

Mitigating Livestock Methane Emissions through Animal Selection Genetics and Genomics

A ‘white paper’ outlining stage 2 of establishing a Global Research Network

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All responsibility for any errors or omissions rests with the author.

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Executive Summary

Background

This 'white paper' identifies an opportunity for NZ to contribute significantly to a proposed Research Network focussed on the goal of reducing methane (CH₄) intensity in ruminant livestock by capitalising on animal to animal genetic variation.

A workshop funded by the New Zealand Government to support the goals and objectives of the Global Research Alliance on Agricultural Greenhouse Gases (the Alliance), was held in Auckland in May 2011. This workshop brought together international researchers with expertise in the area of animal selection, genetics and genomics to discuss the feasibility of setting up "a network on animal breeding approaches to reduce methane emissions".

A key aspect to breeding sheep and cattle which can reduce CH₄ emissions intensity is the identification and estimation of the genetic parameters that can contribute most to cost effective reductions in emissions intensity. The workshop identified considerable synergies in combining data sets; as funding constraints can potentially limit the value of existing national projects. The workshop also identified a need to standardise measurement protocols (or at least have robust calibration and conversion equations), establish common reporting formats and collect contemporary rumen and DNA samples from all current and future studies.

Workshop Findings

Participants at the workshop agreed:

- There was value in establishing a formalised network in the research area of animal selection, genetics and genomics aimed at identifying genetic traits associated with lower emissions intensity in individual animals
- Collection and storage of DNA and rumen samples from all animals tested should be routine
- There is a need for common measurement protocols, regular meetings, information exchange, and collaboration across countries
- There is a lack of scientific clarity around breeding objectives, which is due the potential for genetic interactions with age, physiological status, feedstuff, feeding level and environment (GxE)
- A combined database of information from existing national research programmes is needed
- Reporting of feed intake measurement technology, appropriate breeding objectives and data transformations should include an indication of raw data as well as the more usually reported point measurements since the trait of interest (breeding objective) is typically emissions or intake over a year for a breeding individual and its offspring
- Genetic selection and targeted breeding could reduce emissions per unit product through selecting genetic traits that increase the general efficiency of production (eg milk yield, reproductive efficiency and disease status per animal)
- CH₄ emissions differ between individual animals even after adjustment for intake and that the heritable component of intake that is independent of liveweight and liveweight changes can be changed by selection

- That technologies to rapidly and cost effectively measure CH₄ emissions is a priority research area
- That it would be advantageous if results could be presented in a common format and, where practicable, merged and meta-analyses conducted
- That while there would not be direct translation between species, it is highly likely that information derived from one species would provide a very good indicator for application to another
- That the most appropriate methods for rumen contents collection and storage are difficult to define although experiments are underway assist in the identification of best practice protocols

Agreed Priority Issues and actions arising from the workshop

The workshop agreed on the following priority issues and actions, subject to participating countries and individuals being able to secure necessary funding to deliver on these action points.

1. The establishment of an Animal Selection, Genetics and Genomics Network.
 - Australian and New Zealand scientists would take primary responsibility for the establishment of the Network
2. The establishment of a common database for the storage of phenotype and pedigree data. These data will be available for genome wide association studies.
 - Dr Eileen Wall will explore the possibility of using an existing UK database to progress this.
 - New Zealand would contribute by employing a post-doctoral researcher to work across the Network to facilitate the combining and analysis of these combined data sets;
3. The establishment of protocols for storing samples from all animals measured to be used for subsequent DNA analysis
4. New Zealand would contribute by coordinating establishment of these protocols
5. Continue discussions on defining which co-traits should be targeted and how these would be incorporated into breeding objectives.
 - Dr Julian Hill (Australia) explained that a planned workshop in Australia in 2012 would be an efficient way to progress this. The working title of this workshop 'Defining the low CH₄ emitting phenotype'. Members of the proposed Network would be invited to participate in the workshop.
 - New Zealand would contribute by employing a post-doctoral researcher to work across the Network to facilitate the development of an agreed format for expressing traits and breeding objectives; and
6. Develop a set of common protocols to guide the search for the rapid measurement of CH₄ and intake when repeated measurements on large numbers of animals are required.
 - New Zealand would take a lead in progressing this via the employment of a post-doctoral researcher to work with members of the Network to develop the common protocols.

