Research to mitigate enteric CH$_4$ emissions from ruminants using Animal selection, Genetics and Genomics

A discussion document prepared for participants in a Global Research Alliance International Workshop 16$^{th}$-17$^{th}$ May 2011, Auckland, New Zealand
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Background
Between March 3rd - 5th 2009 a 3 day workshop funded by MAF and PGgRc was held in Wellington, New Zealand under the auspices of LEARN. Twenty-nine participants from 7 countries attended. This brought together for the first time, animal genetics and animal methane researchers and provided a forum to discuss aspects of genetic improvement for enteric emissions.

Then in December 2009, the GRA, which currently has 30 member countries, was launched. At the inaugural Senior Officials Meeting held in New Zealand in April 2010, three research groups were established; ‘Livestock Research’, ‘Croplands’ and ‘Paddy Rice’. That meeting agreed that New Zealand and the Netherlands would jointly coordinate the Livestock Research Group.

The first meeting of the Livestock Research Group was held in Banff, Canada from 8-9 October 2010, immediately following the international Greenhouse Gas and Animal Agriculture (GGAA) Conference from 3-7 October. At that meeting, among the achievements recorded was an agreement that the group should develop topics/projects focused initially on: facilitating data sharing; developing protocols and standardised methodologies and best practice guidelines for measurement techniques; preparing synthesis papers on growing areas of livestock emissions research.

This workshop is a direct progression from the previous meetings.

Target participants
Two groups of participants were targeted:
Delegates: researchers actively generating data of use to the network and researchers acting as links to other work-streams that can use resources created by the workshop
Attendees: interested parties (e.g. end-users or funders) key to investment in the future. It is likely that there will be opportunities highlighted during the workshop that will determine future research priorities, which will require considerable research investment. Potential investment partners must understand the value of a networked research approach.

Workshop Purpose
International collaboration to establish a collaborative network that will maximise efforts to mitigate CH$_4$ via animal selection based on genetics and genomics.

The key to this will be the development of methodologies that allow data collected by diverse groups located in different countries to be coordinated in a way that adds value to each group’s input.

Rationale for initiating the Workshop
The use of genetic and genomic technologies to mitigate CH$_4$ emissions from ruminants requires a huge resource of animals of different species and breeds. This is impossible to achieve by any single country or research organisation.

The most effective way to use the new technologies then, is for key groups to function in a network environment that enables them to maximise the value of their individual resources by pooling data.

In order for this to be meaningful, it is essential that all participants use standardised methodologies for measurement, data capture, data storage and presentation, and that these are accessible in a shared environment.
Emphasis will be placed on ruminant species, which are the major contributors to pastoral agriculture CH₄ emitters.

However, this does not ignore the contributions of intensive farming systems (in particular pigs and poultry) to CH₄ emissions. It is therefore imperative that a watching brief is placed on the literature surrounding these species as well.

The most likely applied technology for mitigating CH₄ emissions from ruminant animals will be through genomic selection.

For this to be successful, thousands of animals per species have to be genotyped and/or sequenced in the research phase. This will only be possible if all international parties pool all their available information.

The default will be that this information will be placed in the public domain free of any IP entanglements.

**Workshop Goal**

The primary goal is to establish and standardise protocols for CH₄ mitigation research within the Global Research Alliance in the areas of animal selection, genetics and genomics. This includes developing consistent data and sample storage protocols in order to underpin that research.

A secondary goal is to ensure that other work-streams are considered at the planning phase to ensure that maximum value is obtained from the research, by as many disciplines as possible.

**Workshop Objectives**

- Identify common protocols for measuring CH₄ emission phenotypes that are suitable for both genetic and genomic research and arrange for suitable calibrations of measurement differences between countries
- Define a list of correlated and productive traits and ensure that these traits are also co-measured on individuals during CH₄ phenotyping
- Evaluate and formalise protocols for collecting and storing DNA from all animals measured and ensure high density SNP chipping and sequencing can be achieved in the future
- Evaluate and formalise protocols for collecting and storing rumen samples from all animals measured to allow differences in rumen micro flora to be explored by other work streams
- Define criteria and establish a secure, subscription based, electronic storage and distribution mechanism for phenotype, genotype and DNA sequence data that is accessible by all collaborators
Discussion points
The workshop organisers have identified the following points as pivotal to progressing research in this area. They are also key to establishing International collaborations. By adopting a coordinated research approach we have the potential to enhance the power of our individual programme results by combining them with those of other groups.

Speakers are therefore asked to consider the bullet points below when preparing their presentations and summaries.

However, the list is not presented as definitive and all participants are free to present other points of discussion that they believe to be valuable to the group as a whole.

