PGgRc®

PASTORAL GREENHOUSE GAS
RESEARCH CONSORTIUM

Annual Report 2010




DEVELOPING SOLUTIONS TO REDUCE NEW ZEALAND AGRICULTURAL EMISSIONS

Foreword

This report is the seventh annual report to the Crown on the Research Programme of the Consortium. It details the progress made during a year where significant financial pressure was felt across the livestock sector. Highlight of our programme this year has been the confirmation that real differences exist in sheep flocks for methane emissions, a further world first for the Consortium and the publication of the first genome sequence of a rumen methanogen.
The development of the New Zealand Agricultural Greenhouse gas Research Centre (NZAGRC) by Government is welcomed and the pastoral industry looks forward to working with them and the Science providers to further accelerate the progress we have made since our commencement in 2002. This initiative coupled with the development of the Global Research Alliance on agricultural greenhouse gas research led by New Zealand should ensure that we continue to be at the forefront of this research and continue to prepare our sector for a strong future in a carbon conscious world.

Mark Leslie
Consortium Chairman
November 2010

Contacts:
Mark Aspin
Consortium Manager
DD +64 4 4716031
Mob. +64 27 2483509
Mark.aspin@pggrc.co.nz
www.pggrc.co.nz

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Glossary of Acronyms used:

FRST	Foundation for Research Science and Technology
MAF	Ministry of Agriculture and Forestry
SLMCC	Sustainable Land Management & Climate Change (a funding initiative directed through MAF)
SFF	MAF Sustainable Farming Fund
NZAGRC	New Zealand Agricultural Greenhouse gas Research Centre
LEARN	Livestock Emission Abatement Research Network

Executive Summary

Founded in 2002 PGgRc is a partnership between New Zealand pastoral industries and the Foundation for Research Science and Technology (FRST).

PGgRc's key focus goal is to decrease total agricultural emissions of greenhouse gases by 10% per unit of output by 2013 relative to 2004. (This is estimated to be a 4Mt reduction in the agricultural greenhouse gas emissions as identified in the National Inventory).

The Consortium aims to position the Consortium and New Zealand as a global leader in agricultural greenhouse gas mitigation.

In the past eight years, the Consortium's investment has grown the science team involved from 12 to 60 and has contracted over $30 million in scientific programmes aimed at reducing agricultural greenhouse gases. With additional funding in 2009 from the participants matched through MAF - SLMCC funding it has accelerated the genomic, vaccine, inhibitor and animal variation parts of its programme, It confidently expects its high-quality science programme to gain momentum and make good progress over the next two years.

One of our major achievements has been the closure of the Methanobrevibacter ruminantium genome – a world first and the subsequent publication of this in the science journal PLoS One. We have completed and applied for 4 patents applications based on this research, with 3 applications at National stage in 44 countries.
With the addition of the closure of *Methanobrevibacter* spp. SM9 genome completed and annotated, we have significantly broadened our scientific base and understanding of the genetic blueprint of methanogens.

A flock of high and low emitting sheep with a mean difference in methane emissions of 7.8% has been created through screening a selection of industry selected recorded sheep. Further research has confirmed the consistency of this difference across differing diets. This is a substantial milestone for the Consortium and opens the way for further development of this flock as a mechanism to ultimately enable selection of low emitting ruminants.

A novel *Methanosphaera* isolate, 3F5, has been obtained in pure culture from the rumen of a pasture-fed sheep. This isolate is being characterised in more detail, and its genome will be sequenced. It is the first isolate of *Methanosphaera* from the rumen. *Methanosphaera* spp. make up 10 – 15% of methanogens in New Zealand ruminants.

We have developed and refined our assays and methods for establishing the population characteristics of the rumen microbiological community. The methods will undergo further refinement and validation in the latter part of 2010 and then be a sound set of methods with which to interrogate animal trial samples and accurately identify any population changes that occur during mitigation trials

We have shown that the lytic enzymes extracted from the phage associated with Methanobrevibacter ruminantium can be induced to act on and degrade the methanogen in pure culture. Final determination of the application and commercial viability of the approach will be assessed in the next 12 months.

The LEARN network has grown to over 600 researchers and interested parties from over 80 countries, and continues to gain momentum through workshops and other activities. In future this activity will be managed through the NZAGRC.

Our Science programme was reviewed by the Science Advisory group in October 2010 and some further improvements identified. Overall they have assessed that our programme remains at the

forefront of this research internationally with the activities in animal variation and development of a methane vaccine regarded as world leading.
PGgRc has produced leading world research on the production of methane and nitrous oxide from grazing livestock, and is internationally acknowledged for managing the most comprehensive science programme of its type in the world.

In 2009 Ben Vlaming completed and was awarded his PhD, the first to come from the PGgRc programme. Another PhD candidate, Natasha Swainson, is also expected to be conferred with her doctorate in the near future. A third PhD candidate Jeyamalar Jeyanathan is very close to submitting her PhD thesis for examination.

The launch of the New Zealand Agricultural Greenhouse Gas Research Centre (NZAGRC) by the Crown in March will broaden and add to the work the consortium has developed to date. PGgRc has worked with its participant AgResearch in the centre's development and will be a member of the NZAGRC with representation on the Steering Group and Stakeholder Advisory Group.

Our programme of work continues to advance as planned and we are confident that by the end of the current contract in 2012 we would have achieved the following:

- Evaluated the effects of selecting livestock for low methane alongside other productivity traits
- Thoroughly evaluated and developed the concept of creating a methane vaccine in ruminant livestock
- Enhanced farmer understanding of agricultural GHG' s in New Zealand farm businesses
- Evaluated 50 possible compounds as Inhibitors through our chemigenomics pathway and identified leading compounds for high throughput screening.
- Identified forage factors that affect emissions
- Evaluated the cost effectiveness of nitrification inhibitors across NZ in reducing nitrous oxide emissions and enhancing pasture production.

PGgRc Annual Report – 2009-2010

1. The Nature and Purpose of the Programme

PGgRc is a partnership between New Zealand pastoral industries and the Foundation for Research Science and Technology (FRST). It was formed in 2002.

PGgRc has been the "centre of excellence" for pastoral-based agricultural greenhouse gas mitigation research both in New Zealand and internationally, and expects to continue its high-quality science programme into the foreseeable future.

Over the past seven years the Consortium has invested over $30 million in scientific programmes aimed at reducing agricultural greenhouse gases. It has produced internationally acknowledged research on the production of methane and nitrous oxide from grazing livestock as part of the most comprehensive programme of its type in the world.

