A Pastoral Greenhouse Gas Research Strategy

Annual Report to the Crown on Progress

For the Year ended 30 June 2004.

Submitted by the
Pastoral Greenhouse Gas Research Consortium
July 2004

Introduction

This report to the Crown on the Investment by the agricultural sector into the mitigation of greenhouse gases covers the requirements as outlined in the Memorandum of Understanding (MOU) dated January 2004. The programme of activity that is reported here is driven by the pastoral greenhouse gas research strategy developed and appended to the MOU between the Pastoral Greenhouse Gas Research Consortium (PGGRC) parties and The Crown. The goals of the strategy which aims to develop safe, cost-effective greenhouse gas abatement technologies that will seek to reduce methane and nitrous oxide emissions from livestock by at least 20 percent by 2012 are as follows:

- 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 and beef cattle and deer, which lower methane emissions from New Zealand ruminants and nitrous oxide from grazing animal systems; and
- To exploit commercial opportunities arising from the science and technologies in a global market.

This document is reported in the manner that PGGRC manages research programmes; the table at the end of this report cross references this document with the strategy as it was compiled and originally reported.

This document represents a total investment into greenhouse gas research of $7m The industry will contribute $4.7 million, more than half of it either recently initiated or new funding.

Pastoral Greenhouse Gas Consortium Partners

The Participants

Fonterra Co-Operative Ltd
Meat and Wool NZ Ltd (formerly Meat New Zealand)
Dairy Insight Inc.
Wrightson Ltd
New Zealand Fertiliser Manufacturers Research Association Inc.
DEEResearch Ltd
AgResearch Ltd

Associate Members

NIWA
MAF

Research Providers

AgResearch
Livestock Improvement Corporation (BoviQuest)
Dexcel
Lincoln University
ViaLactia BioSciences

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Executive summary

This report highlights many of the key areas of research being conducted in developing methane and nitrous oxide mitigation technologies through:

- **Building underlying knowledge of microbe and protozoa populations.**
- **Exploring potential genomic solutions to inhibition of methanogens**
- **Determining the genetic effect on methane production**
- **Forage inhibitors, cultivars with superior genetics in a proof-of-function trial after confirming methane-lowering attributes.**
- **Understanding the extent and seasonal variation of N_2O emissions from cow urine and N fertiliser, to enable the development of best management practices for reducing N losses to the atmosphere.**
- **Determining the effectiveness of a nitrification inhibitor, dicyandiamide (DCD), in reducing nitrous oxide emissions.**
- **Construction of a prototype mega-chamber for scaling-up to measure the effectiveness of DCD in reducing nitrous oxide emissions on-farm**
- **Establishing collaborative studies with international researchers to complement and extend research.**
- **Confirming one PhD scholar who is supervised by AgResearch and Massey University**
- **Expanding the Consortia to include a new party (FMRA) and initiating preliminary discussions with parties to expand it further.**

And represents a total investment into greenhouse gas research of $7m, of which the industry will be contributing $4.7 million, more than half of it either recently initiated or new funding.

1. Methane Research Programmes

1.1 Rumen microbial strategies to lower Methane emissions

- To obtain information on specific microbes involved in methane formation and methanogen survival so that rumen processes can be exploited to provide new strategies for lowering ruminant methane emissions.
- To investigate microbial populations involved in methane emissions and increase understanding of fundamental processes in the microbial ecosystem so that next-generation methane-abatement technologies can be developed.

Progress 03/04

- The diversity of rumen protozoa in grazing deer, cows and sheep in NZ have been described.
- Forages were shown to selectively influence some, but not all, protozoal populations in grazing deer. Some species of protozoa were found to have high association with methanogens suggesting that these could be targeted in methane-lowering strategies.
- Activity from methanogen-specific phage has been found in tests to isolate lytic phage with the potential to destroy methanogens.
- New methanogen isolates were purified and added to our methanogen culture collection to increase this resource which is essential for much of our knowledge discovery, testing, and *in vitro* assessment of methane-abatement options.
- Five clovers were examined but none inhibited methanogens
- A real-time PCR method has been developed to provide a non-culture method for quantifying methanogen populations from DNA extracted from rumen samples.
- PCR analysis of DNA extracts obtained from the gut of lambs showed the presence of the fibre-degrading bacteria *Ruminococcus albus* and *Fibrobacter succinogenes* in the rumen soon after birth and well before forage digestion occurred.

