installed at the National ICBF Bull Beef Progeny Performance Test Centre in Tully, Co. Kildare. Over ~2 year period, detailed measurements of enteric methane output, feed intake, feed efficiency, growth rate and carcass output were recorded on near 300 beef cattle. In addition, rumen fluid samples were also obtained during the last week of the methane measurement period, in order to better understand the key microbes associated with the methanogenic output of an animal. Numerous methods for expressing the quantity of methane an animal produces were assessed including traits such as daily methane (g/day), yield (g/ kg of dry matter intake (DMI)), intensity (g/kg of carcass output) and residual methane emissions (RME; g/day; difference between an animal's actual and predicted level of methane output, based on its DMI and body weight). The relationship of all methane indices with key metrics of animal performance such as feed intake, growth rate, feed efficiency and carcass output, were determined.

Prior to investigating the relationship of the rumen microbiota with methane output, a rumen specific 16S amplicon sequencing standard was developed, in collaboration with colleagues in Wageningen University. This standard, consisting of rumen bacteria and archaea, was sequenced on three separate Illumina MiSeq sequencing runs. The reads generated were then aligned to four different 16S reference databases. findings from this element of the project were then utilized to correlate the rumen microbial composition with methane output.

Project Results

Detailed information on feed intake, growth rate, feed efficiency, bodyweight, carcass output and enteric methane out was measured over a 90 day period. From the data generated at Tully, it was established that for a typical indoor finishing ration, beef cattle produce, on average, 22 g of methane for every kg of feed dry matter consumed (i.e. DMI). In order to better assess animals on their methane emissions vis a vis, growth performance, the relationship of various expressions of an animal's enteric methane output with productivity was investigated. The RME index was the only methane trait observed to be independant of any measure of animal growth performance, but was the best predictor of daily methane emissions. Based on this, cattle were ranked as high, medium or low in terms of RME. Low RME animals (efficient) produced, on average, 30% less methane, despite having the same level of feed intake, feed efficiency, growth and carcass output as their

high (inefficient) ranking RME contemporaries.Results from this study, which were recently published in the American Journal of Animal Science, highlight the potential to breed more environmentally sustainable animals, while at the same time not having a negative impact on the animals' performance, and indeed profitability.

In a bid to identify potential rumen microbial biomarkers associated with the RME trait, rumen microbial samples from 250 beef cattle were successfully sequenced on the Illumina MiSeq. The bioinformatics analysis of this proportion of the study was guided by the findings from the previous analysis performed on the rumen specific standard, whereby it was deemed the RefSeq+RDP database was the most suitable database for the alignment of 16S amplicon reads when studying the rumen microbiome. An increased abundance of lactic acid producing bacteria and bacteria capable of converting lactic acid to propionate, was identified in low RME animals. Furthermore, low RME animals had an increased production of ruminal propionate. Differences in the composition of the rumen archaeal community, between high and low RME ranked animals persisted, with an increased abundance of the Methanobrevibacter RO clade observed in low RME animals. The observed differences in the composition of the bacterial and archaeal rumen community, suggests the potential for an increased abundance of lactic acid producing bacteria to be an effective rumen microbial biomarker for low RME cattle.

Section 2 - Research Outputs

Summary of Project Findings

This project has identified the residual methane emissions index as the most appropriate trait for identifying the true methanogenic output potential of beef cattle. Voluntary feed intake has one of the largest influences on both an animal's level of methane output and growth rate. Residual methane emissions was the only trait observed to be independent of all measures of animal productivity, but strongly correlated with daily methane emissions. Indeed, animals ranked as low for RME, produced 30% less methane but maintained the same level of growth performance as their high contemporaries. An increased ruminal abundance of lactic acid producing bacteria was identified as a potential ruminal biomarker for a low RME phenotype.

The findings from this research project has identified the RME concept as the promising phenotype for inclusion in the national breeding index, to bring about a reduction in the methane output of future generations of livestock via genetic selection. While the concept will need to be further validated at pasture, the RME phenotype has the potential to be readily embraced by the livestock community, due to its ability to identify low methane emitting cattle, without impacting animal productivity and therefore, on farm profitability.