Non-carbon dioxide emissions from pastoral agriculture account for about 48% of New Zealand's total greenhouse gas emissions. Under the Kyoto Protocol, New Zealand is required to reduce its greenhouse gas emissions to 1990 levels, or to pay or take responsibility for any emissions over this target.

There are currently no proven, practical and cost-effective farm practices and technologies to reduce agricultural emissions. However, nitrification inhibitors do show promise for nitrous oxide reduction. There is also a need to improve the accuracy of our estimates of on-farm emissions.

As outlined in the 2004 Memorandum of Understanding between the Crown and the pastoral industries, the government agreed to bear the cost of the agricultural sector's non-carbon dioxide emissions providing the sector contributed to research into ways to reduce greenhouse gas emissions from agricultural activities.

PGgRc has developed, and is now implementing a Research Strategy to reduce greenhouse gas emissions from agricultural activities.

2. The Goals of the Strategy

To identify, establish and develop on-farm technologies to improve production efficiency for ruminants

To identify, establish and develop on-farm technologies for sheep, dairy, beef cattle, and deer, which lower methane emissions from New Zealand ruminants and nitrous oxide from grazing animal systems.

To exploit commercial opportunities arising from the science and technologies in a global market.

3. Current Targets for the Consortium

- *To decrease total agricultural emissions of greenhouse gases by 10% per unit of output by 2013 over business as usual relative to 2005. (This is estimated to be a 4Mt reduction in the agricultural greenhouse gas emissions as identified in the National Inventory).*

- *To have 33% of farmers implementing at least one greenhouse gas mitigation strategy by 2013.*
- *To increase knowledge about climate change and the Kyoto Protocol in the agricultural sector.*
- *To establish New Zealand and the PGgRc as a global leader in agricultural greenhouse gas mitigation.*
- *To ensure national coordination of all greenhouse gas-related investments, while also developing further international collaboration and involvement to increase global capability.*
- *To exploit national and international commercial opportunities arising from the work the Consortium is undertaking.*
- *To help New Zealand farmers adapt to the effects of climate change, and to become competitive in a carbon-constrained global economy beyond 2012.*

4. PGgRc Science Programme

The PGgRc science programme integrates a wide range of science approaches from the microbe to the farm. It encompasses fundamental research to further understand the processes of methane and nitrous oxide formation through to the design of practical, relevant, on-farm greenhouse gas mitigation systems.

The research programme is strongly focused on developing a pathway for farmer adoption. This is critical if the Consortium goals are to be achieved. There are eight research foci:

Rumen Ecology:
Rumen ecology informs and supports research to mitigate rumen methanogenesis by identifying, quantifying and culturing microbes in the rumen, and investigating the impacts of vaccine, inhibitor, and nutritional interventions on the long-term functioning of the rumen ecosystem. It holds strategic importance to all of our methane research as it is the "tool set' that we apply to understand what is going on in the rumen before and after manipulation. It also provides basic understanding of the rumen, offers new leads for interventions arising from that understanding, investigates rumen microflora in animal variation studies, provides methanogens and material for the genomics and vaccine objectives, and studies the consequences of a methanogen-free rumen. Through this objective we will be better able to identify the "market" with respect to the methanogens found in the ruminant populations we target, and assess the likelihood of leads being able to be developed to viable mitigation options. A programme like ours could not have progressed without access to this underpinning knowledge.

Methanogen Genomics:
This objective provides a specific approach that has allowed us to identify the genetic blueprint of rumen methanogens and critically analyse those functions that are unique to rumen archaea and hence offer the most specific targets to chase. The knowledge gained through genomics allows a targeted approach to be applied that would otherwise not be possible. The genomic analyses reinforce not only the understanding of rumen ecology, but directly inform and underpin the vaccine and chemigenomics objectives aimed at reducing methane production in the rumen. It also delivers information at a fundamental level which allows it to be readily protected and strategically applied for commercialisation and collaborative purposes if required. Genomics will have similar value in understanding any other rumen organisms, including homoacetogens and protozoa.

Anti-Methanogen Vaccine:

In this objective we are developing, optimising and testing an anti-methanogen vaccine that will reduce growth and methane production by rumen methanogens. It is likely that an effective vaccine targeting methanogens in the rumen will require a cocktail of antigens targeting the entire array of methanogen species dwelling in the rumen of livestock - that is why there are strong links between this work and the work we are doing in our methanogen genomics and rumen ecology objectives. A vaccine, if successful and administered in the "conventional" manner, offers an approach that would be readily adaptable for many livestock systems. The research approach is very much 'blue sky", as we are trying to induce the host's immune system to work on a defined group of organisms in the rumen. This objective is integrated with the ecology and genomics objectives and could not deliver without that input.

Chemigenomics / Inhibitors:
This objective uses genomic and microbial data to identify compounds (small molecules) that could specifically target methanogens. The objective has developed a "pipeline" drawing on in-silico (computer) and chemical compound library analysis to match these to the specific enzymes and proteins that methanogens require to function in the rumen. The chemigenomics pipeline consists of: target identification, cloning of target enzyme genes, enzyme expression, enzyme purification, assay development, enzyme characterisation, determination of crystal forming conditions, enzyme structure determination, modelling of published enzyme structures, in silico prediction of inhibitors, testing of inhibitors in enzyme assays and/or pure culture, and testing using rumen simulation in vitro techniques. The initial product of this objective will be compounds that can then be tested through Mitigas for their efficacy against methanogens. If they prove effective, then they can be developed further into a mitigation solution delivered through feed supplements, drenching, bolus or some direct delivery mechanism to livestock.

Animal Selection & Breeding:
In this objective, we are building on work undertaken between 2002 and 2006 where we measured methane emissions by 700 dairy cows using the SF_6 technique. It also expands subsequent work, using respiratory chambers, to segregate a population of low emitting sheep from flock mates that were high emitting, by continuing that screening across 4 years to eventually screen 1000+ sheep. The objective draws on knowledge of ruminant nutrition and physiology and genetics to identify the characteristics that make the livestock different. Mitigation solutions arising from this may be low cost tools that farmers can apply to move their livestock to lower emissions, or they may be biomarkers found in blood, saliva, milk, or they may be gene markers or breeding values established from correlations with other traits.