Objectives 04/05

Locate methanogen-specific bacteriophage because they are promising anti-methanogen agents.

Identify specific protozoal populations important in sustaining methanogen's populations, and then develop agents, which inhibit only these protozoa as an abatement strategy. Indications are that certain types of protozoa are more involved than others in forming symbiotic associations with methanogens, and that some forages can selectively alter specific protozoal populations and lower methanogen numbers in deer.

Develop and apply molecular techniques to the rumen microbial ecosystem, establish a robust set of molecular procedures for monitoring target populations, and obtain fundamental information on microbe –microbe and microbe-forage interactions including those with fibre-degrading microbes and with acetogens.

1.2 A genomics approach to identify targets for methanogen inhibition.

Progress 03/04

- The assembly of sequenced pieces of the *Mbb. ruminantium* genome has been completed, and the process of filling in gaps is continuing
- Forty genes were identified as being involved in the methane formation pathway.
- An extraction procedure has been established to provide mRNA of a quality suitable for the preparation of labelled cDNAs.

Objectives 04/05

Work in this objective has identified several features of *M. ruminantium* that are potential targets for methane mitigation. Genes that are involved in methanogenesis have been identified and these will be targeted for inhibition. The remaining work of genome annotation and functional analysis of genes will continue and will identify secondary targets for inhibition of methanogens, and will use a whole genome array to investigate *M. ruminantium* gene expression during growth on H_2 + CO_2

1.3 Forage inhibitors to lower methane emissions

1.3.1 Evaluation of Forage Plants

- Thirty seven extracts from grasses and tannin-containing legumes were tested for their methanogen inhibiting capacity using *Mbb. ruminantium* as the target. None of the extracts had any lasting anti-methanogen effects. One extract had a temporary bacteriostatic effect, but was toxic to microbes essential for rumen digestion.
- Kikuyu grass extracts showed no inhibition of methane production in broth culture of *Mbb. ruminantium*.
- Crude extracts from condensed tannin-containing forage legumes were prepared and none were active against *in vitro* broth culture with *Mbb. ruminantium*.
- The *in vitro* data combined with knowledge from a recent *in vivo* trial would suggest it may be difficult to find a level of dosing for a condensed tannin extract to inhibit methane that does not adversely affect animal performance.

Objectives 04/05

Evaluate cultivars with superior genetics in a proof-of-function trial after confirming methane-lowering attributes with an *in vitro* rumen bioassay. Obtain a seasonal profile of methane abatement from cultivars with the *in vitro* rumen bioassay.

To evaluate agronomically favourable plant forages (optimal crude protein to soluble carbohydrate content) for their potential to reduce methane emissions from grazing ruminants with the aim to select a superior cultivar consistent with meeting both productivity and green house gas emission targets.

1.3.2 Fumarate in Ryegrass – Screen of natural levels in NZ cultivars

Progress 03/04

- This experiment tested the hypothesis that additions of fumarate would increase fibre digestibility and reduce methane in a dose-dependent manner, when ryegrass-dominant pasture was fermented in continuous culture. Addition of organic acids such as fumarate to high grain diets fed to cattle have increased ruminal utilisation of lactate, with consequent increases in pH and propionate concentrations and reductions in methane production.

- Digestion characteristics responded linearly ($P<0.05$) as fumarate increased from 0 to 30 mM. Concentrations of propionate and total volatile fatty acids increased by 74% and 19%, respectively. Increasing fumarate reduced the ratio of acetate:propionate (2.4 v.s. 1.5) and reduced methane production ($P=0.057$) by 37%.

- The increased concentration of propionate in this experiment appears to be a direct response to additional substrate (fumarate), rather than by an indirect improvement in lactate utilisation or fibre digestibility. These results suggest that higher concentrations of fumarate in ryegrass diets could increase energy capture by improving the supply of glucogenic compounds and reducing losses to methane emissions.

