THE NEW ZEALAND PASTORAL GREENHOUSE GAS RESEARCH STRATEGY

3RD

ANNUAL REPORT TO THE CROWN ON PROGRESS

JULY 2006

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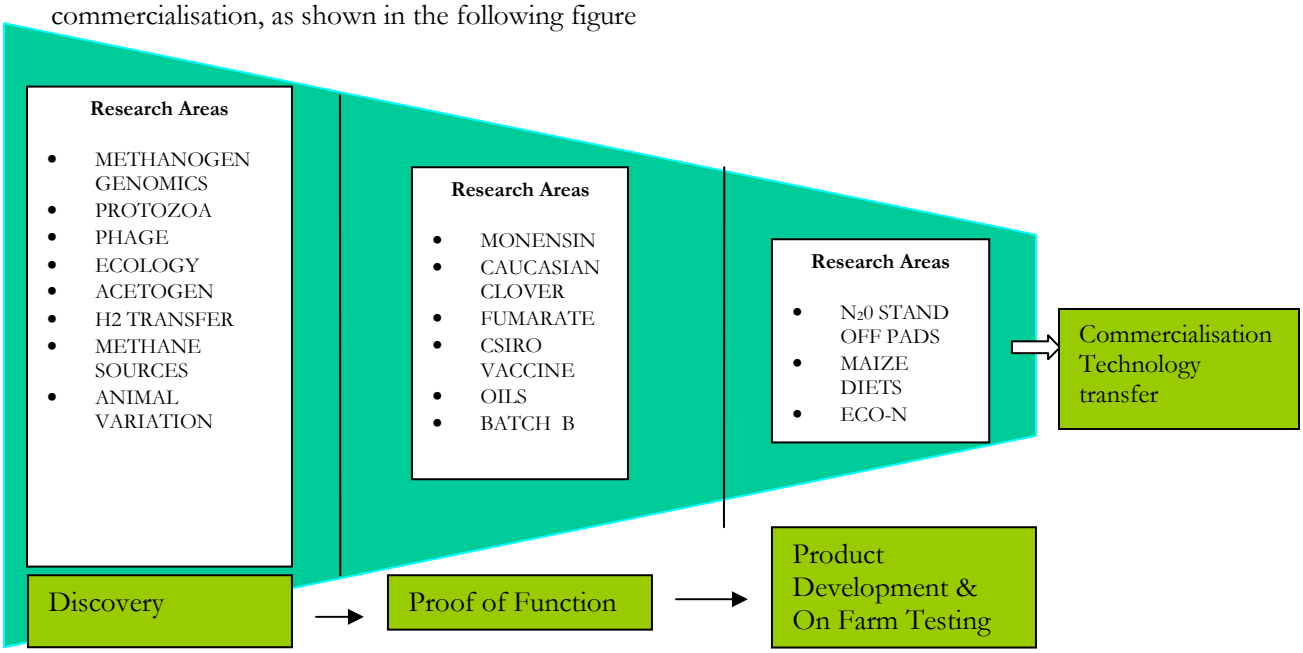
INTRODUCTION

This is the third report to the Crown on the investment by the pastoral sector into the mitigation of agricultural greenhouse gases to June 2006, being the fourth year of the five year initial consortium period and tracks four years of progress. Since the submission of the first report the Kyoto protocol has come into force and is now binding. The recent updating of the expected position for New Zealand at the end of the first commitment period in 2012 identifies the significant challenge the sector faces in assisting New Zealand to meets it commitments under this treaty.

The programme of activity that is reported here is driven by the Pastoral Greenhouse Gas Research strategy developed and appended to the Memorandum of Understanding (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[1] 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. The approach has been to invest along a continuum from discovery through to commercialisation, as shown in the following figure



[1] Note: On establishment of PGGRC in Nov 2002 a 20% reduction was understood as necessary to meet the 1990 target in 2012. Subsequently with refinements to the Inventory this has proved to be incorrect and PGGRC is looking in the future to a target of having mitigation tools available to meet 1990 levels to provide greater clarity of progress.

The last 12 months has seen a number of significant events impacting on the consortium. Firstly the Government review of climate change policy identified the importance of its role in developing research to mitigate for agricultural emissions and emphasizing the need for continued investment.

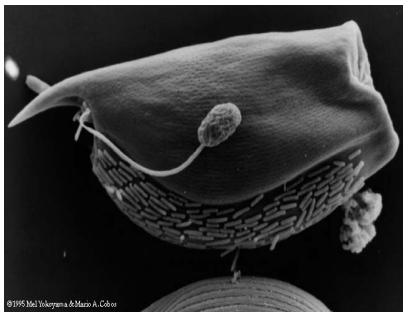
In April the consortium in conjunction with the Foundation for Research Science and Technology (FRST) carried out a full review of its governance and science. This review, involving independent experts, described the PGGRC activities as "World leading research, with excellent productivity for money expended. The Consortium represents the biggest single integrated programme globally responding to issues around livestock greenhouse emissions". This was a satisfying result for the consortium and provides an opportunity to provide a central point for future mitigation research, the review has also challenged the consortium as to the appropriateness of the current goal and questioned whether in its current form it could be solely achieved by PGGRC. In response to this the consortium is developing some more appropriate measures to gauge its progress.

Following on from the review the consortium partners have begun the process of determining their on going involvement with the consortium, PGGRC management has initiated discussions with FRST regarding the continuation of the consortium after June 30 2007. PGGRC is looking to continue to provide a centre of excellence for pastoral based agricultural greenhouse gas mitigation research in New Zealand and potentially globally and looks forward to ongoing discussions with Crown and Industry stakeholders over the next year to ensure alignment of objectives.

Looking to the future, in 2007 and beyond the PGGRC's investment will be focused on building on the knowledge gained to rapidly develop the most promising technologies for mitigation. The strategy will be to build on the knowledge and evolve the research programme accordingly. The challenge of integrating that knowledge in to farm systems is not insignificant and will require continued collaboration with many organisations through out the sector and government.

The consortium believes that it is well placed after 4 years to meet this challenge and looks forward to building the ongoing relationships needed to ensure that we achieve our mitigation targets.

Sheep harnessed to measure methane production using the SF6 tracer technique



Ruminal protozoa – with a single fungal spore and methanogens attached to its underside.

Sampling Lysimeters for Nitrous Oxide production.

EXECUTIVE SUMMARY

The Consortium completed its fourth year of operation with continued progress being made across a broad research front. The highlight of the year was the successful completion of a mid term review of the consortium operation in April. The review team were very complementary of the PGGRC's performance and described it as being; "World leading research, with excellent productivity for money expended. The Consortium represents the biggest single integrated programme globally responding to issues around livestock greenhouse emissions"

Research progress has been excellent in all aspects of the research strategy. Our understanding of the microbial dynamics of the rumen continues to expand. This knowledge and the tools developed will be of immense value going forward and the approach of building underpinning knowledge is in line with the Research Strategy.

Although no methane mitigation technology has been proven, we have strong leads identified towards that end. A mixture of opportunities has been identified and will be developed further within the research strategy. These opportunities span from; specifically targeting the methanogen and rumen microbes to diet manipulation, and through animal selection. We have a solid set of tools and understanding developing of the rumen that will help us to be more effective in identifying effective mitigation technologies and consequently develop cost effective mitigation strategies.

Nitrous oxide mitigation through nitrification inhibitors is well advanced and available to farmers now, as the understanding of these products expands we confidently believe that producers will realise the opportunity they offer to more efficiently manage the nitrogen cycle within farm systems. The more complete understanding of how farm management practices can enhance the management of N_2O emissions will also offer opportunity to farmers to reduce their emissions.

We evaluated a number of promising mitigation strategies throughout the year including Monensin (an anti-bloat chemical) and Caucasian clover. Although neither of these technologies presented us with cost effective reductions in methane the process of evaluation has given us experience in what will be required to make any mitigation technology effective for adoption.

A promising chemical inhibitor for methane was further investigated and was identified as chloroform, a restricted substance. Concerns regarding potential residue accumulation in meat or milk products and negative environmental impacts appear to limit the feasibility of using chloroform as a mitigation strategy.

The sequencing of the rumen methanogen *Methanobrevibacter Ruminantium* has nearly been completed. A number of methanogen specific targets which may be antigenic have been identified, IP protection is being sought for these, and they are being further developed.