Finally, the results from the rumen microbial analysis conducted as part of this study has improved our understanding of the key ruminal microbes contributing to inter animal divergence in methane output. Therefore, this project has identified to the key rumen microbes to target as part of the development of future dietary abatement strategies.

Summary of Staff Outputs

Research Output	Male	Female	Total Number
PhD Students	1	0	1
Research Technicians/ Assistants	1	0	1

Summary of Academic Outputs

Research Outputs	Total Number	Details
PhD Theses	1	One PhD was successfully completed as part of this project. The student recently successfully defended his viva (3/2022) which was titled "Understanding the interactive role of the rumen microbiome with diet and enteric emissions in cattle ".
Publications in Peer Reviewed Scientific Journals	3	Three peer reviewed publications have been produced as part of this project. Two publications have been published, with the most recent publication accepted for publication and currently undergoing typesetting for publication in Frontiers in Microbiology. furthermore, throughout the duration of this project, members of the Irish research consortia were invited to contribute a chapter to the book titled "Improving rumen function". Both published papers, the book chapter as well as a draft version of the paper currently undergoing typesetting, are attached. The titles of the publications are: Effect of divergence in residual methane emissions on feed intake and efficiency, growth and carcass performance, and indices of rumen fermentation and

	methane emissions in finishing beef cattle. Journal of Animal Science. 99 Synthetic sequencing standards: a guide to database choice for rumen microbiota amplicon sequencing analysis. Frontiers in Microbiology. 11. Differences in the composition of the rumen microbiota of finishing beef cattle divergently ranked for residual methane emissions. Frontiers in Microbiology. undergoing typesetting. Role of the rumen microbiome in pasture fed ruminant production systems in Improving rumen function. (ed.) McSweeney C. and Mackie R. (Burleigh Dodds Science Publishing).
	Below is some of the conferences data generated as part of RumenPredicted was presented at. Furthermore, findings from this research project will also be presented at the 8th GGAA – Greenhouse Gas and Animal Agriculture Conference, Orlando, Florida in June 2022. Smith, P.E., Waters, S.M., Kenny, D.A., Kirwan, S.F., Conroy, S. and Kelly, A.K. 2021. The effect of phenotypically ranking beef cattle for residual methane output on daily methane emissions, intensity and animal productivity. Proceedings from the British Society of Animal Science Virtual Conference. April 12-15th, 2021. P18.
Peer Reviewed Conference Papers	Smith, P.E., Gómez Expósito, R., Edwards, J.E., Waters, S.M., McCabe, M.S. 2019. Development of a bovine rumen-specific amplicon sequencing standard. Proceedings from the 7th GGAA – Greenhouse Gas and Animal Agriculture Conference, Iguassu Falls, Brazil. August 4-8th, 2019. P36. Smith, P.E., Kelly, A.K., Kenny, D.A. and Waters, S.M. 2021. Individual members of the rumen archaeal community differ in cattle ranked based on residual

methane production. Proceedings from the 12th International Symposium on Gut Microbiology. October 13-15th, 2021. P115.

Intellectual Property

None

Summary of other Project Outputs

Project Outputs	Details	Total No.
New Processes	This was the first project in Ireland to utilise the GreenFeed System to measure methane emissions from livestock. The expertise gained as part of this project have directly improved the methane mitigation research capabilities of Irish research institutes. Indeed, the expertise acquired throughout this project have been utilsed to establish methane mitigation research programmes across multiple Teagasc Research Centers (Grange, Moorepark, Athenry and Johnstown) as well as the Irish Cattle Breeding Federation bull beef progeny test center.	1

Potential Impact related to Policy, Practice and Other Impacts

Impact	Details
Environmental Sustainability	Findings from this project have the potential to directly benefit national ambitions to reduce enteric methane emissions and achieve carbon neutrality by 2050. By identifying RME as the optimal trait for assessing the methanogenic output of an animal and the ruminal abundance of lactic acid producing bacteria as potential biomarkers for an animals inherent methane output, this project has provided the ground work for future methane mitigation research and breeding objectives for the national herd.