New Zealand Support

To allow New Zealand science to contribute to the priority actions agreed by the workshop participant's support is needed in two areas.

1. Proposed co-coordination of the Animal Selection, Genetics and Genomics Network.

New Zealand scientists will work with Dr Hutton Oddy (DPI, New South Wales) to establish and coordinate the Animal Selection, Genetics and Genomic Network. This would involve defining a vision for the Network, writing a strategic plan and implementing this plan. **Funding of \$50K pa for a minimum of two years is needed to support these activities.**

2. Proposed appointment of a coordinator to facilitate the research undertaken by the Network.

A Post Doctoral position would be established whose goal would be to coordinate the science effort in the four identified priority areas. The appointee will work at the various sites that are contributing data in order to facilitate agreement on storing and analysing contributed data, define and refine common measurement protocols, help identify which co-traits should be targeted and determine how these would be incorporated into breeding objectives. **Funding of \$200K pa for a minimum of two years is needed to support these activities.**

1. Proposed co-ordination of the Animal Selection, Genetics & Genomics Network

Background

The use of animal genetic and genomic technologies to mitigate CH₄ emissions from ruminants through targeted breeding requires access to a very large resource of animals of different species and breeds. This is difficult to achieve for any single research organisation or even country. By operating in isolation, the huge costs involved in this type of research often result in programmes that are in fact sub-optimal in size.

In addition, a high quality DNA sample is required from every animal tested in order to enable DNA variant detection in regions of interest, automated assembly, and SNP, indel and CNV detection, plus analysis software.

Therefore, the most effective way to exploit the new technologies is for key research groups to function as a Network in order to leverage their individual resources by pooling information and resources. This was recognised by the Livestock Research Group of the Global Research Alliance (GRA) by including the establishment of an Animal Selection, Genetics and Genomics Network as a key element of its short term work plan.

Phase 1 of the establishment of an Animal Selection, Genetics and Genomics Network was an international workshop held in New Zealand in May 2011. Invited experts from across the globe attended the workshop to discuss the potential for reducing methane emissions and emissions intensity (emissions per unit product) by capitalising on animal to animal genetic variation in both cattle and sheep. During the workshop participants agreed that there was significant value in establishing a virtual global research Network to facilitate the development of common measurement protocols, encourage regular meetings of international research groups, and provide a channel for the exchange of information, and collaboration across countries.

The Network's objective is enteric methane emissions intensity through animal selection and targeted breeding, by using genetics and genomic tools. To achieve this, thousands of animals per species must be phenotyped, and subsequently genotyped and/or sequenced in the research phase. If all international parties pool information, undertake collaborative research and adopt a common DNA sampling and storage protocol this becomes possible.

The proposed establishment of an Animal Selection, Genetics and Genomics Network provides tacit acknowledgment that its goals could not be achieved in the absence of the Alliance.

Network development

The workshop participants clearly identified the value of a formal Animal Selection, Genetics and Genomics Network. Australia and New Zealand, as Network coordinators will establish and implement a formal plan for the network, including a vision for its long term success. This document is expected to outline how the network will:

- be administered and led
- communicate
- increase the membership to include all Alliance member countries
- determine what activities it will undertake
- share data and resources
- relate back to the Alliance's Livestock Research Group
- manage IP expectations

Proposed Network structure

The Network would meet twice per year. It is anticipated that one of these meetings would be face to face over two days and involve all of the science leaders and other relevant scientists/policy makers. The format will be similar to the Auckland Workshop held in May 2011. The meeting will be formal, and a report would be prepared after each meeting. New Zealand's participation in this workshop would be critical and it is anticipated that funding would be sourced at a later date for scientists to attend.