The PGGRC participated in a workshop in Australia in April 2006 to develop the concept of a methanogen vaccine further. Initial proof of concept work leading on from the original trials will be included in the 2006-07 work programme.

We identified previously unknown families of Methanogens within the developing rumen, demonstrating that some change in population occurs as the ruminant ages. The turnover of populations offers another opportunity for intervention if the mechanisms can be understood.

Attendance at the 2nd Greenhouse gas and Animal Agriculture (GGAA) conference in Switzerland confirmed that our programme is unique in the world in its comprehensive approach. The hosting rights for the next conference in 2007 was passed to New Zealand and Australia. The PGGRC will work with other NZ and Australian organisations to develop this conference to be held on 27-29 November 2007 in Christchurch.

The first known incident of a ruminant acetogen out competing a methanogen in early stage ruminants, was identified within our programme. The replacement of methanogens as the dominant hydrogen sink within the rumen is one of the strategies that could lead to reduced methane and increased productivity from livestock.

A new programme looking at the transfer of hydrogen within the rumen has developed from the identification of common elements found during a comparison of the methanogen genome with a fibre-degrading bacteria genome. Research into this will offer up further specific mechanisms that can be targeted and may also lead to a better understanding of how rumen digestion could be enhanced.

A method was developed to quantify one common species of ruminal acetogen in the rumen, and used together with culture counts of total acetogens to measure acetogens in dairy cows on different diets

Comparison of the methane production from high and low susceptible cows to bloat has identified some initial differences that may be worth further investigation.

The analysis of the N2O research for Dairy farm systems has identified that the stand-off farm system gave slightly lower N2O emissions and total greenhouse gas emissions per $ of economic farm surplus, compared with the control farm system.

Application of *eco-n* gave a 73% reduction in the total emissions from urine
patches under lysimeter studies even when applied 18 days after the urine deposition in winter. Further to this the increase in annual DM yield resulting from *eco-n* application was estimated to be 21 % on a whole paddock basis. Although achieved under optimum

conditions these results give indications of the potential mitigation possible using nitrification inhibitors.

The first year of research jointly funded with MAF into the nitrous oxide emissions of hill country farming systems showed that the use of DCD appears to reduce emissions in a similar way to its use in dairy farm systems

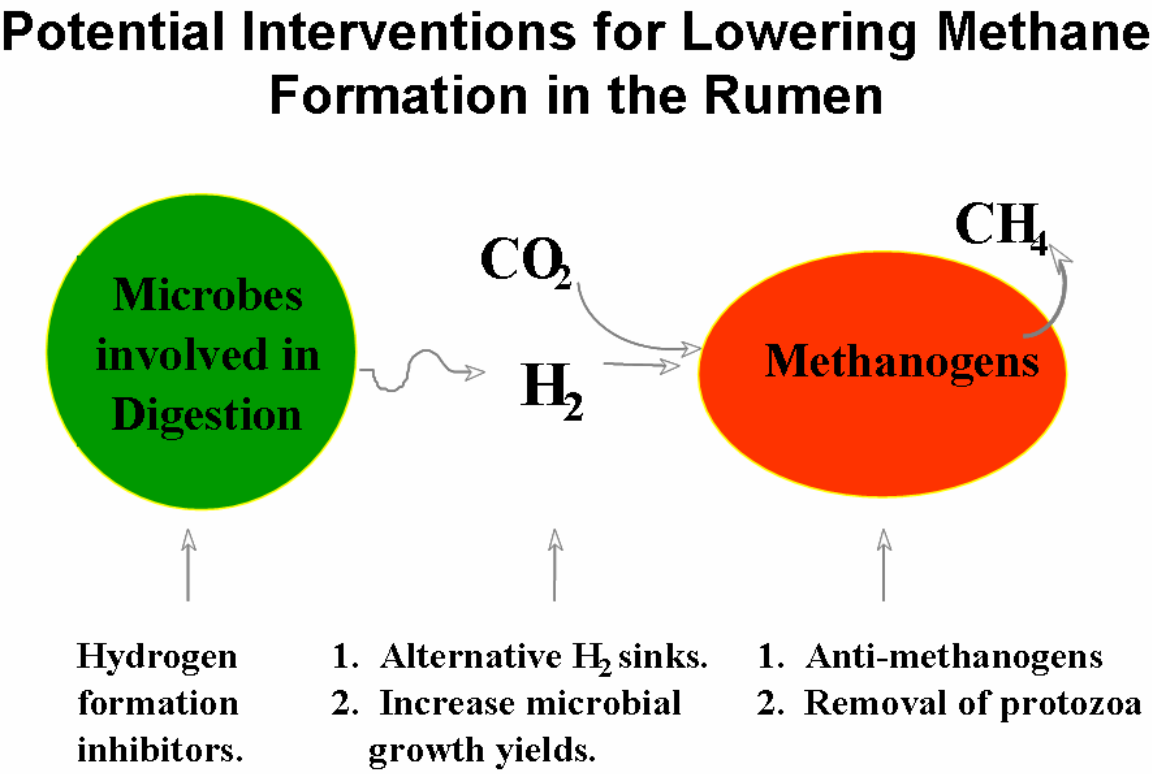
A trial is planned for 2006 /07 where methanogens will knocked down in lactating dairy cows and the effects on rumen function and animal productivity monitored. This trial will run for at least 70 days and is expected to give insights in to the issues that will arise when a methane mitigation technology is introduced.

The PGGRC in recognising the challenge to adoption has initiated a review of nitrification inhibitors and other established nitrogen cycle moderators. The review to be carried out in August 2006 will cover all aspects of their application and aims to identify the gaps in our knowledge and subsequent barriers to adoption. The review will be carried out by an international expert and will involve interviewing all of the interested parties within New Zealand, along with a comprehensive search of the international literature.

2. THE MAIN ACHIEVEMENTS OF THE PROGRAMME

METHANE

The programme of research into methane mitigation is broad and represents a comprehensive and integrated effort to understand how methane is produced by our ruminant livestock and develop this knowledge for use in its abatement. It covers aspects of methanogen genomics, rumen ecology, forage plant interaction and selection for animal variation.



The research strategy identified that the complexity of the rumen would require a integrated approach across all of these areas to deliver sustainable abatement solutions, along with validation in farm systems before they could be released to industry. As our understanding develops the integration of this knowledge will become a feature of the research strategy.

This section of the strategy investigates the microbial population focusing on aspects that affect not only methane production but also importantly its effect on plant digestion. The rumen is a complex ecosystem which consists of large numbers of different microbial species and genera. Our research strategy has been to develop a set of tools for characterizing microbial populations, the relationships between the different populations, identify those inhibiting methanogens, and identify the opportunities for other microbes to act as a alternative hydrogen sinks to replace methanogens.

RUMEN MICROBIAL STRATEGIES TO LOWER METHANE EMISSIONS

Methanogens

Methanogens are bacteria like organism that belong to the kingdom Archea. They utilise hydrogen and combine it with carbon dioxide to produce Methane. They have evolved to carry out this specific function in the rumen environment. They have unique features that set them apart from other ruminant microorganisms and it is these features that offer up the opportunity to target them in a specific manner or understand the impact of mitigation tools. The methanogen tool development is a critical step for future confirmation of the potential of mitigation tools

Progress this year includes:

- A study to identify bacteria competing with methanogens during the primary colonisation of the rumen in new born calves was completed. Of three bacteria isolated, purified and identified, the most likely competitor was found to be an acetogen. This is the first finding of an acetogen apparently out competing methanogens in the gut of ruminants. Potentially providing mitigation solutions based on the evolution that occurs naturally.

- An earlier study showed that there were 2 different patterns of methanogen establishment of in new-born dairy calves removed from cows. Further investigation of this has revealed the presence of the methanogens previously detected by isolation, together with *Methanosarcina mazei*, a methanogen we have not noted before**.**

- The population densities of ruminal methanogens in dairy cows fed different diets were investigated using two methods. Diet appeared to have little effect.

- In efforts to find natural anti-methanogen agents in the rumen, two species of methanogens isolated from NZ ruminants were grown together with two different ruminal mycoplasmas (bacteria-like organisms), in a series of co-cultivations. Results showed that the test methanogens were resistant to attack by mycoplasmas, and that methanogen activity was not affected by their presence, discounting this as a future opportunity.