Dissemination Activities

Activity	Details
Media Events	National media: https://www.irishexaminer.com/farming/arid-40762221.html https://www.independent.ie/business/farming/news/some beef-cattle-emit-30pc-less-methane-with-no-loss-of-productivity-study-finds-41124169.html https://www.independent.ie/business/farming/forestry-enviro/environment/promising-results-from-research-into-breeding-cattle-with-loweremissions-41112353.html https://www.farmersjournal.ie/methane-emissions-30-lower-for-some-beef-cattle-665685 https://www.agriland.ie/farming-news/methane-measurement-study-reveals-positive-findings-for-the-beef-sector/ https://www.farmersjournal.ie/tully-now-climate-testing-all-beef-progeny-467950 International media: https://phys.org/news/2021-12-results-ireland-large-scale-methane-emissions.html https://www.thecattlesite.com/news/57767/ireland-breeding-for-lowmethane-beef-cattle-possible/ https://www.alphagalileo.org/en-gb/Item-Display/ItemId/215367?returnurl=https://www.alphagalile .org/en-gb/Item-Display/ItemId/215367 https://eurocarne.com/noticias/codigo/52754/kw/Teagasc-analiza+c%C3%B3mo+reducir+la+producci%C3%B3n+de+m tano+en+ganado+vacuno https://www.eurasiareview.com/06122021-promising-results-from-irelands-first-large-scale-measurement-of-methane-emissions-in-beef-cattle/https://thatsfarming.com/beef/methane-emissions-in-beef-cattle/
Seminars at which results were presented	Paul Smith, Ruth Gómez Expósito, Joan Edwards, Cia Carberry, Sinead Waters , Matthew McCabe. Amplico Sequencing - The Positives of Being a Control Freak . The 9t Annual Irish Next Generation Sequencing Meeting, Dublir Ireland. 30th May, 2019. Paul E Smith, Stephen Conroy, David A Kenny, Alan K Kelly Matthew S McCabe and Sinéad M Waters. Methan Emissions from Suckler Beef - An update from RumenPredic

	ICBF and Sheep Ireland Genetics Conference, Laois, Irelanc January 17th, 2020. Paul E Smith and Sinead M Waters. Relationship of fee efficiency with enteric methane emissions. BovINE E webinar on Why feed efficiency matters. 4th April, 2022. Paul E Smith, David A Kenny, Alan K Kelly, Matthew S McCabe Stephen Conroy and Sinéad M Waters.Reducing Methan Emissions from Irish Agriculture-The role of the rume microbiome. C-Lock Inc Webinar series. 12th February, 202 Sinéad M. Waters, Stuart F. Kirwan, Steven McLoughlin an Paul E. Smith. Understanding the role of the rume microbiome in reducing enteric methane emissions. Rume Microbial Genomics Workshop. 15th October, 2021 Sinéad M Waters, Paul E Smith, Emily McGovern, Alan K Kell and David A Kenny. Role of the rumen microbiome i enhancing feed efficiency and reducing methane emissions i ruminants. SRUC-Teagasc Research Seminar. 6th Decembe 2019. Sinéad M Waters. Strategies to reduce enteric methan emissions from agriculture. The Signpost Series Webinar 4th of February, 2021. November 10th, 2021. Side event at COP26 organised b WUF. The governments of the Netherlands, New Zealand, Colombi and Kenya, the International Livestock Research Institut (ILRI) and Wageningen University and Research (WUF organized the UNFCCC COP26 side-event 'Toward Sustainable livestock systems'. Livestock systems play a crucial role in reaching global goal We discussed how can we stimulate action towards a climate neutral, biodiversity friendly and food secure livestock secto SW invited due to role as co-chair of the LRG or the GRA an member of EC expert group on methane (DG-AGRI) - a opportunity to discuss our Irish actions/strategies bein developed or under research on reducing emission particularly methane from agriculture.
Workshops at which results were presented	Paul E Smith, Ruth Gómez Expósito, Joan E Edwards, Sinéa M Waters, Matthew S McCabe. Rumen Microbial Amplico Sequencing - Positives of Being a Control Freak. CR Genomics mini symposium on Animal, Plant and Marin Genomics. October 16th, 2019. Paul E Smith, Stephen Conroy, David A Kenny, Alan K Kelly Matthew S McCabe and Sinéad M Waters. An update fror the Irish consortium of RumenPredict. Feed and Nutritio