Objectives 04/05

Further research aimed at evaluating the concentration of fumarate in ryegrass is now been conducted through a fumarate and malate variation screen on 25 ryegrass cultivars. The screen will be conducted in two stages. The initial screen will be a low cost go/no go indicator. The second, using populations grown under strictly controlled conditions will be progressed based on the success of stage one to determine if commercial ryegrass cultivars could provide increased fumarate levels to capture the methane abatement achieved through addition of fumarate

1.4. Animal Factors Investigation to identify Low and High Emitters

1.4.1 Determination whether differences are due to animal genetics or other factors.

Progress 03/04

- Swapping rumen contents between dairy cows and measuring methane emissions before and after the swap has indicated that the animal may be an important regulator of methane production.
- A lower variance in specific rates of methane production for clones compared to non-cloned cohorts suggests that this may be a heritable trait.
- This research includes a PhD scholar supervised by personnel from AgResearch and Massey University.

Objectives 04/05
Identify the most important animal (or other source) characteristic(s) responsible for the between-animal variation in methane production in pasture-fed animals, and to understand the mechanisms via which these differences occur (feed digestibility, digestion process, microbial population, grazing and ingestive behaviour).

In parallel develop a simple index that could account for the relevant physiological and/or behavioural characteristic, an essential first step in selecting animals for low methane production.

1.4.2 Boviquest trial - Quantifying genetic variation in methane production utilising the Friesian-Jersey crossbred trial herd

Progress report 03/04:

- A trial was conducted to measure methane production in Cohort 1 (n=301) of the Friesian Jersey crossbred trial herd. The aim of this project was to prove that methane production could be successfully measured in a large group of animals and to confirm the existence of between animal variation in methane production
    - The cows were successfully phenotyped for methane production
    - Significant variation was found between cows for this phenotype

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Objectives 04/05
Utilising the second cohort of the trial herd is planned, to provide sufficient animal numbers to estimate the genetic effect on methane production and identify any QTL that may be linked to this variation in methane production and ultimately utilised if successful in an animal breeding programme.

1.5 Proof-of-function of possible methane-reducing technologies.

1.5.1 Anti-methanogen vaccines

Progress 03/04

- Two anti-methanogen vaccines were tested on sheep and the results indicate that they did not reduce methane emissions. This work was done in collaboration with CSIRO. A final report has been presented to PGgRC, and results were reported at the NIWA/PGgRC Workshop in March.
- Analysis of rumen fluid samples taken pre and post vaccination indicated that the vaccine formulations did not eliminate the target methanogens from the test animals.

1.5.2 Effect of maize silage on methane emission in dairy cows fed a base diet of pasture

- Pasture-fed cows with 0%, 13%, 23% or 37% of diet as maize silage.

- No significant effect on methane production per unit intake.
- Shows that farmers can feed considerable proportion of diet as maize silage without any negative effects on methane emission.

1.5.3 Effect of white clover on methane emission in dairy cows fed a base diet of pasture

- Pasture-fed cows with 0%, 15%, 30% or 60% of diet as white clover.
- Increasing proportion of white clover in diet reduced methane production per unit intake by up to 20%.
- Some on-farm potential although feeding 60% clover may not be a practical option due to negative effects on dry matter production

1.5.4 Effect of condensed tannins in birdsfoot trefoil (*Lotus corniculatus*) on methane production by lactating dairy cows

- Total methane production similar for cows grazing ryegrass (360.6 g CH_4 / d) or lotus (343.2 g CH_4 / d)
- Methane production per unit intake lower on lotus (19.9 g CH_4 / kg DMI) than ryegrass (24.2 g CH_4 / kg DMI). 66% of the difference due to action of condensed tannins
- **Question: Is it practical to use CT-containing legumes on-farm and will production and environmental benefits indicated in short-term trials still be viable?** This will be addressed in a Forage Mixed Ration (FMR) Farm Systems trial starting June 2004 as the yields of these CT-containing legumes is significantly lower than pasture**.**

- Birdsfoot trefoil agronomic (grazing) trial, final measurements made in April 2004 with the crop in its 2nd season showed on average 7t DM/ha was consumed. Grazing management resulted in greater plant densities found on the hard grazed treatments than on the lax grazed treatments.