- A new method (DGGE) for analysing methanogens in the rumen was developed and successfully tested, providing a valuable tool for determining potential of mitigation tools. This provides for the first time a process for fingerprinting and monitoring different methanogen populations in the rumen. Tests on samples from sheep and dairy cows revealed a complexity of methanogens together with a new range of methanogens previously unrecognised in the biosphere. A manuscript was submitted for publication.

Previous achievements from 2003-05 include:

- New methanogen isolates have been purified and added to our methanogen culture collection over the term of the PGGRC.

- A real-time PCR method has been developed to provide a non-culture method

for quantifying methanogen populations.

- Extracts obtained from the gut of lambs showed the presence of the fibre-degrading bacteria in the rumen soon after birth and well before forage digestion occurred, which led to the research in dairy calves.

- A new molecular method for fingerprinting methanogen populations in DNA extracts from rumen samples was established and applied. This will be used to determine factors affecting methanogen survival, and to measure the diversity of methanogens in NZ ruminants.

- A robust and reproducible culture assay to accurately screen anti-methanogen agents and plant tissues was established and developed.

- The first investigation on methanogens establishing in NZ newborn calves has shown that methanogens are present in the developing rumen 1-2 days after birth. Several of the methanogens were identified and the results indicate a succession of different methanogen species as the rumen develops.

- A molecular method for quantifying methanogens in DNA extracted from rumen samples using DNA extracts was established and confirmed and will be used to check effects of diet on methanogen levels.

- To obtain information on the relationship between fibre-degradation and methane formation, molecular methods were developed for quantifying major fibre-degrading bacteria in rumen samples and successfully tested.

Methanogen Genomics

In order to inhibit methanogens without affecting other useful microorganisms within the rumen, it is essential to have methanogen-specific targets for inhibitors. We have analysed *Methanobrevibactor. ruminantium* genome and have identified a subset of methanogen (archaeal)-specific features. Several strong candidate approaches have been identified which are being progressed as mitigation options. The merging of the sequence data with the microbial information (generated from above) on methanogens continues to be a major focus of the ongoing work in this area.

We have also targeted the use of Peptide Nuleic Acids (PNA) which are mimics of DNA that retain nucleotide-specific binding, but have a peptide backbone that is not degraded by enzymes that breakdown DNA or peptides. PNA's bind strongly to both DNA and RNA and have been used to specifically inactivate gene expression in *E. coli* .To function as specific inhibitors of genes mediating methanogenesis PNA's must be able to:

i) Bind strongly and specifically to genes involved in methanogenesis

ii) Prevent transcription and/or translation of methanogenesis genes

iii) Get inside the methanogen cell

Progress this year includes

- Proteins that have been previously identified as possible intervention targets have been further described and synthesized for use as antigens in a vaccination/antibody production programme in sheep. Continuing this protein work is key to developing delivery techniques.

- A gene encoding a putative methanogen phage lytic enzyme has been identified and cloned into a protein expression vector.

- High density genome sequencing of the *M. ruminantium* genome has been completed and assembled.

- A gene from a rumen bacterium which may be involved in hydrogen transfer with methanogens has been identified and is being further researched.

Previous achievements from 2003-05 include

- 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.

- Archaeal-specific genes within the M. ruminantium genome are being investigated as potential secondary targets for inhibition

- Cell surface proteins from sequence analysis are being investigated as potential targets for a methanogen vaccine

- Peptide nucleic acids are being investigated for potential inhibitory activity against M. ruminantium genes.

- Other M. ruminantium sequences identified from the M. ruminantium genome are being investigated for potential anti M. ruminantium activity.

- The completion of the genomic sequence of M. ruminantium was initiated.

Phage

Ruminant phages offer a real opportunity for inhibiting and reducing methanogen populations in the rumen. They have been shown to be active against methanogens but consistent activity and an effective delivery mechanism will need to be developed. Phage which attack microbes exist in the rumen and have the potential to be used to manipulate ruminal

fermentation. Phage are natural and are an alternative to chemicals and antibiotics and thus easily meet regulatory requirements.

The aim of this research is to:

- Find a phage for potential to lyse methanogen hosts.
- Isolate and characterise methanogen phage and/or lytic enzymes.
- Determine host specificity.
- Test phage/ lytic enzyme in *in-vitro* assays.
- Test phage/ lytic enzyme in ruminants maintained in metabolic crates.

Progress this year includes:

- In a continuation of the goal to locate methanogen-specific enzymes the phage genome was re-analysed and an additional domain was discovered.
- Re-analysis of the *M. ruminantium* phage genome to find degenerate PCR priming sites as well as other methanogen cell surface proteins with peptidase domains indicated that the technique had been successful.
- The previous literature search regarding methanophage has been broadened in an effort to find alternative methods for cloning targeted genes or sequencing entire phage genomes. Two new meta-genome strategies, has produced phage-specific DNA sequence data. Assembly of the first full-length phage genome should become available in the near future.

Previous achievements from 2003-05 include:

- Activity from methanogen-specific phage has been found in tests to isolate lytic phage with the potential to destroy methanogens.
- Fingerprint profiles of total phage populations present in rumen samples from NZ sheep and cows were determined for the first time. This showed that phage populations in the rumen appear to bloom and change over short periods of time.
- Two methanogen–methanogen phage systems were established and tested using imported (non-rumen) methanogens and methano-phage. This confirmed that lytic enzymes rapidly degrade and destroy host methanogens.

Protozoa

In the rumen protozoa directly support methanogen growth and are a potential target in methane-abatement strategies. Because their role in ruminant digestion is not thought to be critical, strategies to reduce their populations may lead to reduced methane production with little or minimal effect on rumen digestion. Our aim is to determine which groups of rumen protozoa are most important in methane emissions from NZ livestock. Protozoa are involved in sustaining some methanogens in the rumen. Overseas studies suggest that:

- Removal of protozoa from the rumen results in a decrease in methane.
- The presence of protozoa is beneficial to overall rumen function and animal productivity.

Our work suggests that some types of ruminal protozoa are more involved in methane production than others. Protozoa populations have the potential to be manipulated by the forage diet and/or selective anti-protozoal agents.

Aim of research:

- Identify the protozoa most important in methane production in NZ ruminants ('target protozoa').
- Evaluate methane production in animals without target protozoa but with otherwise normal rumen populations.
- Evaluate methane production in protozoa-free animals.

Progress this year includes:

- Work to establish robust cultures of protozoa for use in tests to identify interactions with methanogens showed that some species can be maintained for several days, but so far methods have not been successful in achieving growth *in vitro*. A method was established using a new born lamb as an incubator.
- The procedure for picking single protozoal cells, and fixing of cells with formalin to ensure PCR amplification has been modified and improved. It is now possible to routinely pick individual cells, photograph them, identify them, purify their DNA and perform PCR.
- Sequence analysis of rumen protozoa has been performed and alignments re-evaluated. New PCR primers have been ordered and will soon be trialed with single protozoal cells. Based on the new alignments and review, new primers will be ordered to enable rapid total protozoa fingerprinting of rumen samples.
- Sequence analysis of methanogens has been undertaken, and previously published methanogen-specific PCR primers and probes reviewed.

Previous achievements from 2003-05 include:

- The diversity of rumen protozoa in grazing deer, cows and sheep in NZ has been described.

- Some species of protozoa were found to have high association with methanogens suggesting that these could be targeted in methane-lowering strategies

- Fifteen genes from protozoa predominant in NZ were cloned and sequenced to give the first information on the molecular phylogeny of protozoa in NZ ruminants and a phylogenetic tree showing inter-relationships was constructed.

- PCR primers were designed for use in studies in 2005/7 on methanogens attached to protozoa.

- Connections were made with a European Union consortium studying ruminal protozoa, and 5 species of protozoa imported for use in studies on methanogen-protozoa interactions.

Acetogens

These are regarded as a promising alternative sink for hydrogen in the rumen once a methanogen mitigation is applied (as per diagram). The relationship between acetogens and methanogens is not well understood but progress has been made in identifying the species involved, to ensure that if a knock out for methanogens is achieved an alternative sink for hydrogen is available to ensure the ongoing rumen function.

Progress this year includes:

- Acetogens in the rumen offer a potential alternative to methanogens as a H_2-sink, but little is known about factors affecting their populations. A real-time PCR method was developed to quantify one common species of ruminal acetogen in the rumen, and used together with culture counts of total acetogens to measure acetogens in dairy cows on different diets. Culture estimations suggested that total ruminal acetogens increased strongly when the diet was supplemented with grain.