Ruminant Nutrition:
The ruminant nutrition objective examines whether forages or dietary manipulations offer the opportunity for reducing methane emissions and to understand the interactions between animal or plant factors that are responsible for the differences observed. The objective also includes the Mitigas evaluation system, which is a fundamental tool for the sequential evaluation of mitigation solutions from batch culture to the grazing animal.

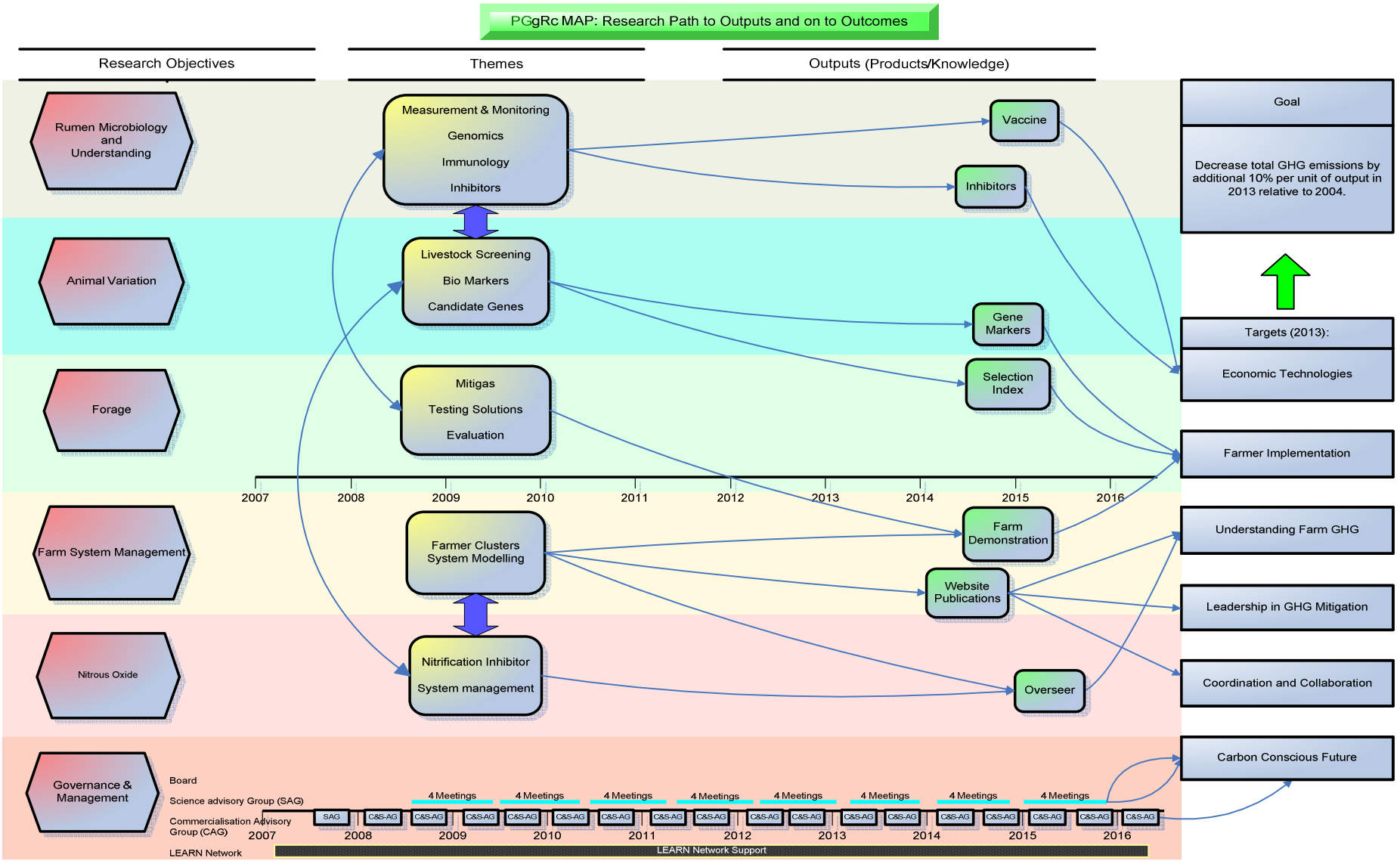
Low Greenhouse Gas Emitting Farm Systems:
This work combines research on the components of farm systems (e.g., dietary manipulation, management of nitrogen inputs). It looks at the development of novel and best farm management technologies, the integration of improved technologies into greenhouse gas friendly farm systems, and provides an extension programme designed to increase awareness and encourage adoption of greenhouse gas reduction measures. Ultimately a systems approach will need to incorporate any or all of the mitigation solutions developed. This objective also supports the work developing understanding amongst farmers so that when we deliver a solution they will be able to recognise its applicability and the opportunity it represents.

Nitrous Oxide Mitigation:
In this objective we have completed the evaluation of the opportunity for system manipulation in dairy systems and are managing a programme of work across 4 major dairy areas evaluating the

full effects of nitrification inhibitors on nitrous oxide, nitrate leaching and pasture production, initially focusing on the product based on dicyandiamide (DCD).

A path to market will be identified for all of the mitigation solutions identified, and the Consortium will be endeavouring to ensure that it can be completed either directly or by on selling, licensing or release of information to support that path. This is the primary role of the Commercialisation Advisory Group (CAG) and the commercialisation activity of the consortium.

PGgRc Map: The diagram on the next page shows pictorially the map of the Consortium operation and how the science programme is designed to deliver the outputs supporting our targets and goal. The Consortium has consciously taken an integrated approach across science disciplines, commercial delivery and industry governance.