1.5.5 Novel lipid supplements to reduce methane production

- Pasture-fed cows supplemented with 500g fish oil / sunflower oil mixture per day

	No oil (control)	Oil
g CH_4 / cow / d	242.4	176.4
g CH_4 / kg DMI	18.5	13.5
g CH_4 / kg MS	150.8	119.3
CH_4 per GEI (%)	5.84	4.25

- This trial suggests supplementing pasture-fed cows with certain oils has beneficial effects regarding methane emissions but questions remain regarding use on-farm due to negative effects on milk fat concentration.

1.5.6 Effect of Monensin on methane emission by pasture-fed identical twin dairy cows

- Trials with pasture-fed cows at end of lactation and during dry period showed sodium monensin delivered by intra-ruminal capsules reduced methane production by 12% and 10% respectively.
- Some potential for on-farm use as also used as bloat-control measure

1.6 Small Molecules

As part of a review of its science plan, PGgRC has contracted AgResearch to collaborate with a range of research providers to determine if there are chemicals available or alternative mitigation ideas for methane or nitrous oxide emissions. The question raised is whether a small molecule or other chemical additives could interfere beneficially with methane production in the rumen.

2.0 Nitrous Oxide Research Programmes

2.1 Nitrous Oxide Emission from Resource Efficient Dairying (RED) Trial

Progress 03/04

- There has been no published field research in New Zealand evaluating strategies to mitigate N_2O emissions.
- A study was carried out to determine N_2O emission factors from cow urine and fertilizer urea following applications onto dairy farmlets. Understanding the extent and seasonal variation of N_2O emissions from cow urine and N fertiliser is important for refining the N_2O emission factors for urine and fertilizer from dairy farm systems to enable the development of best management practices for reducing N losses to the atmosphere.
- Results to date indicate that N_2O fluxes from both urea and urine treatments increased after application, reaching peak fluxes after about 13 days. The N_2O fluxes from the urea treatment had declined to a level similar to that from the control area by approximately 20 days after application, while the N_2O fluxes from the urine treatment had declined to the control level by approximately 40 days.

The estimated N_2O emission factor for urea for the measurement period was 1.27% of the urea-N applied, which is similar to the current New Zealand inventory value of 1.25%. The estimated emission factor for urine was 0.59%, which is lower than the current New Zealand inventory value of 1% and the IPCC "default" value of 2% for an annual average emission factor from N excreted. However, these emission factors should be viewed as preliminary data from one season only.

Objectives 04/05

Continue the reseach programme to confirm the initial findings.

2.2 Nitrification inhibitor's on different Soils

Background

There are two key emission pathways for nitrous oxide:

1. Direct emissions from the land surface following the deposition of animal excreta or the application of N fertilisers; and
2. Indirect emissions from nitrate leached through the soil profile following subsoil denitrification and the re-emerging into streams.

Both sources are important and it is critical to make sure that in developing any mitigation technology to reduce nitrous oxide emissions that we do not simply decrease one source by increasing the other.

Progress 2003/04:

- A research project has been successfully established to determine the effectiveness of a nitrification inhibitor, dicyandiamide (DCD), in reducing nitrous oxide emissions from cow urine and urea fertiliser on a free-draining pasture soil (Lismore) using chamber methods on undisturbed soil monolith lysimeters. The initial results indicate that DCD has significantly reduced nitrous oxide emissions from cow urine patches. These results are in line with our previous research that showed that a 75% reduction in nitrous oxide emissions could be achieved by treating grazed pasture soil with a nitrification inhibitor.
- New large gas measurement chambers have been successfully constructed and attached to large Templeton lysimeters to enable gas collection for nitrous oxide measurement.
- Measurements of the effectiveness of a nitrification inhibitor, DCD, in reducing nitrous oxide emissions from cow urine and urea fertiliser have been started on a second pasture soil (Templeton) using chamber methods on established undisturbed soil monolith lysimeters.
- Preliminary design concepts have been developed for construction of prototype mega-chamber for scaling-up to measure the effectiveness of DCD in reducing nitrous oxide emissions on-farm.