Previous achievements from 2003-05 include:

- A joint study with Australia comparing acetogens in ruminants with those in kangaroos has been completed. All 4 kangaroo acetogens, but only 2 of 4 ruminal acetogens tested, utilised H2 at a high rate in in-vitro assays.

- Specific primers for quantifying a major acetogen species were designed and successfully tested.

METHANE MITIGATION EXPLOITING ANIMAL-TO-ANIMAL VARIATION

Earlier work has shown that methane emissions from different animals under the same conditions can vary significantly depending upon the animal and offers an opportunity for genetic selection for this trait. Genetic solution is a method that can be incorporated rapidly into many livestock systems. However for this to be adopted the trait of low methane production will need to demonstrate value over other selection traits. This year's research included analysis of the large scale Boviquest trial coupled with in depth studies of high and low emitting animals. Results were as follows:

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

Progress this year includes:

- In order to understand uncertainties surrounding the measurement of methane emissions using both SF_6 and calorimetry techniques, a 2-day workshop involving scientists from Canada, Australia and New Zealand was held at Ellinbank Research Station, Victoria, Australia.

- The consensus view from the workshop was that the SF_6 tracer technique is a reliable and relatively accurate method for estimating methane emissions.

- Results from a trial conducted at the Ellinbank calorimeters suggests that variability in methane emissions on a day to day basis is far lower when assessed using calorimetry than when it is assessed using the SF_6 technique. This implies that between and within animal variability is exaggerated by the SF_6 technique.

Previous achievements from 2003-05 include:

- 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.

- A repeat measurement of methane emission was undertaken using 9 cows obtained from the Boviquest trial. The correlation (r) between emissions from animals grazing fresh pasture and the repeat measurement from the animals fed molassed-lucerne was 0.76 for absolute methane emission and 0.51 for methane emission per kg feed consumed.

- An indoor trial was carried out with the same 9 cows to identify mechanisms responsible for between-cow variation in methane emissions. Correlations of these specific CH_4 emissions with those at two previous measurements were nil.

- These results suggest that specific methane emissions either are highly variable for any animal or that the SF_6 technique may be too variable to identify low and high emitting characteristics.

- Methane was found in urine and faecal material but did not correlate with enteric methane emissions. The development of a simple test for high and low emitters will not be successful until high and low emitters can be reliably identified

Research Programme

	PGGRC work		Related work
Year 1 July 2002- June 2003	Cloned animal trial		Dexcel twin trial
Year 2 July 2003-June 2004	Influence of SF_6 permeation tube flow rates on CH4 emissions	Repeatability of between animal CH_4 emissions in LIC trial	LIC QTL trial
Year 3 July 2004 – June 2005	Influence of SF_6 permeation tube flow rates on CH_4 emissions	Determinants & repeatability of CH_4 emissions in high, average and low emitting cows	LIC QTL trial, AGO/MAF SF_6 and calorimetry comparisons
Year 4 July 2005 – June 2006	Influence of SF_6 permeation tube flow rates on CH_4 emissions	Within animal variation in CH_4 emissions Methane emissions from high & low bloat susceptible cows	AGO/MAF SF_6 and calorimetry comparisons
Year 5 July 2004 – June 2005		Within animal variation in CH_4 emissions Species comparisons	

Identifying Gene markers

A trial has been conducted to measure methane production over two years in the dairy industry funded Friesian Jersey crossbred trial herd. The number of methane measurements that has been collected over the two years of the trial (700+) has also presented the opportunity for the calculation of more accurate emission factors for grazing cattle, this will be submitted for inclusion into the next national inventory calculation. 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 variations in methane production.

Progress this year includes:

- A report was received on the genetic control of the trait. However the initial analysis has recommended that a final conclusion can not be drawn or an economic assessment of the trait developed until a further genotyping of the Boviquest herd is completed. This is expected to be reported on in September 2006. Once this assessment has been made it will indicate whether further research to identify a gene marker for the trait will be worthwhile.

Previous achievements from 2003-05 include:

- The cows were successfully phenotyped for methane production

- Successful phenotyping of 680 cows which showed significant variation between cows for this phenotype

- Evidence of genetic variation for methane production and QTL have been detected

- The second year of large scale methane measurements in the Boviquest herd has been completed and the analysis confirming or otherwise the opportunity for genetic selection in dairy cattle has commenced. We have shown that average methane emissions per cow/day and per Kg Dry Matter Intake (DMI) are consistent across years and are highly repeatable within years.

- The initial data demonstrates significant variation in methane production between animals.

- The initial analysis has shown that methane emissions were strongly and negatively correlated with milk production. If confirmed this will mean that selection for low methane production will not lead to lower milk production.

Bloat susceptible cattle

Bloat is a condition of the rumen that results from the inability of some livestock to remove the gases produced during digestion from the rumen, leading to excessive pressure and discomfort in the abdomen, in extreme cases the condition can be fatal. Work in the 1980's had identified genetic variation in susceptibility to Bloat in cattle. These cattle also show differences in the enzymes found in their saliva. A line of these cattle has been maintained within AgResearch. An opportunity was taken in 2006 to test these high and low bloat susceptible cattle for methane production and determine whether any difference may be present.

- An experiment using 24 four mixed-age female animals (12 Low bloat and 12 High bloat) were selected from the bloat susceptibility herd. Each of the selected groups was composed of three, six and three 1-yr-old, 2-yr-old and 3-yr-old, respectively.

- Methane was measured on all animals on two occasions. Methane emissions were higher from the Low bloat lines than the High bloat lines but were very similar when compared on a live weight basis.

- Initial estimates, obtained using energy balance equations, suggest that emissions per unit of intake will be lower in the Low bloat animals but this cannot be confirmed until the indigestible marker data, which provide a better estimate of intake, are available.

FORAGE TO LOWER METHANE EMISSION.

Research Programme

	Direct work						Related work
Year 1 July 2002-June 2003	CSIRO anti-methanogen vaccine	Organic acids review			In-vitro testing of forages		
Year 2 July 2003-June 2004	CSIRO anti-methanogen vaccine	Fumaric acid trial		Oil supplementation (Woodward et al Dexcel)	In-vitro testing of forages		Dexcel in-vitro & in-vivo sodium fumarate
Year 3 July 2004 – June 2005				Oil supplementation (Woodward et al, Dexcel)	Caucasian clover trial	Chemical inhibitor review	Monensin literature review (MAF)
Year 4 July 2005 – June 2006			Monensin trials cattle & sheep		Caucasian clover trial	Batch B	PIRVic monensin trial
Year 5 July 2004 – June 2005							

Evaluation of Caucasian clover

Previously Caucasian clover has demonstrated low methane production in in-vitro assays compared to other clovers. Samples of Caucasian clover, both the commercially available and some unreleased series were collected from November 2004 through to April 2005 and evaluated with the *in vitro* rumen assay to determine a seasonal profile. In a follow up trial the best cultivars were to be tested in a grazing trial.

Progress this year includes:

- A trial using 40 5-6 month old wethers was undertaken at the AgResearch Lincoln Research Centre farm and compared methane emissions from wethers fed pure Caucasian clover or a Ryegrass: Caucasian clover mixture with those from wethers fed pure Ryegrass, White clover, or a Ryegrass: White clover mixture.

- DMI was higher for wethers fed White clover (0.98-1.04 g/day) than those fed Caucasian clover (0.89-0.90 g/day) in both periods with the DMI of the Ryegrass and Ryegrass: clover mixtures being similar to Caucasian clover. The digestibility of Caucasian clover was higher (68.4-68.8%) than the digestibility of the other feeds (62.2-65.0%).

- There was no evidence from this *in vivo* trial that sheep fed Caucasian clover had lower methane emissions than White clover. In fact the methane emissions were

higher from the sheep fed Caucasian clover than those fed Ryegrass which in turn had higher methane emissions than sheep fed White clover. The inclusion of Ryegrass and either Caucasian or White clover in a 70:30 mixture into the feed was complimentary and reduced the methane emissions from that obtained with feeding Ryegrass or Caucasian clover alone.

Previous achievements from 2003-05 include:

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.
- The use of Maize silage in dairy herds has also been shown to reduce Nitrous Oxide emissions as well when used over the autumn winter period.