PGgRc MAP: Research Path to Outputs and on to Outcomes
Research Objectives
Themes
Outputs (Products/Knowledge)
Rumen Microbiology and Understanding
Animal Variation
Forage
Farm System Management
Nitrous Oxide
Governance & Management
Measurement & Monitoring
Genomics
Immunology
Inhibitors
Livestock Screening
Bio Markers
Candidate Genes
Mitigas
Testing Solutions
Evaluation
Farmer Clusters System Modelling
Nitrification Inhibitor
System management
Vaccine
Inhibitors
Gene Markers
Selection Index
Farm Demonstration
Website Publications
Overseer
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
Board
Science advisory Group (SAG)
Commercialisation Advisory Group (CAG)
LEARN Network
4 Meetings
SAG
C&S-AG
LEARN Network Support
Goal
Decrease total GHG emissions by additional 10% per unit of output in 2013 relative to 2004.
Targets (2013):
Economic Technologies
Farmer Implementation
Understanding Farm GHG
Leadership in GHG Mitigation
Coordination and Collaboration
Carbon Conscious Future

5. Consortium Funding

5.1 PGgRc is funded by:

- *Fonterra Co-operative Ltd*
- *Beef + Lamb New Zealand Ltd (B+LNZ)*
- *DairyNZ Inc*
- *PGG Wrightson Ltd*
- *The New Zealand Fertiliser Manufacturers' Research Association Inc (NZFMRA)*
- *DEEResearch Ltd*
- *AgResearch Ltd*
- *Landcorp Farming Ltd*

5.2 Associate members are:

- *The National Institute for Water and Atmosphere (NIWA)*
- *The Ministry of Agriculture and Forestry (MAF).*

5.3 FRST and MAF Contracts: (Through which matched funding provided)

- *METH0701 PGGRC II*
- *METH0801 Protozoa, Low Methane Rumen and Mitigas.*
- *METH0802 Accelerated ruminant methane mitigation*
- *METH0901 Sheep, cattle, and methane predictors*

5.4 Research providers and contributors include:

- *AgResearch Ltd*
- *DairyNZ*
- *Via Lactia Biosciences*
- *Lincoln University.*
- *Landcare Research Ltd*
- *University of Otago*

5.5 Table of Funding for 2009/10

	Original core	Acceleration	Total
AgResearch	**350,000.00**		**350,000.00**
FRST (government)	**2,250,000.00**	**1,400,000.00**	**3,650,000.00**
DairyNZ	**468,000.00**	**400,000.00**	**868,000.00**
DEEResearch	**35,000.00**		**35,000.00**
NZFMRA	**94,500.00**		**94,500.00**
Fonterra	**500,000.00**	**650,000.00**	**1,150,000.00**
B+LNZ	**370,000.00**	**250,000.00**	**620,000.00**
PGG Wrightson	**125,000.00**		**125,000.00**
Landcorp Farming	**100,000.00**	**35,000.00**	**135,000.00**
Total	**4,292,500.00**	**2,735,000.00**	**7,027,500.00**

6. Research Progress and Success

The Consortium continues to develop its broad programme of work with good progress across many of the objectives and the acceleration of the genomic and associated vaccine and inhibitory compound development through additional participant funding along with the SLM&CC funds from MAF.

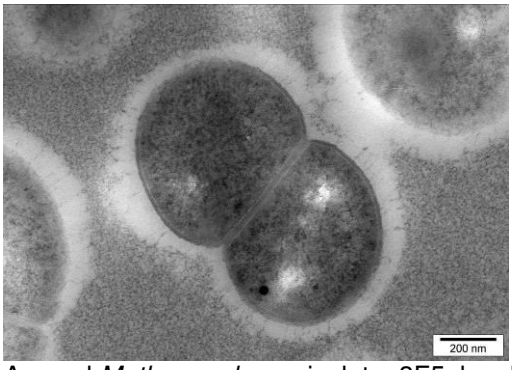
Objective 1: Rumen microbial ecology

Objective Leader: Dr Peter Janssen

Objective 1 covers 5 broad areas. These are (1) census methodology refinement, (2) isolating new methanogens, (3) microbiological censuses in animal trials, (4) the microbiology parts of the study of the methanogen-free rumen, and (5) the microbiology parts of vaccine development.

Census methodology refinement and microbiological censuses in animal trials
We have developed and refined our assays and methods for establishing the population characteristics of the rumen microbiological community. The methods will undergo further refinement and validation in the latter part of 2010 and then be a sound set of methods with which to interrogate animal trial samples and accurately identify any population changes that occur during mitigation trials.

Isolating new methanogens
We have been isolating representatives of groups of methanogens that are not available in culture but appear to be abundant in New Zealand ruminants.



A novel *Methanosphaera* isolate, 3F5, has been obtained in pure culture from the rumen of a pasture-fed sheep. This isolate is being characterised in more detail, and its genome will be sequenced. It is the first isolate of *Methanosphaera* from the rumen. Our research indicates that *Methanosphaera* spp could make up 10 – 15% of methanogens in New Zealand ruminants, and they have been detected in all animals (sheep, cattle and deer) on all diets.

Progress has also been made in understanding the growth requirements of the currently uncultured 'Rumen Cluster C' clade of methanogens. There are indications that this methanogen family could account for a significant proportion of the methane produced in NZ ruminants. Without the ability to culture these methanogens and subsequently sequence their genome it becomes challenging to understand them in sufficient detail to be able to identify suitable specific mitigation targets.

Study of the methanogen-free rumen
Anti-methanogen agents have been tested for their ability to inhibit pure cultures of methanogens, and tested against homoacetogens and other rumen bacteria.

A trial involving sheep in respiratory chambers will commence in October 2010 using an inhibitor to evaluate the effects on the rumen population when methanogens are inhibited.
This is the second trial of this nature undertaken by the PGgRc in an effort to better understand the affects that methane mitigation will have, building and extending on the knowledge from a similar trial carried out in 2007 using chloroform as the inhibitor.

Homoacetogenic bacteria are alternative users of hydrogen to methanogens and could if conditions are favourable take the roles of methanogens and convert hydrogen to acetate –an energy source for the ruminant instead of methane.

The feasibility of production of homoacetogens in volumes sufficient for inoculating animal trials of up to 12 sheep has been ascertained. Depending on the size of the trial, addition of up to three different homoacetogens isolates to each animal could be attempted to increase the likelihood that at least one will establish itself in the rumen. This approach is will be used in the methane free trial and is an example of an ecological approach to changing the rumen microbe population so that less methane is produced.

Objective 2: Methanogen genomics

Objective Leader: Dr Eric Altermann

Since its conception in the first PGgRc programme, methanogen genomics has established itself as the core foundation for many allied science programmes providing key information to our vaccine inhibitor and rumen ecology programmes

We published the first rumen methanogen genome enabling access to the information developed through the consortium to all researchers:
"The genome sequence of the rumen methanogen *Methanobrevibacter ruminantium* reveals new possibilities for controlling ruminant methane emissions. PLoS One, 5:e8926 Leahy, S.C., Kelly, W.J., Altermann, E.H., Ronimus, R.S., Yeoman, C., Pacheco, D.M., Li, D., Kong, Z., McTavish, S., Sang, C., Lambie, S.C., Janssen, P.H., Dey, D., and Attwood, G.T. 2010."