If you consider that a major area has been overlooked please email your suggestions for change/inclusion to grant.shackell@agresearch.co.nz as soon as possible.

Session 1 Measuring CH₄ Emissions

Chambers

Experimental design
- Animal resources, number, age, sex, genotypes?
  - How animals should be randomised to match the available facility?
  - Minimum length of acclimatisation of animals to exptl. conditions?
  - Benchmarking between facilities?

- Length of measurements (hourly, daily?)
  - How many measurements per animal
  - Periods, days per period?
  - Repeatability of measurements

- Expressions of emissions phenotype (g/d, g/kg DMI, g/kg DDMI)

- What sort of diet, feeding level, frequency of feeding
  - Feed intake and feed intake rate
  - Diet standardisation. How meaningful is it for pasture-based production systems?

Cattle
- Chambers or head boxes
- Automatic feed weighing

Sheep
- What sort of chambers
- Automatic feeding

Alternative CH4 measurement techniques
- Survey of potential and problems with a summary of the best
  “Butter boxes”
  - Calibration versus chambers
  - SOPs
  - SF6
  - Intra rumen devices
  - Lasers
Session 2 Measuring RFI

Experimental design
- Animal type – growing animals, mature animals?
  What measurements are required?
  What is the minimum habituation period?
  Benchmarking between facilities? – ‘link sires’ internationally

Length of feeding period
- How long should animals be recorded for?
- Does season (temperature/day length) have an effect?

Type of feed
- Should feed type be standardised?
- Is standardisation of feed analysis (ME, protein etc...) enough?
- Hard feed v cut and carry pasture?

Individual variation
- Is individual feeding variation an issue – should some or all of the following also be recorded routinely?
  - Feed bout duration?
  - Feed bout frequency?
  - Individual feed bout intake?
  - Feeding source preferences?

Species
- What are the differences in how to approach sheep vs cattle?
  - Can the same facility be designed for both?

Session 3 Genotyping and Analysis

Sample types and storage

Tissue/Blood for DNA
- Quantity and quality
  - Genotyping
  - Sequencing
  - Sample type?
  - Storage method?

DNA
- Should calibration samples be swapped between labs?
- Should a common sample be analysed by all labs to benchmark?

Blood for metabolic parameters
- Quantity
- Sample type
- Storage

Rumen contents
- Quantity
- Sample type
- Collection method
- Storage

Analysis methods

SNP chips
- Joint purchase
Bioinformatics requirements
- Data storage format
- Preferred analysis packages

Correlated Traits
- How are RFI and CH₄ emissions correlated?
  Phenotypically and genetically

Liveweight
- Liveweight and intake correlations i.e. field measurements use liveweight as an intake predictor, as do chamber measurements for amount offered. Is this sensible?

Growth rate
- How is Growth rate correlated to intake?
- Do animals growing at the same weight each the same amount?

Body composition
- Does Body Condition may influence intake?

Predictor traits
- List of predictor traits and estimated correlations with CH₄ emissions
- Protocols for measuring those of potential interest

Rumen microflora (inhibitors/manipulation)
- Can rumen microflora composition be used as a predictor?

Session 4 Moving Forward
Prioritisation
- Which traits?
- Which order?

Protocol Development
- SOPs
- International genetic linkage – what are the options

Intellectual property
- What are the implications of collaboration and data sharing?
  A likely method of application is genomic selection and for this to be successful, thousands of animals per species must be genotyped and/or sequenced in the research phase.

  This will only be possible if all international parties pool their available information.

  Can information be placed in the public domain free of IP entanglements?
The Future – International collaboration

Regular contact
- How do we establish and formalise a network to enable continued input through maintaining regular contact.

At a minimum an annual face-to-face meeting at an appropriate International Conference by as many Network members as possible. In addition, provision could be made for formalising a regular ‘meeting’ procedure using conference calling /video conferencing. At a wider level the LEARN network is an obvious starting point.

Data sharing
- How do we best establish and host a shared database while maintaining data security for those contributing?

The logical structure is a secure, subscription access website, which allows discussion and access to data on a permissions basis. In order to do this agreement will be needed on data input format.

The Future – Domestic collaboration

Within New Zealand, on-going collaboration and interaction with other researchers will be facilitated through discussions and face to face meetings as part of the Sustainable Land Management and Climate Change initiative
- What/do options exist for other countries?

Interim reporting:
Immediately following the workshop a working document will identify how the research effort can be coordinated across countries in such a way that results can be shared within and between both species and breeds.