The expansion into this field by PGgRC has created the opportunity of a potential new partner with matched funding. The Foundation is currently evaluating the research programme and once we have support for the consortium will be in a position to expand the scope of this work further.

3.0 Collaborations

PGgRC had set a target to establish and initiate collaborative studies with international researchers to complement and extend research funded by PGgRC building on previous collaborations with CSIRO in Australia;

- with QDPIF, Queensland, Australia by 30 Dec 2004,
- with Japanese researchers by 30 April 2005.

3.1 Australia

Dr A. Klieve, a rumen microbiologist with the Queensland Dept of Primary Industries and Fisheries (QDPIF), visited Dr Joblin in April 2004 supported by the PGgRC to formulate a 2004/05 collaborative work programme for a study on reductive acetogens from kangaroos and from ruminants. A Statement of Proposed Work was prepared and has been submitted and accepted by PGgRC. An exchange agreement signed to transfer expertise on profiling viral populations in the rumen, and on the measurement of acetogen hydrogen affinities between laboratories, has been executed and the project commenced on May 28, 2004.

3.2 Japan

Professor Hisao Itabashi, Tokyo University of Agriculture and Technology, together with 2 scientists from the National Institute of Livestock and Grassland Science and 2 officials of Ministry of Agriculture, Forests and Fisheries of Japan visited New Zealand for discussions on possible collaborative efforts between Japan and NZ on reducing ruminant methane emissions. After an exchange of information at a formal meeting between the visitors and PGgRC representatives, both sides agreed to proceed and are at present considering possibilities for funding and considering areas for joint projects.

4.0 Inventory

4.1 Methane emissions from growing dairy calves and mature cows

- Treat calves as separate entity to cows up to 6 months regarding contribution to methane inventory.

		g CH_4 / day		**G CH_4 / kg DMI**		
	Calf age	**Calf**	**Cow**	**Calf**	**Cow**	**p value**
November 2002	4 month	50.8	327.7	19.75	23.32	0.002
March 2003	8 month	115.6	350.3	25.35	24.92	0.678
September 2003	14 month	140.0	302.2	18.89	17.62	0.137
December 2003	17 month	232.1	436.9	26.50	24.71	0.134

5.0 Modeling

5.1 Decision support modeling:

Decision support modeling – Work by Simon Woodward (Woodward Research) under a FRST sub-contract in "A systems approach to reducing methane emissions" (DRCX0207) has made progress in developing a decision support model for methane emission from dairy farms using a restricted range of management options. Further work is needed to expand the options and include nitrous oxide in this model.

- The Dexcel Whole Farm Model is a research framework that has a methane output component but does not include a wide range of cropping options.
- The OVERSEER model is a nutrient budget model that calculates GHG emissions from ruminant enterprises but does not allow the integration of cropping and livestock on one farm or predict the effect of management changes on animal production.
- The EcoMod model developed in Australia incorporates much of the scientific knowledge on pasture growth, soil nutrients and soil water relations but is relatively weak in animal intake and methane emission areas.

- Dexcel has submitted a proposal to PGgRC to develop their methane decision support model ("METHADS") to extend to estimate farm Nitrous Oxide and Carbon Dioxide emissions associated with alternative land management and technology options for a particular farm, including on-farm cropping (METHADS already estimates methane and economic outcomes of generic NZ dairy systems). In addition, estimates of production and environmental risk due to unpredictable variation in biological responses and weather will be modeled. PGgRC is currently considering this proposal.

6.0 Attachments

6.1 NIWA / PGGRC Workshop Proceedings

A workshop jointly hosted by NIWA and PGGRC in March 2004 with the express aim of providing a forum to:

- provide an opportunity for scientists and science users to interact

- make progress towards a common strategy for collaboration between science research groups and science users, and;

- smooth out misunderstandings and increasing the overall effectiveness of the specialist research work.

The Papers are arranged in four themes

- Pastoral greenhouse gas emission processes – microscopic and paddock scales – options for mitigation

- The New Zealand greenhouse gas emissions inventories – methods and new compilations – how good are they?

- New measurements of trace gases – concentrations and fluxes - Pacific, NZ, Antarctica.