With the new *Methanobrevibacter* spp. SM9 genome now completed and annotated, we have significantly broadened our scientific base and understanding of the genetic blueprint of methanogens.

Both genomes will serve extensively to aid in the analysis of other methanogens (e.g. *Methanobacterium formicicum* BRM9, *Methanosarcina* sp. CM1, and *Methanobrevibacter* sp. YLM1) and facilitate in the refinement and definition of methane mitigation targets.

Promising leads discovered from the *Methanobrevibacter ruminantium* genome project are now being subjected to *in vitro* testing. Therefore, we are pursuing the optimisation of growth environment conditions, with the aim to create a versatile, easy-to-handle *in vitro* culture system.

We have been successful in getting the phage associated with *Methanobrevibacter ruminatium* M1 to express, indicating that it could be feasible to utilise this natural association if these conditions could be triggered through an effective intervention.

Similarly, we are characterising non-ribosomal peptide synthase genes found in *Methanobrevibacter ruminantium* M1 and *Methanobrevibacter*. sp. SM9, to determine if the compounds these enzymes produce may offer options to modulate methanogen growth

We are utilising a combinatorial *in silico* (computer) and *in vitro* (laboratory) approach to discovering new gene targets, which may have application as antigen targets for a methanogen specific vaccine. These two complementary approaches have been pursued in parallel and has

led to a new collaboration between our AgResearch based team and Prof. Greg Cook at University of Otago.

Objective 3: Methanogen vaccines

Objective leader: Dr Bryce Buddle

Our progress in screening for antigenic targets in the main methanogens found in New Zealand ruminants continues. This approach to identify targets has been extended by using both the original sub-cellular fractions and additional fractions. Separation of proteins in the various fractions, together with analysis of proteins from our genomic programmes has so far identified more than 10 different immunogenic proteins in *Methanobrevibacter* ruminantium M1.

Using this approach, and as part of the accelerated programme, a number of proteins have been identified as being immunogenic from two additional methanogens, *Methanobrevibacter* sp.SM9 and *Methanosarcina barkeri* CM1.

Serum containing antibodies (antisera) against a number of the sub-fractions has been tested to determine their ability to reduce methane production. Some reduction in the production of methane in *in vitro* pure cultures of *Methanobrevibacter ruminantium* M1 was observed and is encouraging, indicating that the process of refining and testing sub-cellular fractions from methanogens should help in identifying potential vaccine antigens.

An adjuvant that promotes strong antibody responses at mucosal surfaces will be required to formulate an anti-methanogen vaccine. A trial using a model antigen, Tetanus toxoid was performed in sheep to identify an optimal adjuvant for inducing mucosal antibody responses. Strong and persistent serum antibody responses and moderate salivary antibody responses were induced by injection of antigen.

Objective 4: Exploiting animal to animal variation

Objective Leader: Dr Cesar Pinares

Exploiting animal-to-animal variation in methane emission may be a cost-effective mean to reduce enteric methane emissions from ruminant livestock. For this approach to work it is necessary to demonstrate that methane emission is a heritable trait and that this trait has no negative association with production and functional traits.

In 2008 a flock of 105 ewe lambs were screened for their methane yields (i.e. emissions per unit feed dry matter intake, DMI) on a standard forage. This screening resulted in a flock of 10 high and10 low emission sheep with a mean difference of 7.8% that have been drafted and maintained as a stand alone flock.

In 2010 repeated measurements were conducted indoors with the selected sheep and confirmed that the flock were consistent in their methane emission rankings across a range of diets (pasture through to concentrate pellets) with the differences tending to be greatest under concentrate diets

Further evaluation of these animals will continue to better understand what they differ for this trait. Meanwhile they have been mated to rams screened for high and low emissions and will lamb in the spring 2010.

On the basis of this we have expanded the activity in this objective with a new sheep screening process for methane emission to obtain more accurate estimates of heritability and repeatability and screened 360 sheep during 2009–2010 year. The consortium will continue this screening

over the next two years so that in total 1000+ sheep will have been screened resulting in a flock of 100 sheep in the PGgRc methane flock by 2012.

In parallel we have also continued the our studies with cattle, further evaluating the data collated on 700 dairy cows in 2004/05, to establish whether there are candidate genes or markers that can be utilised to identify this trait in commercial herds. Both the sheep and cattle studies will be run as an integrated programme, with the intention of applying any results across both species.

Objective 5: Farm system and adoption

Objective Leader: Dr Simone Hoskin (now lead by Dr David Pacheco)

This objective comprises three major strands of work; (1) evaluating nitrous oxide management practices, (2) modelling of GHG friendly farm systems and raising awareness of GHG issues and profile of the PGgRc to sheep and beef, dairy and deer sectors and (3) nutritional approaches to reducing methane emissions.

Evaluating nitrous oxide (N_2O) management practices
For the last three study years, the annual nitrous oxide (N_2O) emission rate in dairy farmlet studies associated with the use of nitrification inhibitors and restricted grazing was 20% lower, on average, than from the control farmlet (with current standard practices).

Total calculated annual emissions for the sum of the three major greenhouse gases, nitrous oxide, methane and carbon dioxide, from the dairy system with the use of nitrification inhibitors and restricted grazing were 6% lower than that from the control system. Reducing emissions can potentially have economic benefits when carbon costs are imposed.

Modelling of GHG friendly farm systems and raising awareness of GHG issues to sheep and beef, dairy and deer sectors. (Jointly funded by MAF through SLMCC, SFF)
Ten existing farmer cluster groups (4 sheep and beef, 4 dairy and 2 deer), each consisting of up to approximately 15 farmers, were identified to represent a range of climatic and geographic areas and different farming systems across the country. The learning process, based on adult learning theory principles, was structured to be flexible and evolutionary so that the learning would follow the needs, interests and questions of the farmer participants and not those of the facilitators or project management team.

Key findings from the case study modelling:

- For all sectors (sheep and beef, dairy and deer), changes to farm management practices had little impact on either total or the intensity of emissions.
- Sheep and beef farms have substantially reduced their intensity of emission in the last 25 years and many are likely to have limited future options to further reduce their intensity of emissions while still farming livestock.
- Dairy systems have potential to benefit from very high breeding worth (BW) cows if systems changes are also implemented. To maximise the GHG benefits of very high BW cows, a lower stocking rate is required, the practical implications of that system require further consideration.
- The use of a nitrification inhibitor was not economically viable for sheep and beef or deer farmers but was a promising option for dairy farms in areas suitable for its use.