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

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 CH4 / d) or lotus (343.2 g CH4 / d)
- Methane production per unit intake lower on lotus (19.9 g CH4 / kg DMI) than ryegrass (24.2 g CH4 / kg DMI). 66% of the difference due to action of condensed tannins
- 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
- 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.

- Five clovers were examined but none inhibited methanogens

- The low methane production appears to correlate with higher soluble sugar and starch content.

- In the assays, seasonal Caucasian clover cultivars were significantly different to ryegrass and white clover at 12h in % accumulated-methane.

- No strong indication of seasonal or location effects on methane production were observed.

METHANE MITIGATION TECHNOLOGIES EVALUATED FOR PROOF OF CONCEPT

Fumarate

An 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 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 and reduced methane production 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.

Impact of Grain or Oil supplementation on methane production

Two trials have been carried out in previous years, these trials were undertaken primarily to measure the effect of dietary oils on rumen methane production. Oils derived from plant or animal sources are reported to reduce rumen methane production by up to 80% in vitro (Fievez et al., 2003) and about 25% in vivo (e.g. Machmuller et al., 2000). Although the degree of methane suppression is variable, unsaturated oils or fats should always reduce methane production because the process of rumen bio-hydrogenation (creating harder fats) provides a sink for hydrogen ions derived from fermentation, reducing the amount of hydrogen available for methane production.

- In a 2003/04 trial pasture-fed cows were supplemented with 500g fish oil / sunflower oil mixture per day

	No oil (control)	Oil
g CH4 / cow / d	242.4	176.4
g CH4 / kg DMI	18.5	13.5
g CH4 / kg MS	150.8	119.3
CH4 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.

- Currently economics are marginal, however PGGRC is developing a Commercialisation model to understand the cost / benefits including a cost associated with carbon to better understand viability

- The 2005 trial involved 30 cows grazing pasture ad libitum., 10 also received a grain supplement and 10 a protein and oil supplement (Oil). Methane production was measured over four consecutive days from all cows.

- The trial did not identify any significant differences for methane production between treatments which were in contrast to the previous trial carried out in 03/04.

- It is concluded that daily supplementation with fish and flaxseed oil for an extended period does not necessarily lower methane emissions, expressed in terms of feed DM intake, but oil supplements may lower voluntary feed intake. Future evaluation of oil or other dietary compounds for methane mitigation should be accompanied by measurements of rumen digestive physiology and microbiology.

Anti-methanogen vaccine

Progress this year includes:

- .In April 2006 a workshop was held in Brisbane to develop the concept of a methanogen vaccine further. Initial proof of concept work leading on from the original trials will be included in the 2006-07 work programme.

Previous achievements from 2003-05 include:

- 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.

- 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.

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

- Re economics comment above maybe should pick it up generically.

2006 Trial

- A trial was undertaken with 60 pasture fed cows in early lactation, to measure the effects on Monensin on methanogenesis, milk production, dry matter intake, liveweight change and other aspects of digestion over an 80 day period. The long duration of the trial was designed to test the persistency of any reduction brought about by the administration of monensin.

- The administration of Monensin did not significantly affect dry matter intake (DMI), methane production and milk yields (Table 1). Monensin did not affect fat: protein ratios, cow live weight change, milk somatic cell counts or rumen parameters (pH, NH_3). Methane emissions averaged 19.2 g/kg dry matter intake, a value that is approximately 10% lower than values used in current national inventory calculations.

	Control	Monensin
DMI (kg/day)	17.03	15.71
Methane (g/day)	328.4	313.7
Methane (g/kg DMI)	19.31	20.06
Milk yield(kg/day)	21.37	22.00
Milk solids Kg/day	1.678	1.694

- These data demonstrate no significant benefits for greenhouse gas mitigation or animal productivity.

Small Molecule Methane Inhibitors

The identification and application of chemicals that could inhibit methanogens directly offers a opportunity to "fast track" methane reduction by removing methanogens. To identify suitable chemicals a literature search was commissioned.

- The review was completed and a report provided to the PGGRC. The literature search was extended to chemical inhibitors of methane production from sludge and other anaerobic environments related to research in water quality.

- The review was sent to an International expert for an independent assessment.

- Some promising chemical inhibitors were identified but currently the consortium is considering business cases before further progressing selected options.

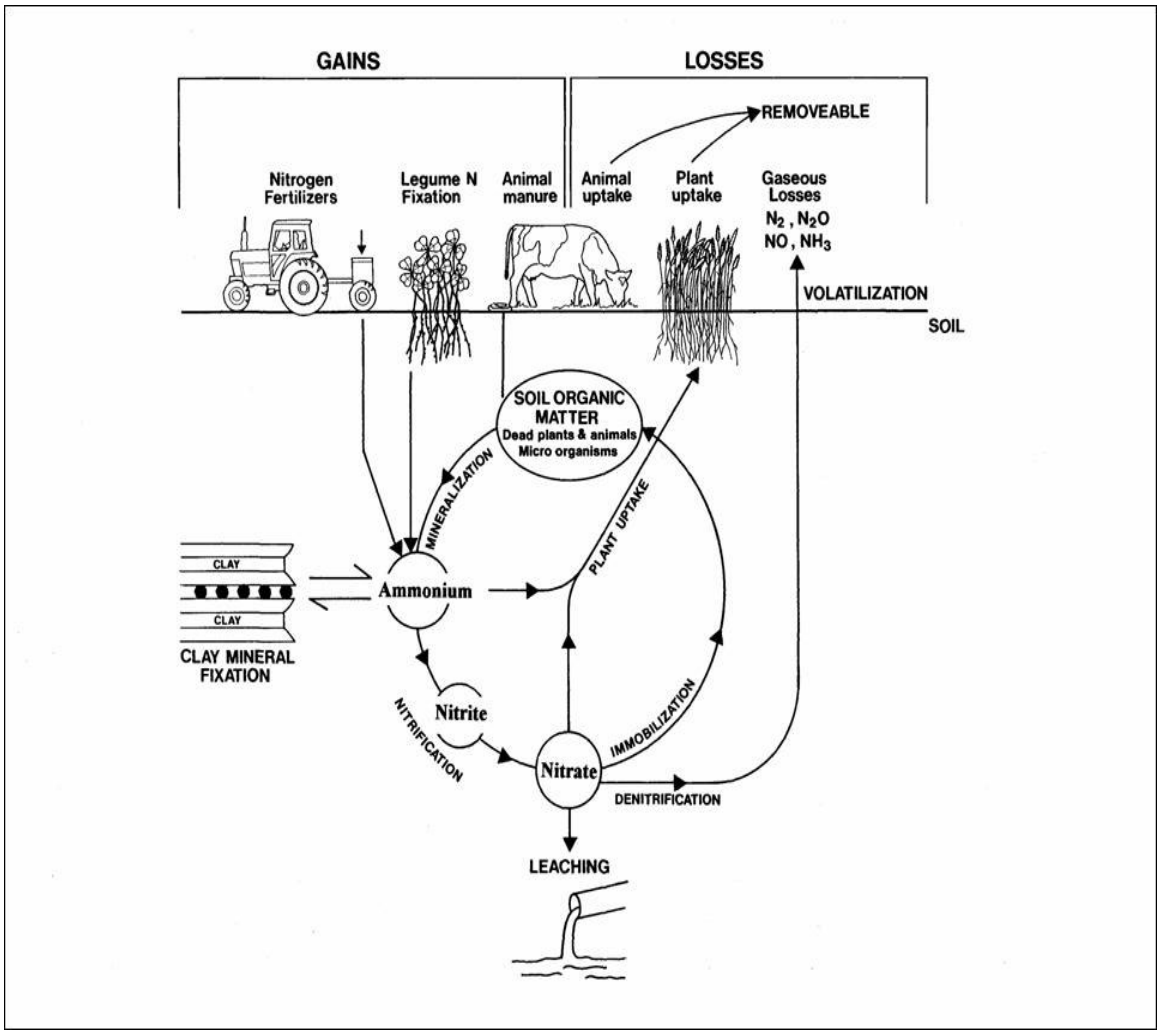
2006 Chemical inhibitor trial

- In a previous trial in which methane emissions from sheep were measured, one batch of the treatment chemical indole-acetonitrile was found to inhibit methanogenesis. The compounds in the chemical batch were investigated and the methanogen-inhibiting component was identified as chloroform.