The second meeting will take place on an opportunistic basis, and would be arranged when members of the ASGGN are attending a significant International Conference. This would be an informal meeting and would not result in a formal report, although those present would contribute to an informal summary that would be available to all Network members.

Benefits of the Network

- enhance collaboration across the Network
- avoidance of unnecessary duplication of effort
- the opportunity to lobby for and leverage international funding
- the exchange of protocols and material
- optimised and common techniques and protocols
- wider access to technical knowledge and training
- improved communication between Network members
- international exchange of staff, knowledge and training

Outcomes of the Network

- the creation and agreement of guidelines/protocols for data acquisition and analysis that would be available to Network members
- standardised methods that scientists will use in their research
- centralised DNA storage facility
- international access to a world class DNA lab
- a web portal to display and promote research activities of ASGGN members

- international exchange of staff, knowledge and technologies

Contributors to the Animal Selection, Genetics and Genomics Network

One of the key tasks for the Network would be to encourage the participation of researchers from all Livestock Research Group member countries. The following table identifies the countries and organisations initially involved in the Network.

Country	Research Organisations
New Zealand	AgResearch DairyNZ AgResearch PGgRC Vialactia AgResearch Abacus Biotech AgResearch
Australia	Beef CRC CSIRO Murdoch University Sheep CRC University of New England University of WA
Canada	Agriculture & Agrifood Canada Alberta Ag & Rural Development University of Alberta University of Guelph
France	INRA
Ireland	Teagasc
Netherlands	Wageningen University
Brazil	EMBRAPA
UK	Abacusbio Agri-Food & Biosciences Inst Roslin Institute Rothamsted Research Scottish Agricultural College University of Edinburgh University of Nottingham University of Reading
USA	University of Montana University of Wyoming

Benefits of New Zealand's participation in the Network

The network will provide New Zealand with a great opportunity to integrate its animal selection, genetics and genomics activities in the CH₄ mitigation area with activities worldwide. The Animal Genetics team at AgResearch Invermay is world class, has an enviable international animal genomics presence and is well placed to manage New Zealand's contribution to the network. It is anticipated that a key role for New Zealand will be intimately involved in setting the vision for the Network and actively participating in its administration in order to help ensure that the Network will achieve its aims.

Expected cost

To successfully participate in the network New Zealand scientists would need an investment from the NZ government for:

- Funding of 0.15 FTE for at least 2 years (**50k** per annum; including overheads) for New Zealand to contribute to the Network. This would permit
 - co-coordination of the Network with Australia (Dr Hutton Oddy, UNE, Australia has offered to co-coordinate the Network).
 - writing and implementation of the plan for the Network
 - administration and co-coordination of Network activities including fostering of joint research projects and exploiting opportunities for research exchanges
 - establishing communication tools
 - establishing protocols for DNA storage throughout the network, to maintain the potential for reanalysing samples should 'new' traits be discovered

Other Network member countries that have agreed to participate will provide time and resources to facilitate the success of the Network. All available research funding opportunities would be used to facilitate the exchange of knowledge, scientists and technicians; for example LEARN, GRASS, Borlaug Fellowships

2. Proposed coordination and facilitation of research undertaken by the Network.

Background

Further to endorsing the Network, the participants at the workshop agreed to support and collaborate across three specific areas which would give a significant boost to science in the Animal Genetics space, and deliver outcomes that enable a significant advancement in the quest to deliver mitigation options for ruminant animals. By being a cornerstone participant in these projects, New Zealand has an important opportunity to use the Network to showcase its science strengths in the Animal genetics area.

It is proposed that a post doctoral fellow be appointed by a relevant New Zealand research institution, to work with scientists across the Network to co-ordinate the following three research priority areas.

A Storing, combining and analysing data sets;

The most effective way to use genomic technologies is for key groups to function in a networked environment that enables them to maximise the value of their individual resources by pooling information. In order for this to be meaningful, it is essential that all participants use standardised methodologies, data capture, data storage and presentation, and that the measurements recorded within these constraints are

accessible in a shared environment. The logical way to achieve this is through secure, managed access to a common database.