The report will contain copies of presentations and/or transcription summaries of discussion related to
- CH4 measurement methodologies
- RFI and additional correlated traits of interest
- DNA sample collection and storage
- genotyping technologies
- sampling and storage of rumen contents
- rumen content analysis

The Workshop Report Draft submission date is June 10th 2011

White paper
The definitive outcome from the workshop will be a ‘white paper’ that proposes ways to consolidate and collaboratively advance work in this field internationally.

It is expected that this document will standardise
- protocols for measurement of CH4 emissions
- Calibration SOPs to make results comparable between countries
- lists of correlated & productive traits to be co-measured
- protocols for collecting & storing DNA from all animals measured
- protocols for collection & storage of rumen samples
- criteria & protocols for data storage that can be shared internationally

The draft White Paper will be circulated to all delegates at the workshop for review/comment and an agreement in principal of the accuracy of the content.

Submission date for the White Paper Draft is July 30 2011

NOTES
## Appendix 1  GRA Workshop Programme

### Monday 16th May 2011

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>09.00</td>
<td>Welcome/Housekeeping/Scene setting</td>
<td>Grant Shackell</td>
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<tr>
<td>09.15</td>
<td>Introduction</td>
<td>Dr Harry Clark (NZAGRC)</td>
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<tr>
<td>10.30</td>
<td>Session 1: Measuring CH4 Emissions</td>
<td><strong>Chair</strong>: Graeme Attwood</td>
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<td></td>
<td><strong>Chair</strong>: Dr Roger Hegarty (Australia)</td>
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<tr>
<td>09.30</td>
<td>Cattle</td>
<td>Dr Cesar Pinares (New Zealand)</td>
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<td>11.00</td>
<td>Sheep</td>
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<tr>
<td>12.00</td>
<td>Discussion</td>
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<tr>
<td>13.00</td>
<td>Lunch</td>
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<tr>
<td>14.00</td>
<td>Session 2: Measuring RFI</td>
<td><strong>Chair</strong>: Prof Phil Vercoe</td>
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<td></td>
<td><strong>Chair</strong>: Dr Donagh Berry (Ireland)</td>
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<tr>
<td>14.30</td>
<td>Cattle</td>
<td>Prof Stephen Moore (Canada)</td>
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<tr>
<td>15.00</td>
<td>Discussion</td>
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<tr>
<td>15.30</td>
<td>Afternoon Tea</td>
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<tr>
<td>16.00</td>
<td>Sheep</td>
<td>Dr Kristy Cammack (USA)</td>
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<tr>
<td>16.30</td>
<td>Strategies to improve feed efficiency &amp; reduce methane production</td>
<td>Andrew Thompson (Australia)</td>
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<tr>
<td>17.00</td>
<td>Discussion</td>
<td>Dr Garry Waghorn (NZ)</td>
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<tr>
<td>18.00</td>
<td>Break</td>
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<tr>
<td>19.00</td>
<td>Dinner and Networking</td>
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</tbody>
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### Tuesday 17th May 2011

<table>
<thead>
<tr>
<th>Time</th>
<th>Session 3: Genotyping and Analysis</th>
<th><strong>Chair</strong>: John McEwan</th>
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<tbody>
<tr>
<td>08.30</td>
<td>Scene setting</td>
<td>Dr Peter Amer (New Zealand)</td>
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<tr>
<td>09.00</td>
<td>Rumen contents, sample types and storage</td>
<td>Dr Sandra Kittelmann (NZ)</td>
</tr>
<tr>
<td>09.15</td>
<td>DNA, sample types and storage</td>
<td>John McEwan (NZ)</td>
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<tr>
<td>09.30</td>
<td>Analysis Methods</td>
<td>Dr Dominique Francois (France)</td>
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<td>10.30</td>
<td>Morning Tea</td>
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<tr>
<td>11.00</td>
<td>Correlated Traits</td>
<td>Dr Dorothy Robinson (Australia)</td>
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<tr>
<td>12.00</td>
<td>Wrap up</td>
<td>Dr Eileen Wall (United Kingdom)</td>
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<tr>
<td>12.30</td>
<td>Lunch</td>
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<td>13.30</td>
<td>Session 4: Moving Forward</td>
<td><strong>Chair</strong>: Mark Aspin</td>
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<td><strong>Chair</strong>: John McEwan (New Zealand)</td>
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<td>14.00</td>
<td>Protocol Development</td>
<td>Dr Alexandre Caetano (Brazil)</td>
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<td>15.00</td>
<td>Afternoon Tea</td>
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<tr>
<td>15.30</td>
<td>Data Sharing (10 min + Discussion)</td>
<td>Dr Roel Veerkamp (Netherlands)</td>
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<td>16.30</td>
<td>Roundup Discussion</td>
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<tr>
<td>17.30</td>
<td>Close and Farewell</td>
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