- A brief review on the literature relating to chloroform and its toxicology was collated. Concerns regarding potential residue accumulation in meat or milk products and negative environmental impacts appear to limit the feasibility of using chloroform as a mitigation strategy.

NITROUS OXIDE

Nitrous Oxide (N_2O) is a gas that is produced in soils from the breakdown of Nitrogen resulting from livestock urine and dung and to a lesser extent application of nitrogenous fertilisers. A powerful greenhouse gas it has a Global warming potential (GWP) of 310 and accounts for 17% of the total national emissions. Consortium research for N_2O focuses on three aspects; dairy farm emissions, nitrification inhibitors and hill country farm emissions.



DAIRY FARM NITROUS OXIDE EMISSIONS

A study initiated in 2003 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 fertiliser is required to enable the development of best management practices in farm systems and Nitrogen management in the environment.

The collection of this data will also enable more accurate calculation of the emission factors for N_2O and subsequent use in the national inventory calculations.

Progress this year includes:

- For the years 2003-2004 and 2004-2005 the averaged total annual N_2O emissions (including both the field measured and calculated emissions from all components of the farm systems studied, including dairy farmlets, maize growing land and stand-off pad) were 7.91, 8.21 and 7.10 kg N_2O-N per hectare of dairy farm on the control, maize supplement and stand-off farm systems respectively.

- Total N_2O emission per kg of milk solids production from the maize supplement farm system was 22% lower than that from the control system, and total N_2O emission per kg of milk solids production from the stand-off farm system was 10% lower than that from the control system.

- Total calculated annual emissions for the sum of the three major greenhouse gases, (N_2O, CH_4 and CO_2,) were 11,045, 13,557 and 10,645 kg CO_2-equivalent per hectare from the control, maize supplements and stand-off dairy farms, respectively.

- Economic farm surplus depends on milk production, milk price and associated costs. Maize silage had a variable effect between years on N_2O emissions and total greenhouse gas emissions per $ of economic farm surplus. The stand-off farm system gave slightly lower N_2O emissions and total greenhouse gas emissions per $ of economic farm surplus, compared with the control farm system.

Previous achievements from 2003-05 include:

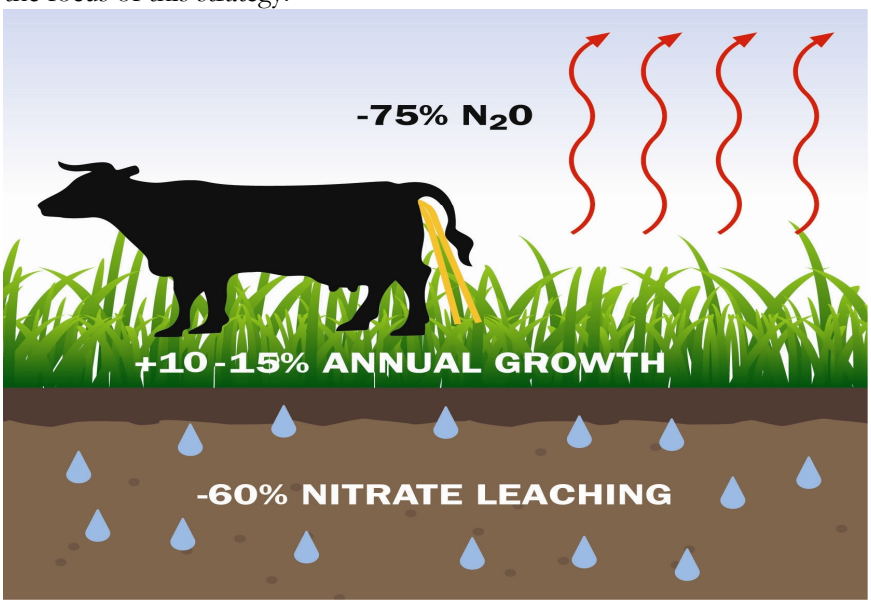
- Results indicate that N_2O fluxes from both urea and urine treatments increased after application, reaching peak fluxes after about 13 days. The N_2O fluxes from the urea treatment had declined to a level similar to that from the control area by approximately 20 days after application, while the N_2O fluxes from the urine treatment had declined to the control level by approximately 40 days.

- N_2O emissions from a stand-off pad at Dexcel's Scott Farm were measured as an adjunct to the Resource Efficient Dairying (RED) trial. Data from this measurement will be incorporated with results from the farmlet study to estimate the total N_2O emission associated with "whole" farm systems, which include stand-off pads.

- N_2O emissions from the dairy farmlets at Dexcel's Scott Farm during the winter and spring seasons of 2004 and the summer and autumn seasons of 2005 were measured. The preliminary data indicates that both the use of maize silage and use of winter stand-off pads have potential for reducing N_2O emissions.

NITRIFICATION INHIBITORS

The application of nitrification inhibitors offers farmers an opportunity to not only reduce their N_2O emissions but also losses due to nitrate leaching and provide an opportunity to better utilise nitrogen in the farming system. These products are already available on the market but there effectiveness across differing soil conditions and nitrogen loads is not completely understood and is the focus of this strategy.



Summary benefits from the use of DCD in Dairy farm systems (Lincoln University/Ravensdown Ltd)

A research programme jointly funded with Ravensdown Ltd and carried out at Lincoln University since 2002, 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 & Templeton) using chamber methods on undisturbed soil monolith lysimeters. The initial research indicated that DCD (applied and marketed under the name eco-n) showed that a 75% reduction in nitrous oxide emissions could be achieved by treating grazed pasture soil with a nitrification inhibitor. The validation of this result across a number of NZ soils and several seasons is the focus of the programme of work.

Progress this year includes:

Templeton Soil Lysimeters studies:

(a) Nitrous oxide emission

- The large amounts of N_2O gas emitted from this winter runoff block experiment on a Templeton soil were significantly reduced by treating the soil with *eco-n*, even with a delay of 18 days after the grazing of the forage.

- Application of *eco-n* reduced the total emissions from urine patches to 5.7 kg N_2O-N/ha (equivalent to a 73% reduction) even when applied 18 days after the urine deposition in winter.

(b) Nitrate leaching

- Application of *eco-n* was effective in reducing nitrate leaching from cow urine patches even when applied 18 days after the deposition of the urine in the winter.

- Total nitrate-N leaching losses were reduced from 352 kg N/ha to 212 kg N/ha. This reduction of 140 kg N/ha was equivalent to a 40% reduction.

(c) Pasture Yield

- The application of *eco-n* 18 days following the application of urine in June increased pasture dry matter yield by 1.6 t/ha from 10.5 t/ha to 12.1 t/ha.This represents a 15% increase in pasture dry matter yield.

1. Lincoln University Dairy Farm - Temuka soils Drainage Plot Pasture Trial

- *Eco-n* significantly and consistently increased pasture yield in both the urine patch, and 'inter-urine' areas of the pasture in all four years of the trial. Mean annual dry matter (DM) yields over 4 years for 'inter-urine' areas was 10.3; 'inter-urine+*eco-n*' was 12.4; 'urine' patch areas was 12.4 and 'urine+*eco-n*' was 16.0 t DM ha^{-1}.

- These average DM yield increases with *eco-n* were equivalent to 20% for the inter-urine and 29% for the urine patch areas.

- Expressed on a whole paddock basis, the increase in annual DM yield resulting from *eco-n* application was estimated to be 21 %.

- The concentrations of N, calcium (Ca), magnesium (Mg) and potassium (K) in the pasture were unaffected by treatment with *eco-n*.

- Pasture DM, protein, carbohydrate, metabolisable energy (ME) and fibre levels, and sward clover content, were not affected by treatment with *eco-n*.

- The four years of pasture yield results have been summarized in a draft paper for submission to an international science journal.

2. Lincoln University Dairy Farm -Templeton soil Pasture Yield Trial

- Pasture on the free-draining Templeton soil showed a significant annual DM yield response to *eco-n*, with increases of 17% and 28% for non-urine and urine patch areas, respectively when compared to control plots.

3. Waikato Horotiu soil lysimeters (**Dexcel) trial**

- *Eco-n* applied to the Waikato Horotiu soil lysimeters was effective in reducing nitrous oxide emissions. Total N_2O-N emissions from the urine treated lysimeters without *eco-n* reached 6.2 kg/ha. This was reduced to 2.4 kg/ha, representing a 61% reduction.