A database will be developed that

1. has sufficient storage capacity
2. is secure, but can be accessed globally
3. has input specifications and is compatible with a variety of computer platforms
4. has dedicated administration

Such a database is relatively costly to set up and maintain, and has the potential to generate IP issues. Dr Eileen Wall (SAC) has indicated that she has funding to establish a database for the UK. Dr Wall will work with the postdoctoral fellow to develop this version into a Database that is feasible for the Network to use.

B Developing an agreed format for expressing traits and breeding objectives;

Knowledge on the heritability of methane emissions and its genetic associations with other performance traits is key to predicting the expected responses to selection. Establishing how these parameters change with environment (i.e., interactions between the genotype and environmental conditions) is important for incorporation into breeding objectives. Workshop presentations identified the need for a defined trait before breeding objectives can be addressed.

A common task for all members of the Network is therefore to clearly define the optimal approach for reducing emissions intensity. Possible approaches include

1. breeding for improved biological and financial efficiency of the animal
2. breeding for improved biological and financial efficiency of the system
3. breeding for reduced GHG emissions of the animal
4. breeding for reduced GHG emissions of the system

Once the optimal approach has been identified a priority will be to identify and subsequently measure the best predictor traits for achieving the desired outcome. This will need to be done across a broad range of situations to obtain robust estimates of these predictor traits. This will be a topic for discussion at a workshop being organised in Australia in 2012. The working title for the workshop is 'Defining the low emitting phenotype.'

C Developing protocols for measurement of the CH₄ so that low emitting phenotypes can be rapidly and reliably identified.

The workshop participants recognised that the third priority was to optimise short term measurement procedures by:

1. establishing parameters for short term measurement protocols
2. determining whether information derived from one species provides an indicator for application to another

3. determining how best to measure the correlation between CH₄ emissions and intake
4. determining which food intake measurement is most appropriate

It is expected that research in relation to these questions would continue to be core funded in each country. However, a key task for the network to be successful is to determine as a priority the most suitable rapid measurement technologies. This will be a topic for discussion at a Network meeting in Australia, proposed for 2012. If standardisation across countries proves to be too difficult, it will become imperative to at least have robust calibration and conversion equations. It is proposed that New Zealand takes a lead role in this crucial project.

Proposed Role of the postdoctoral fellow

A key aspect to breeding sheep and cattle which produce less CH₄ is the estimation of the genetic parameters found in low-emitting animals. There are considerable synergies in combining data sets as the small size of national data can potentially limit the value of existing national projects.

In order to achieve synergies from combining datasets from different countries there is a need to standardise measurement protocols (or at least have robust calibration and conversion equations), adopt common reporting formats and collect contemporary rumen and DNA samples.

The Network would address some of these issues, but for the actual combining and analysis of the data sets a Post Doctoral fellow would be perfect for this task. The person would visit and work in sites contributing data; initially in New Zealand (Invermay), Australia (UNE) and Scotland (SAC). In addition, the fellow would help establish a format for trait (or traits) expression and breeding objective in order encourage subsequent industry adoption across the contributing countries.

In essence the Post Doctoral position would act as “glue” holding the various programmes together, and focussing the Network’s targets.

Benefits:

- better understanding of complex datasets around measurements of CH₄ emissions and co-related traits
- efficient analysis of data from across countries
- accelerated progress towards the long term goal of identification of potential new genetic and genomic tools for reducing methane emissions by livestock
- funding leveraged across species and countries

Outcomes:

- commonly accepted breeding objectives and trait definitions
- establishment of measurement protocols that are consistent across countries
- better knowledge of the relationship of between emission traits and production traits

- identification of potential new genetic and genomic tools for reducing methane emissions by livestock
- an optimised rapid and cheap method of ranking animals in industry
- a set of DNA samples that offer the potential for implementation of genomic selection in a wider range of animals on a routine basis.