Nutritional approaches to reducing methane emissions.
The past year has focussed on evaluating alternative fresh forages to perennial ryegrass-based pasture (pasture) for reduced methane emissions. Both white clover and chicory have proved

inconsistent at reducing methane yields in comparison with pasture in multiple calorimetry studies with sheep. Therefore, neither of these forages can be considered as viable alternative forages to pasture for mitigating methane emissions in pastoral systems.

Some progress has been made in terms of investigating possible nutritional mechanisms which may influence methane emissions, such as rumen pH and rumen turnover. However, this has been hampered by lack of a difference in methane emissions between white clover or chicory and pasture, and technical difficulties in trying to manipulate these rumen processes without a confounding effect of feed intake.

Objective 7: Chemigenomics

Objective Leader: Dr Ron Ronimus

Assessment of the commercial viability of lytic enzymes
We have shown that the lytic enzyme (PeiR) extracted from the phage associated with Methanobrevibacter ruminantium can act on the methanogen in pure culture situations. Final determination of the application and commercial viability of the approach will be assessed in the next 12 months.

Chemigenomics targets identified and assessed via systematic pipeline
Substantial progress has been made in cloning, expression, purification, crystal screening and assay development for the 40 targets within the PGgRc Programme. Seven of these have been purified and 4 have formed crystals. These developments will form the basis of rational discovery of inhibitors of methanogens that can be delivered using existing bolus or drench technologies.

Objective 6: Nitrous Oxide

The consortium activity in mitigating Nitrous oxide is centred around the project management of the Nitrous Oxide Mitigation Research (NOMR) set of trials run in the dairy sector. These trials are funded by MAF along with Fonterra, DairyNZ and FMRA and are an evaluation of the nitrification inhibitor Dicyandiamide (DCD) for nitrous oxide emissions, nitrate leaching and pasture growth across 4 major dairy regions (Waikato, Manawatu, Canterbury, South Otago). These trials have been running since 2009 and will continue through to 2011.

The funding for these trials is not included in the total funding for the consortium but represents a significant contribution from the consortium partners noted above. The total investment by all parties over the life of the trial series is expected to be $10m, with 50% from industry matched by MAF.

SUMMARY

Our programme of work continues to advance as planned and we are confident that by the end of the contract we would have achieved the following:

- Evaluated the effects of selecting for methane in livestock
- Carried out a proof of concept of the anti-methane vaccine
- Enhanced farmer understanding
- Evaluated 50 possible compounds as Inhibitors
- Identified forage factors that affect emissions
- Evaluated Nitrification Inhibitors across NZ

7. Other Consortium Achievements

Advisory groups
We maintain two independent advisory groups to provide advice for the Board on science and commercial strategies. The science group includes seven research experts, five of whom are from outside of New Zealand. The Commercialisation Advisory Group includes four New Zealand-based experts with international experience in the commercialisation of science.

We have developed comprehensive tools for commercialisation and assessment that will act as a guide as we move our research through to solutions for the sector.

Our science programme was reviewed by the Science Advisory Group in October 2010 and some further improvements identified. Overall they have assessed that our programme remains at the forefront of this research internationally with the activities in animal variation and development of a methane vaccine regarded as world leading.

Patents Applications
We have developed four patent applications on the basis of the utility discovered through the genome sequencing of *Methanobrevibacter ruminantium*. M1. These applications cover the major targets identified and are currently being examined. The four patent applications are as follows:

WO 2009/041831 A1	*Phage φmru polynucleotide's and polypeptides and uses thereof*
WO 2009/041830 A2	*Cell-permeabilising peptides and polypeptides for microbial cells*
WO 2009/041832 A2	*Vaccines and vaccine components for inhibition of microbial cells*
PCT/NZ2010/000169	*Complete genome sequence of the methanogen Methanobrevibacter ruminantium*

LEARN
The Consortium has managed the Livestock Emission Abatement Research Network (LEARN) (www.livestockemissions.net) website. This is an initiative championed by MAF, AgResearch and the Consortium to draw global greenhouse gas researchers together using the internet. The network has attracted over 600 researchers and interested parties from over 80 countries, and continues to gain momentum through workshops and other activities. In future this activity will be managed through the NZAGRC.

Protein from Pasture
In 2009 the consortium contributed with MAF Sustainable Farming fund, towards an evaluation of the feasibility of extracting protein from pasture to increase the value by delivering a high value by product and a ruminant feed from the residue that could provide a low methane emitting diet. The study concluded that on current costings this alternative use for pasture is not viable, and therefore studies to evaluate the feed for methane emissions did not proceed. Although this may appear a disappointing outcome the study has provided a thorough method for evaluating this opportunity should the economies change in the future.

The MAF – PGgRc jointly funded farmer understanding programme launched a website *www.farmersandclimate.org.nz* to provide topical and useful information to farmers in support of the project

Over the last two years, both the Consortium Manager and the Chairman have written and presented a significant number of briefing reports and presentations for stakeholder and sector-interest groups. The PGgRc produced a newsletter in February 2010 to background the consortium and provide updates across the programme; these will be produced throughout the remainder of the contract period.

Media Coverage
We have received continued media coverage throughout the last year within in New Zealand across radio, television and print media. In addition internationally our science was profiled in:
New Scientist
Wall street Journal
Al Jazerah Television
BBC TV

8. Partnership Initiatives

NZAGRC
The launch of the New Zealand Agricultural Greenhouse Gas Research Centre (NZAGRC) by the Crown in March will broaden and add to the work the consortium has developed to date. PGgRc has worked with its participant AgResearch in the Centre's development and will be a member of the NZAGRC with representation on the Steering Group and Stakeholder Advisory group. PGgRc has also entered into an agreement with MAF as the funder of NZAGRC to make our accumulated Intellectual property available to the Centre for on going research.

The Consortium looks forward to making the most of these opportunities for our pastoral farmers as the work programme for the NZAGRC gets underway and gains momentum.

During the year the Consortium has initiated negotiations with several organisations about potential solutions for the abatement of methane. The development of the proof of concept facilities utilising SLMACC funding will further enhance our ability to evaluate these opportunities.

9. Human Capital Building

Since its formation in 2002 the Consortium has increased the number of researchers it contracts from 12 to a team of 60 currently working across our programmes.

In 2009 Ben Vlaming completed and was awarded his PhD, the first to come from the PGgRc programme. Another PhD candidate, Natasha Swainson, is also expected to be conferred with her doctorate in the near future A third PhD candidate, Jeyamalar Jeyanathan, is very close to submitting her PhD thesis for examination.