4. Temuka soil lysimeter nitrous oxide trials

- The *eco-n* treatment on the heavy Temuka soil reduced N_2O-N emissions in the urine treatments from 12.5 kg N_2O-N/ha to 7.1 kg N_2O-N/ha, equivalent to a 43% reduction.

- The total N_2O-N emissions from this heavy Temuka soil were not as high as expected, probably due to more complete denitrification, which produced N_2 gas rather than N_2O gas.

5. Construction of mega-chamber for N_2O measurement on-farm

- The development of a large scale lysimeter which may better reflect the conditions in a farm paddock scale was commenced with some initial design features being tested. This has had a number of engineering challenges to overcome, however a mega-chamber has been successfully constructed to measure N_2O emissions on-farm. The chamber is currently being field tested.

6. Scientific paper

- A scientific paper that summarizes key N_2O research results from the Ravensdown/PGGRC funded research programme has been submitted to an international journal for publication.

Previous achievements from 2003-05 include:

- Large gas measurement chambers were 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 was 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.

Templeton Deep Sandy Soil Lysimeter Trial

- Application of *eco-n* to the large, deep sandy Templeton soil lysimeters significantly reduced N_2O emissions by over 55%.

- The applications of *eco-n* in May were similarly effective in reducing N_2O emissions irrespective of whether they were applied immediately after urine application, or 10 days later.

Lismore Shallow Stony Silt Loam Soil Lysimeter Trial

- Application of *eco-n* at 7.5, 10 and 15 kg DCD/ha all reduced N_2O emissions by between 65 and 73% (confirming previous results) Lincoln University Dairy Farm Drainage Plot Trial

- Average data for the past 3 years shows that *eco-n* increased annual pasture yield in both the urine patch areas (23% increase) and the non-urine patch areas (21% increase).

- *Eco-n* reduced nitrate leaching losses from the large-scale on-farm Temuka drainage plots.

These results from the on-farm plots confirm the earlier lysimeter results.

Waikato Lysimeter Trial at Dexcel, Hamilton

A new lysimeter facility was successfully installed on the Scott Farm, Dexcel Hamilton

WISE USE OF NITROGEN IN HILL COUNTRY

This programme of work is measuring the N_2O emissions from an industry trial that is studying the production and environmental effects under increase use of nitrogen fertilisers and intensification in sheep and beef farms. The work is jointly funded with the Ministry of Agriculture. Monitoring is carried out on two research sites "Ballantrae" in the southern North Island and "Invermay" near Dunedin in the South Island. The trials involve various treatments of different levels of fertiliser and urine application to give as wide as possible nitrogen input and also involve the application of DCD as a nitrification inhibitor.

Progress this year includes:

- The work is on target and the measurements completed at Ballantrae and Invermay as scheduled. Artificial sheep urine (at a rate of about 300 kg N ha^{-1}) was applied to the four N treatment paddocks of each site, and N_2O emission measurements were carried out at frequent intervals over a six week period.

- Interim results from Ballantrae indicated that total N_2O emission over the 6 weeks period were highest under the urine treatment and that DCD was effective in reducing emissions to background levels. In the 300 N treatment, DCD reduced emissions to below background levels, suggesting that at this level of nitrogen fertiliser application N_2O emissions from background soil N were also reduced by DCD. The results also showed that both background and urine-induced N_2O emission losses increased with N fertiliser application rate.

- Interim results from Invermay suggest that N_2O emission losses over the 6

week period were higher than at Ballantrae. However, the effects of N fertiliser and DCD use on N_2O emissions were generally similar to those at Ballantrae.

- The N_2O emission factors for sheep urine (expressed as N_2O-N lost as % of N applied) for the measurement period ranged from 0.05 to 0.8% with the higher values found in the high N fertiliser treatments. At the lower N fertiliser rates the emission factors were similar to those estimated from sheep urine applied to flat land pastures.

3 THE SUCCESS OF THE RESEARCH

The research programme has developed as planned over the first four years of the consortium with effective progress being made across all research objectives.

MID TERM REVIEW

The mid term review of the Consortium by the Foundation was completed in April and has presented PGGRC with an opportunity this year to gain an independent viewpoint on the focus and progress being made. The review focused on both the science and governance aspects of the consortium activities, with the most emphasis being on the science programme. The executive summary of the report drew the following conclusions:

Quality of Research
In the panel's view the quality of the research was first class and highly relevant to the objectives of the Consortium. Some research is already reaching end-user application but it must be appreciated that some of the projects are still in the discovery phase which is necessary to provide a platform for future sustainable impacts.
Business Strategy
The Consortium is now at a stage where greater attention needs to be paid to the business strategy, commercial realities of product development, implementation and incentives for the uptake of the more promising aspects of the work.
Science Strategy
Science strategy is well developed in line with the Consortium objectives but now needs to be focused in line with real commercial opportunities that have been more rigorously evaluated in terms of feasibility, commercial impact and industry or public good. While not compromising the basic underpinning science required, the science programme should be focused on fewer development projects going forward.

The Review has identified several areas of activity that the consortium partners will be developing further in the next 12 month, particularly with reference to commercialisation of promising options.

METHANE

Within methane research, the genomic sequence of *Methanobrevibacter ruminantium* has provided solid data on the potential sensitivities of a predominant methanogen. Microbial ecology studies have accelerated the microbiological understanding of the rumen and we now have a comprehensive set of tools to identify and monitor changes that may occur in the rumen when we apply mitigation technologies. The understanding of the rumen biology through studying the population components has continued as planned. The results of the research programmes will be collated into a series of reports by June 2007 that consolidates the knowledge across all of the rumen physiology we have targeted in the last 5 years.

As yet no clear methane mitigation technologies have been confirmed, however promising information from the study of ruminant phage along with a detailed understanding of the methanogens biological pathways to produce methane had identified target approaches. These are being progressed and will be reported on in the next 12 months. This will underpin the proof of

concept work on a vaccine approach that commences this year. The process to protect the intellectual property of these targets is in progress.

Central to the activity of methanogens is their ability to identify and utilise hydrogen. The new programme that developed throughout the year from the identification of common elements found in methanogen and a plant degrading bacteria genome comparison, may offer up further specific mechanisms that can be targeted. The understanding of this mechanism may also reveal a better understanding of how rumen fermentation could be enhanced.

The identification of acetogenic bacteria that was able to successfully compete with methanogens in populating a young ruminant's rumen is a significant event and offers the opportunity that this could be exploited further as a mitigation approach.

A small scale investigation to identify whether the rumen is the only source of methane that comes from livestock has been initiated and progresses well. Knowledge of this will ensure effective targeting of mitigation technologies.

The delayed trial with Caucasian clover was completed but unfortunately the results did not reflect what had been previously been shown in the laboratory screen. The opportunity to develop further forage options will be discussed with Pastoral Genomics as they have identified an opportunity to incorporate some of the condensed tannin characteristics previously found to lead to lower methane production, into clover plants that are agronomically better to incorporate into farm systems.

The research to identify whether it is possible to use animal selection to reduce methane output has continued. Research has focused on understanding the variances that arise from the the SF_6 technique used to identify methane production in grazing animals. A further set of trials are planned for the current year to complete that investigation. Once these studies are finished we will then know if identifying high and low emitters from the SF_6 technique is valid. The trial to investigate whether cattle previously selected for susceptibility to Bloat was completed and is being analysed.

The investigation into a contaminate compound that had stopped methane production in a food flavours trial was completed. The compound was identified as chloroform which has previously been shown to effectively stop methanogen activity in laboratory experiments even at very low levels of contamination. Although it is very effective in doing this chloroform is a restricted compound and can not be used in the animal food chain.

Leading on from all of this a new programme planned for the next 12 months is to take the acquired knowledge across all of our methane work to date and apply it to a situation where methanogens are removed from the rumen of lactating dairy cows for a period of 70 days. This will enable a comprehensive monitoring of both the rumen function and productive effects of this to be undertaken.

NITROUS OXIDE

The research into the nitrification inhibitor *Eco-N* has continued to confirm its effectiveness in reducing Nitrous Oxide emissions and Nitrate leaching. Nitrification inhibitors are already available to producers but the uptake of them has been modest. A challenge for the future will be the

development of appropriate policies that recognises nitrification inhibitor use. These will be required if the advantage in reductions are to be realised.

The programme of work on the dairy farm systems has been analysed and management options identified for farmers to minimize the nitrogen losses from farm systems. Further refinement of these options will be developed in a series of farm trials focused on restricting nitrogen use and maximizing productivity.