Expected cost:

AgResearch occupies a unique position globally to be the host for this position. John McEwan a world recognised expert in the sequencing of the bovine (cattle) and ovine (sheep) genomes has significant involvement in New Zealand's sheep methane emission research in the area of establishing divergent methane emissions selection lines and so would serve as an ideal mentor for the post doctoral fellow.

To successfully host the post doctoral fellow, New Zealand scientists would need an investment from the New Zealand Government for:

- A stipend for 2 years and operational funds (**\$200k** per annum) to
 - enable the fellow to travel to collaborating countries
 - source the data
 - identify the samples
 - understand the key players in the Network

Appendix 1

Background to the priority research areas

Area 1: Ruminant Methane Mitigation Trait Measurements

The workshop agreed that reducing emissions intensity (units CH₄/unit product) is a logical end goal to pursue. However, there was no agreement on either, which of the co-traits that would eventually contribute to developing a unified breeding objective should be measured routinely, or how that breeding objective would be defined.

There is good evidence that CH₄ emissions differ between animals, both before and after adjustment for intake or liveweight, and that these differences are repeatable. What is not clear is the heritability of the trait, its relationships with other traits and how it can be best and most cheaply measured.

Current results suggest that short (~1hr) measurements repeated several times over a period of weeks or months are most suitable for breeding purposes. Such a measurement regimen offers the potential for data to be collected immediately off pasture. However, these procedures have yet to be optimised.

In the absence of an agreed CH₄ emissions trait, liveweight/carcass weight is used as a proxy for intake which in turn is used as a proxy for methane emissions in IPCC inventory calculations. The Workshop focused on CH₄ and feed intake measurement technology and identified a clear need to standardise protocols for measurement where ever possible. While this is a legitimate objective, there remain a number of issues (e.g. geography, breed) that can cloud experimental results. Preferably, a rapid CH₄ measurement should be able to be related to intake. However, there can be considerable variation in intake (up to 30%) between individual animals of the same liveweight that are growing at the same rate.

In order to understand this, one measure is Residual Feed Intake (RFI - the difference between actual and expected intake for the animal's liveweight). Unfortunately, RFI rankings do not remain consistent across feed types. In order for Intake data to be validated as a proxy for CH₄ emissions it needs to be correlated with actual methane production. It is therefore necessary to determine which intake measurement (e.g. RFI, DMI, Feed efficiency) is the most appropriate, and how this can be co-measured in relation to rapid CH₄ measurements.

Respiration chambers are acknowledged as the “gold standard” for measuring CH₄ emissions, but suffer from cost, numerical power and time constraints. A major obstacle therefore is establishing, and accounting for, between animal variation and repeatability of measured emissions. Several rapid measurement technologies are available (Portable chambers, C-lock, lasers), but these need to be evaluated alongside, and calibrated to, respiration chambers.

The workshop participants recognised that the first priority was to optimise short term measurement procedures by

- establishing parameters for short term measurement protocols
- determining whether information derived from one species provides an indicator for application to another
- determining how best to measure the correlation between CH₄ emissions and intake
- determining which intake measurement is most appropriate

It is expected that research in relation to these questions will continue to be core funded in each country. However, a key task for the network to be successful is to determine which of the rapid measurement technologies is a priority. This will be a topic for discussion at 2012 Australian meeting. If standardisation across countries proves to be too difficult, it will become imperative to at least have robust calibration and conversion equations.

Area 2: Ruminant Methane Mitigation Breeding Objectives

The general aim of animal breeding is to select animals to produce offspring that ensure that each generation is genetically superior to its antecedents. This imparts a 'generation interval' component to genetic improvement. To determine whether an individual has passed on a specific trait to its offspring involves, at a minimum, age at puberty and gestation length for traits that can be measured at birth (e.g. Birthweight), and at a maximum age at puberty, gestation length and lifespan for traits that are measured at the end of life (e.g. Longevity).

Livestock genetic improvement has a role in reducing greenhouse gas emissions. In order to make continued improvement it is necessary to have defined breeding objectives targeting the trait(s) of interest. Breeding goals (and associated tools) can help to reduce emissions intensity per country, per animal and per unit product. The biggest hurdle to developing establishing breeding objectives is the variability between farming systems, animals and measurements, and unravelling the part that genetics play in this.