	Network Workshop - LRG/GRA GLOBAL NETWORK projec Brazil. August 4th, 2019. Paul E Smith. The Rumen Microbiome and Methan Emissions. National University of Ireland Galway researc seminar. 10th March, 2021. Paul E Smith and Sinéad M Waters. Estimating Enteri Methane Production from Cattle. World Wildlife Fund -Th Potential for Seaweed as Livestock Feed. 10th October, 2020

Knowledge Transfer Activities	At numerous national and international arranged events, data from this project was disseminated to the general public. Furthermore, this project has featured in the Teagasc beef newsletter,
Identify knowledge outputs generated during this project.	TResearch articles and the Teagasc Signpost series newsletter (all attached in proceeding sections). Furthermore, as part of the Teagasc Beef Open day in 2020, numerous pieces for social media (both videos and written posts) were disseminated.

Identify any knowledge transfer activities executed within the project.	Teagasc Beef Open Day 2018, 2020 Agricultural Science Association-Meat Myth Busters event. February 2020. Science Week open day at Teagasc Grange 2018, 2019 Agri-aware open day Teagasc Grange 2018, 2019 IFA livestock committee farm visit to Teagasc Grange. March 2022. C-Lock Inc webinar series 2020. ICBF and Sheep Ireland National Conference 2019.
List any impacts resulting from the knowledge transferred during the project.	None

Section 3 - Leveraging, Future Strategies & Reference

Leveraging Metrics

Type of Funding Resource Funding Summary

EU R&I programmes	€600,000.00	EU Horizon project 'MASTER' funded in 2019 (€10M in total - led by Teagasc): PI of Rumen Predict (SW) partner in WP3 of MASTER with other partners of RumenPredict and extended our objectives/task to add power and value to our original study. Focused on understanding the rumen microbiome in cattle and sheep with a particular interest in reducing GHG emissions.
Exchequer National Funding	€1,200,000.00	METH-ABATE - funded by DAFM - focused on developing and evaluating feed additives for ruminants to reduce methane emissions. Used skills and infastructure in Rumen Predict project.
EU R&I programmes	€1,023,000.00	EU project 'HoloRuminant' (10M euro project led by INRA in France) - focused on understanding microbiomes associated with performance and GHG output from cattle and sheep. SW is a WP leader and gained €1.02M for the laboratory in GE.
Exchequer National Funding	€350,000.00	SeaSolutions - reducing methane emissions using seaweed and their extracts.

Future Strategies

Our future work is focused on the development of breeding strategies with ICBF to enhance feed efficiency and reduce GHG emissions.

Future works is also very focused on the development of feed additives for reducing methane emissions from ruminants particularly in pasture based systems.

Refinement of our national inventories for methane emissions beef and dairy cattle - working with EPA

Project Publications

Waters S. M., Kenny D. A., Smith P.E. 2020. Role of the rumen microbiome in pasture fed ruminant production systems in Improving rumen function. (ed.) McSweeney C. and Mackie R. (Burleigh Dodds Science Publishing).

Smith, P.E., Waters, S.M., Expósito, R.G., Smidt, H., Carberry, C.A. and McCabe, M.S. 2020. Synthetic sequencing standards: a guide to database choice for rumen microbiota amplicon sequencing analysis. Frontiers in Microbiology. 11.

Smith, P.E., Waters, S.M., Kenny, D.A., Kirwan, S.F., Conroy, S. and Kelly, A.K. 2021. Effect of divergence in residual methane emissions on feed intake and efficiency, growth and carcass performance, and indices of rumen fermentation and methane emissions in finishing beef cattle. Journal of Animal Science. 99

Smith, P.E., Kelly, A.K.,Kenny, D.A. and Waters, S.M. 2022. Differences in the composition of the rumen microbiota of finishing beef cattle divergently ranked for residual methane emission. Frontiers in Microbiology. currently undergoing typesetting.

Supporting documents

Teagasc-Beef-Newsletter---March-2022
Author's Proof (3)
TResearch_Winter2020_CuttingGHGs_Web
TResearch_Spring2019_RumenMicro_p20-21_proof
Chapter Improving Rumen Function Waters Kenny and Smith
Author's Proof (3)
fmicb-11-606825 (3)
skab275 (3)