Our programme of work has been the basis for several LEARN Fellowship scholars to visit from developing countries. Bought here for a fixed term through our contracting research providers the scholars are a mixture of PhD and post-doctoral researchers who have been involved with our programme in both methane and nitrous oxide

Kirsty Hammond a PhD student working in the Rumen nutrition objective was named winner of the "Young Dairy Scientist Communication Award" in 2010. Kirsty research has focused on identifying nutrition factors in forages that influence methane emissions.

10. Future Plans and Challenges 2010–12

We expect the Consortium research programme to continue its momentum and make good progress over the next 2 years. The emergence of the NZAGRC initiative from Government has provided further impetuous to the sector to make the most of the significant investment since 2002. As the plans for this develop PGgRc will be working in partnership with the Crown and its entities to make the most of this opportunity for New Zealand.

We have accelerated our most promising research on inhibitory compounds and vaccines and animal variation through the MAF- SLMCC. In tandem with this, we will support the additional sequencing of more methanogens and testing of promising mitigations through the Mitigas process.

The Consortium will continue to manage with Industry and MAF in the Nitrous Oxide Mitigation Research (NOMR) programme of work that will more clearly demonstrate opportunities for cost effective use of nitrogen inhibitors to farmers. It will confirm if these mitigation solutions can enhance productivity through pasture growth and contribute to reducing their own on-farm greenhouse gas inventories. We believe these will be the two key drivers for future uptake. This programme will have completed 2 years of trials by the end of 2010 and is expected to continue through to 2011.

We will continue to utilise and draw on independent advice from our Science and Commercial Advisory Groups.

As the research programme progresses, the Consortium expects an increasing emphasis on commercialisation. We are moving rapidly to appraise our identified “mitigation products” and evaluate them against market requirements to develop effective commercialisation plans for there deployment.

Given the confirmation of the Emissions Trading Scheme as a means of dealing with domestic carbon costs, and identification of the scheduled entry by Agriculture into this in 2015, we will continue to build the likely effects of the ETS on our sector into our commercial plans as they develop.

The Consortium has confidence that its continued investment in pastoral greenhouse gas research will place New Zealand in a better position to counter the effects of climate change. We believe we are excellently placed to achieve our goal of providing cost-effective solutions to greenhouse gas emissions to the pastoral livestock sector.

The development of the solutions needed to achieve this goal will require broad knowledge and integration across many disciplines. These will seldom be found at any one institution, so the PGgRc will be actively looking for, and supporting, collaboration nationally and internationally. With this will come other challenges in harnessing the combined resources and focusing them to deliver cost-effective solutions that allow our pastoral industries to continue to grow and prosper.

The Consortium will be striving to deliver solutions that support this change, be they products targeting livestock, microbes, changes to the farm environment, or knowledge that needs to be adopted. The approaches taken will be based on sound science, and will build on the knowledge and skills developed so far.

11. Appendices

11.1. PGgRc Management

Consortium Manager – Mark Aspin
Commercial Manager – James Li
NOMR Project Manager – Dr Allan Gillingham

11.2. Principal Investigator

Dr Harry Clark – Feb 2010, Dr Peter Janssen has taken this role since July 2010.

11.3. PGgRc Board

Mark Leslie – Fonterra Ltd (Chairman)
Leon Black – B+LNZ
Rick Pridmore – DairyNZ
Peter Benfell – AgResearch Ltd
Hilton Furness – Fert Research (NZFMRA)
Damian Lynch – PGG Wrightson Ltd

11.4. Observers

Mark O'Connor – DEEResearch Ltd
Collier Isaacs – Landcorp Farming Ltd
Mike Jebson – MAF
Murray Poulter – NIWA
Harry Clark – NZAGRC

11.5. Science Programme Teams & Key Publications

1) Microbial strategies to reduce methane emissions

Objective Leader – Dr Peter Janssen

Presentations
Kenters N, Henderson G, Kittelmann S, Janssen PH (2009) Rapid isolation of novel anaerobic bacteria. Poster presentation. 68th Annual Assembly of the Schweizerische Gesellschaft für Mikrobiologie, 4 – 5 June 2009. University of Lausanne, Lausanne, Switzerland

Henderson G, Naylor GE, Leahy SC, Janssen PH (2009) Identification of new, potentially homoacetogenic bacteria in the rumen after analysis of formyltetrahydrofolate synthetase gene sequences. Conference presentation. COMBIO 2009, 6 – 10 December 2009. Christchurch, New Zealand

Janssen PH (2010) Sustainable Land Management and Climate Change research at AgResearch. Presentation to Combined Meeting of the Peak Group and Technical Working Groups for Sustainable Land Management and Climate Change, 18 March 2010. Pastoral House, Wellington, New Zealand

Janssen PH (2010) Methane mitigation: targeting rumen methanogens. Presentation to Global Research Alliance on Agricultural Greenhouse Gases Senior Officials, 10 April 2010. Bio Commerce Centre, Palmerston North, New Zealand

Janssen PH (2010) Methane mitigation: targeting rumen methanogens. Presentation to Meat and Wool New Zealand Board, 4 May 2010. New Zealand Agricultural Greenhouse Gas Research Centre, Palmerston North, New Zealand

Janssen PH (2010) New Zealand Agricultural Greenhouse Gas Research Centre. Presentation to GreenDrinks Meeting, 18 May 2010. Steeple Conference Centre, Kingsgate Hotel, Palmerston North, New Zealand

Janssen PH, Buddle BM (2010) Methane mitigation: targeting rumen methanogens. Presentation to Understanding Climate Change and Greenhouse Gas Emissions, Impacts, Adaptations, and Mitigation Options on Farms – Farmers Project Summit, 25 May 2010. AgResearch, Palmerston North, New Zealand

Published Reports
Henderson G, Naylor GE, Leahy SC, Janssen PH (2010) Presence of novel, potentially homoacetogenic bacteria in the rumen as determined by analysis of formyltetrahydrofolate synthetase sequences from ruminants. Applied and Environmental Microbiology 76: 2058-2066.