Gains in economically based production have been shown to reduce emissions intensity in both sheep and cattle. There is need to quantify these gains. For example, an increase in NLB (the number of lambs born per ewe) would increase a total emissions equation per ewe due to an increase in number of animals but reduces emissions intensity per kg of meat produced. The latter is more important economically and also allows equivalent meat production on a smaller area and with less overall emissions.

Knowledge around the heritability of methane emissions and its genetic associations with other performance traits is key to predicting the expected responses to selection. Establishing how these parameters change with environment (i.e., genotype by environmental interactions) is also important to understand before incorporation into breeding objectives. Workshop presentations identified the need for a defined trait before breeding objectives can be addressed.

The first priority therefore is to decide whether the target is

- breeding for improved **efficiency (biological and financial) of the animal**
- breeding for improved **efficiency (biological and financial) of the system**
- breeding for **reduced GHG** emissions

Preliminary evidence from NZ farming systems suggests breeding for improved financial efficiency of the system (given a known cost of carbon equivalent emissions) would also reduce GHG emissions per unit product and improve biological efficiency. However, optimisation is required as is general acceptance across countries.

Traditionally, results in this area have been reported as point measurements. Evidence was presented as to the need to report an indication of raw data alongside data on CH₄ emissions and/or feed intake measurements, and data transformations since the trait of interest (breeding objective) is typically emissions or intake over a year for a breeding individual and its offspring.

The ASGGN will address some of these issues but there is an urgent requirement for combining and analysing international data sets.

The biggest recent development in genetics is the emergence of genome wide selection to accelerate genetic gain. For reasons of cost reduction it holds significant potential for GHG traits. More recently there has been the emergence of a layered genotyping approach based on sequencing, 50K genotyping, 5K genotyping and a parentage-plus (100 SNP product). The first 3 techniques are held together by computer based imputation of genotypes using parentage and linkage-disequilibrium. It is important that any research into genetics of GHG is therefore linked with its subsequent potential application via genomic selection. In the simplest form this means DNA samples must be collected from all animals measured for GHG traits, and stored appropriately.

The primary aim is to identify predictor traits for methane emissions for both species and breed. To improve the accuracy of the co-efficient estimates and make them relevant across a greater range of breeds, more detailed phenotype resources are required that represent a greater number of breeds.

It is expected that research in relation to these questions would continue to be core funded in each country. However, a key task will be to determine which trait(s) should be measured routinely in all core research programmes.

Area 3: International Database of Trait Measurements

A key task will be facilitating agreement on how animal measurements of CH₄ emissions, Dry Matter Intake, RFI and other associated traits (reproduction, disease etc) is presented and how it is stored. The data recorded will be used to estimate genetic parameters (heritability, genetic correlations, phenotypic correlations)

around CH₄ emissions and production traits, that contribute to reducing CH₄ emissions intensity by increasing the productive performance of animals.

The most effective way to use genomic technologies is for key groups to function in a networked environment that enables them to maximise the value of their individual resources by pooling information. In order for this to be meaningful, it is essential that all participants use standardised methodologies, data capture, data storage and presentation, and that the measurements recorded within these constraints are accessible in a shared environment. The logical way to achieve this is through secure, managed access to a common database.

Such a database is relatively costly to set up and maintain, and has the potential to generate IP issues. Dr Eileen Wall has indicated that she has funding to establish a database for the UK. Dr Wall has offered to discuss and coordinate the potential for this to become the Database for the ASGGN. Potentially, this could be extended to include all members of the GRA.

A Database will be developed that

- has sufficient storage capacity
- is secure, but can be accessed globally
- has specified input specifications is compatible with a variety of computer platforms
- has dedicated administration

In addition to the measurements recorded on each animal, co-samples of DNA and rumen contents will be collected for downstream host genomic and rumen metagenomic studies. New Zealand scientists support this initiative and will be interested in how it progresses through the Network.