- Human and economic dimensions of greenhouse gas issues – effects on pastoral industries - the progress of the Kyoto Protocol, within and beyond New Zealand

6.2 Proceedings of the 2nd Joint Australia and New Zealand Forum on Non- CO2 Greenhouse Gas Emissions from Agriculture.

This forum, sponsored by the Australian Greenhouse Office was held in Melbourne in October 2003 and attended by the PGgRC Chairman.

The proceedings can be found at:
www.greenhouse.crc.org.au/crc/products/nonco2forum.pdf

Appendix 1.0

Pastoral Greenhouse Research Strategy

A Summary of Methane Research

Result Area	Areas of investigation	Funded programme objectives	Section
Discovery	Methanogen genomics	Genome sequences Identification of methanogen inhibitors	1.2
	Rumen microbes	Strategies to modify hydrogen and methane production by microbes Novel methanogens in other species	1.2
	Animal factors	Investigate whether between-animal differences are genetically determined or due to other factors Explanation of apparently low emissions in deer	1.4
	Methane inhibitors-Forage	Isolation of condensed tannins/plant inhibitors with anti-methanogenic properties. Desk-top study of literature on inhibitors	1.3
	On-farm testing protocols	Desk study protocol for evaluation of potential rumen function modifiers before trialling. Malate / fumarate review completed	1.3.2
Proof of concept/ function	Vaccination	Test CSIRO vaccine in sheep	1.5.1
	Condensed tannins	Feeding trials with Lotus corniculatus, kikuyu and sulla	1.3.1
	Diet Manipulation	Evaluation of mitigation options using grains, oils, fats	1.5.5
	Forage mixes	Design of rations to economically improve efficiency by reducing methane output per kg DDMI and through diluting the animal's maintenance requirements.	1.5.2 1.5.3
	Monensin	Monensin (Rumensin™) may reduce methane by 20% - some acceptability concerns with feeding an antibiotic. Evaluation is needed in NZ farming conditions.	1.5.6
Develop-mental / on-farm testing	Farm scale modeling / resource accounting		5.0
	Inhibitor screening bioassay	Anaerobic culture methods used to test methanogen survival following addition of plant extracts with suspect anti-methanogen properties.	1.3
	Increased Legume Content	On-farm evaluation to increase CTs feeds available (clover establishment). Development of management guidelines to assist farmers growing/feeding Birdsfoot trefoil and Sulla	1.5.4

A Summary of Nitrous Oxide Research

Result Area	Areas of investigation	Funded Programme objectives	Section
Discovery	Nitrification inhibitors		2.2
	Fundamentals of N2O production, transformation and transfer.	Identify factors which affect Pasture plant uptake of N Processes and drivers of N loss from soils and emission of N2O	2.1
	Research into effect of timing of fertiliser applications that produce the required agronomic result on N2O emissions	Set guidelines on N fertiliser use and timing of application in relation to rainfall and soil wetness to minimise N2O emissions from soils.	2.1
	Input response functions for excreta	Definition of the relationships between quantities of N in urine and N2O output together with outputs from dung affected soil. (Increase N retention and increase the dung-N to urine-N ratio)	2.1
	Diet manipulation (N levels that more closely match animal needs) Manipulate dietary C/N ratio to increase N retention	Trial will look at, effect of use of stand off pads and maize silage inputs.	2.1
	Research into product gas ratio (N2O vs. N2)	Investigate product gas ratios (trap nitrate and favour the emission of N2 rather than N2O in riparian and other wet areas), including determination of CH4 emissions from these areas.	6.1
Proof of concept/ function	Delivery of nitrification inhibitors to target sites	Ongoing development of cost effective ways to use DCD Developing early DCD results in the field	2.2
Development/ on-farm testing	Farm scale modelling / resource accounting		5.0
	On-farm testing protocols	Desk study protocol for evaluation of potential inhibitors of N2O production before trialling.	
	On-farm testing of mitigation options through managing critical source areas of N2O	Management practices for improving soil aeration and drainage, reducing soil compaction, and/or application of effluent and sewage.	
Technology Transfer	Development of soil management guidelines	Wet soils best management practices Best management practices for irrigated soils	