Science Team involved:
AgResearch: 6

2) Methanogen Genomics

Team Leader: Dr Eric Altermann

Presentations
Kelly WJ, Leahy SC, Altermann E, Ronimus RS, Pacheco DM, Li D, Yeoman CJ, Kong Z, McTavish S, Sang C, Lambie S, Janssen PH, Dey D, Attwood GT (2009) The genome sequence of the rumen methanogen Methanobrevibacter ruminantium: identifying targets for controlling ruminant methane emissions. Conference presentation. COMBIO 2009, 6 – 10 December 2009. Christchurch, New Zealand

Lambie SC, Whitley H, Kelly WJ, Leahy SC, Attwood GT, Altermann EH. 2009. ømru: a prophage of the rumen methanogen *Methanobrevibacter ruminantium*. Conference presentation. COMBIO 2009, 6 – 10 December 2009. Christchurch, New Zealand

Altermann EH, Lambie SC, Whitley H, Kelly WJ, Leahy SC, Attwood GT. Identification non-ribosomal peptide synthetase genes in *Methanobrevibacter ruminantium* M1. Conference presentation. COMBIO 2009, 6 – 10 December 2009. Christchurch, New Zealand

Published Reports
S. M. Leahy, W. J. Kelly, E. H. Altermann, R. S. Ronimus, C. Yeoman D. M. Pacheco, D. Li, Z. Kong S. McTavish, C. Sang, S. C. Lambie, P. H. Janssen, D. Dey, and G. T. Attwood. 2010. The genome sequence of the rumen methanogen *Methanobrevibacter ruminantium* reveals new possibilities for controlling ruminant methane emissions. PLOS One, 5:e8926.

Science Team involved:
AgResearch: 12
University of Otago: 1

3) Development of an Anti-methanogen Vaccine

Objective Leader: Dr Bryce Buddle

Presentations
Wedlock N, Pedersen G, Dey D, Janssen PH, Denis M, Buddle B (2009) Development of a sub-unit vaccine to reduce methane emissions in ruminants. Poster presentation. 39th Annual Meeting of the Australasian Society for Immunology, 6 – 10 December 2009. Broadbeach, Queensland, Australia

Pedersen G, Wedlock N, Dey D, Janssen PH, Denis M, Buddle B (2009) Development of a sub-unit vaccine to reduce methane emissions in ruminants. Poster presentation. COMBIO 2009, 6 – 10 December 2009. Christchurch, New Zealand

Neil Wedlock. Development of a vaccine to reduce greenhouse gas emissions from ruminants. Presentation to media via Science Media Centre, Tackling methane emissions – the science involved, 13th August 2009. http://www.sciencemediacentre.co.nz/2009/08/11/tackling-methane-emissions-the-science-involved/

Published Reports
Wedlock DN, Pedersen G, Denis M, Dey D, Janssen PH, Buddle BM (2010) Development of a vaccine to mitigate greenhouse gas emissions in agriculture: Vaccination of sheep with methanogen fractions induces antibodies that block methane production in vitro. NZ Vet. J. 58:29-36

Science Team involved:
AgResearch: 7

4) Animal Selection & Breeding

Team Leader: Dr Sarah Berry / Dr Cesar Pinares (AgResearch)

Published Reports
Pinares-Patiño C.S. and Clark H. (2010). *Rumen Function and Digestive Parameters Associated with Methane Emissions in Dairy Cow. Pp. 86–93, in: G.R. Edwards and R.H. Bryant (Eds.) Meeting the Challenge for Pasture-Based Dairying. Lincoln University, Christchurch, New Zealand.*

Pinares-Patiño C.S., Muetzel S., Molano G., Hunt C. And Clark H. (2010). *Emissions of hydrogen gas from sheep fed fresh perennial ryegrass and pelleted diets*. Pp. 447–448, in: G.M. Crovetto (Ed.) Energy and Protein Metabolism and Nutrition. Wageningen Academic Publishers, Wageningen, The Netherlands.

Hegarty, R.S. and McEwan J.C. (2010).*Genetic Opportunities to Reduce Enteric Methane Emissions from Ruminant Livestock.* Proceedings of the 9th World Congress on Genetics Applied to Livestock Production.

Pinares-Patiño C.S., Waghorn, G.C., Hegarty, R.S., Hoskin, S.O. (2009). *Effects of Intensification of Pastoral Farming on Greenhouse Gas Emissions in New Zealand*. New Zealand Veterinary Journal (invited Feature Review) 57: 252–261.

Science Team involved:
AgResearch: 11
Via Lactia Biosciences: 3

5) Low Greenhouse Gas Emitting Farm Systems

Objective Leader – Dr Simone Hoskin (Dr David Pacheco)

Presentations
NZIPM Conference (R. Dynes and F. Kelliher) October 23, 2009

MAF Australia and New Zealand Climate Change Conference (M. Brown and R. Dynes) November 16-17, 2009

Managing climate change conference (R. Dynes)
February 10-11,2010 - Fertiliser and Lime Research Centre Conference (R. Dynes and T. Rhodes) November 18-20, 2009

R. Dynes presentation to senior executives from TEAGSC (Ireland) March 24, 2010

R. Dynes presentation to Australian methane modelling workshop. April 19-20, 2010

Women in Dairying, Workshop on NZ ETS and farming systems
GHG emissions (2 presentations) (R. Dynes and D. Smeaton) May 13, 2010

Taranaki Federated Farmers AGM (M. Brown) 21st May 2010

Published Reports
Hammond, K. J. Muetzel, S. Waghorn, G. G. Pinares-Patino, C. S. Burke, J. L. Hoskin, S. O. The variation in methane emissions from sheep and cattle is not explained by the chemical composition of ryegrass. Proceedings of the New Zealand Society of Animal Production. 2009. 69: 174-178.

Hammond, KJ (2010) Methane from dairy cows: tax, wasted energy, the future. Large Herds Conference 2010, Invercargill, 15-18th March 2010. Winner of the "Young Dairy Scientist Communication Award"

Swainson N. M., 2009: Methane emissions and mitigation technologies in cattle, sheep and red deer. A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy (PhD) in Animal Science at Massey University, Palmerston North, New Zealand. Thesis submitted November 2009.

Hammond KJ (2010) Methane and what it is costing you. New Zealand Dairy Exporter, May 2010 Issue.

Understanding Climate Change and Greenhouse Gas Impacts adaptations and mitigation options on Farm - Case studies as presented in the Project Summit (May, 2010)

Science Team involved:
AgResearch: 12
DairyNZ: 3
PGGWrightson: 1
Farm Consultants: 3

6) Chemigenomics

Objective Leader – Dr Ron Ronimus

Science Team involved:
AgResearch: 5