The evaluation of Eco-N has continued on track with the N_20 emissions and NO_3 leachate still being dramatically reduced even if the product is applied well after nitrogen is applied. The building of a mega chamber to measure larger samples of pasture for emissions is now being field tested.

The first year of a trial to establish emission levels in hill country situations has been completed and partially analysed. The pattern of emissions is similar to those found in dairy farms albeit at a lower level in keeping with decreased nitrogen loadings of these systems. The incorporation of DCD as a nitrification inhibitor appears to reduce the level of emissions in a similar manner to dairy systems.

The PGGRC in recognising the challenge to adoption has initiated a review of the knowledge that is available for DCD and other established nitrogen cycle moderators. The review to be carried out in August 2006 will cover all aspects of their application and aims to identify the gaps in our knowledge and subsequent barriers to adoption. The review will be carried out by an international expert and will involve interviewing all of the interested parties within New Zealand, along with a comprehensive search of the international literature.

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2003-2005 papers

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5 FUNDING CONTRIBUTION 05/06

The funding listed here represents the direct funding invested through the PGGRC

Organisation	**Funding (GST excl)**
Meat and Wool NZ Ltd	390,000
Fonterra Ltd	350,000
Dairy InSight Inc.	450,000
PGG Wrightson Ltd	125,000
DEEResearch Ltd	35,000
Fertiliser Manufacturers Research Association	90,000
Ravensdown Ltd	220,000
Foundation Research Science & Technology	1,929,000
MAF	57,000
AgResearch	350,000
Total	3,996,000

6 PARTNERSHIP INITIATIVES

INTERNATIONAL COLLABORATIONS

Activities this year include:

Support for 2005/2006 for collaboration with Japanese researchers at the Rumen Microbiology Laboratory, National Institute of Livestock and Grasslands Science (NILGS), Tsukuba, was obtained from the GIF Aichi-leveraging Fund administered by NZ Trade and Enterprise for the Ministry for Foreign Affairs and Trade. Scientist exchange visits were arranged. Preliminary tests with a prototype of a monitor for measuring hydrogen and methane in exhaled breath were carried out with sheep in metabolism crates. Continued support for this study in 2006/7 has recently been obtained from NZ Trade and Enterprise.

Attended the Greenhouse Gas & Animal Agriculture conference in Zurich Switzerland in September 2006. New Zealand and Australia subsequently gained the right to host the next conference in Christchurch in 2007. The PGGRC will participate fully in the development of this conference.

Joint Workshop on SF6 in Australia: In order to understand uncertainties surrounding the measurement of methane emissions using both SF_6 and calorimetry (respiration chambers) techniques, a 2-day workshop involving scientists from Canada, Australia and New Zealand was held at Ellinbank Research Station, Victoria, Australia.

To further develop whether it is feasible to develop a vaccine for methanogens a workshop was held in Brisbane involving AgResearch and CSIRO. The key scientists involved with the earlier work that evaluated the CSIRO vaccine within the PGGRC programme were all present. The outcome from this has been a proof of concept project to be carried out in the current year.

Previous activities from 2003-05 include:

Connections were made with a European Union consortium studying ruminal protozoa, and 5 species of protozoa imported for use in studies on methanogen-protozoa interactions

A joint study between the Queensland Dept. of Primary Industries and Fisheries (QDPIF) and PGGRC comparing acetogens in ruminants with those in kangaroos has been completed and a report sent to the PGGRC

Collaboration with Japanese scientists on molecular techniques for monitoring methanogens has been initiated using funds obtained from the Aichi-leveraging fund of the Ministry of Foreign Affairs and Trade (MFAT) administered by NZ Trade and Enterprise. In the establishment phase, a Japanese scientist from the National Institute for Livestock and Grassland Science (NILGS) in Tsukuba visited in March 2005 with a reciprocal visit to Tsukuba in June 2005. Efforts are being made to continue the collaboration in 2005/6.

Consortium partner Meat & Wool New Zealand joined the Australian Beef Cooperative Research Center (Beef CRC) as a core partner. The lowering of methane in beef cattle is one of the foci of the CRC and will be developed over the next seven years. This will offer the opportunity to work closely with Australian researchers and if appropriate collaborate fully in on –going research.

NATIONAL ACTIVITIES

Other activities in which PGGRC personnel have been involved include;

- Participated in the MethoNet and NZOnet workshops that focus on the inventory aspects of agricultural greenhouse gases.
- The Manager attended the Climate change and Governance conference in Wellington in March 2006
- The Manager attended and acted as a panel discussant on climate change at the Sustainable Farmland conference in Wellington in June 2006.

7 HUMAN CAPITAL BUILDING INITIATIVES

PHD STUDENTS

Ben Vlamming

Commenced 2003

In his second year, the main area of his study being the measurement of methane produced by livestock. Main activities for the year are as follows:

- Involved in the planning and running of an animal trial in November and December 2004 for the measurement of SF6 and CH4 in cattle urine, and faecal material. Also recorded eating/ruminating behaviour by visual observation for 48 hours.

- A technique was been developed to extract and analyse CH4 and SF6 from faecal gas samples. Calculations have been developed to ascertain the quantities of both SF6 and CH4 being excreted per day in the trapped faecal gas. Small quantities of both SF6 are released but they are insignificant compared to that released from the mouth.

- In conjunction with NIWA a method was developed to measure CH4 and SF6 concentrations in urine and blood. Both SF6 and CH4 are present in small quantities in the urine but the quantities released per day are insignificant compared to that released from the mouth. The blood samples are still being analysed.

- An experiment was undertaken to ascertain if eating behaviour could be responsible for the variance between animals in CH4 production. A visual procedure was established for measuring eating, and ruminating behaviour was established after consultation with a scientists experienced in behavioural measurements. The data are still being analysed.

- An analysis was undertaken of the methane database to ascertain if there was any relationship between SF6 permeation rate and absolute methane emissions. The analysis of the methane database was completed in conjunction with a statistician, and a paper written and submitted to the NZSAP. This was presented in May 2005. The conclusion reached was that in cattle there does appear to be a positive relationship between CH_4 emitted per kg DMI. The same relationship does not apply to sheep probably because of the low range of permeation rates in the tubes used for sheep.

2005-06

- Studies to estimate in vitro the fate of SF6 released from permeation tubes suspended in a range of fluids (water, rumen liquor, rumen solids). From this data construct an SF6 mass balance and determine whether non-gaseous SF6

absorption/adsorption could be a major factor in the large animal-to-animal variations found in methane emissions measured using the SF6 technique.

- The PhD is due to be completed by December 2006.

Natasha Swainson

Commenced June 2005

Received a Enterprise Scholarship from TEC with the industry sponsors being PGGRC, Elanco and DEEResarch, while the supporting research project is wholly funded by the consortium.

The aims of the associated research project is to gain a better understanding of how dietary manipulation affects methane emissions and nitrogen retention in sheep, cattle and deer, to investigate the potential of an existing cattle mitigation technology (sodium monensin) in sheep and to examine the impact of combining potential mitigation approaches. This research will identify the forage components that influence methane emissions and nitrogen retention and provide new information on mitigation technologies that may be employed under New Zealand's efficient farming systems

Sodium monensin, which affects both energy supply and protein utilisation has shown some potential as a possible GHG mitigation tool. In beef cattle fed concentrate based diets methane emissions have been reduced by up to 25% and nitrogen retention has been increased. New Zealand data collected on dairy cows in a PGGRC trials in 2003/04 on pasture indicated a more modest reduction of 10-12% in methane production. However, there is some controversy regarding the longevity of monensin effect and in addition, all data in the literature refer to the effects of monensin on cattle while in New Zealand, sheep are the biggest single source of methane emissions.

2006.

An indoor experiment investigating the effects of monensin on methane emissions, nitrogen retention and energy metabolism in lactating ewes, was undertaken in September. Interim results show that monensin had no effect on methane emissions (expressed on a daily or a unit of dry matter basis), energy balance or ewe and lamb liveweight change.

A report detailing the results of studies on methane emissions from growing red deer has been submitted to MAF and written up as a paper which will be submitted to the New Zealand Journal of Agricultural Research.

Submitted 6 monthly reports to Massey University as per requirements of PhD enrolment.

SECTION II

OTHER AGRICULTURAL GREENHOUSE GAS RESEARCH

I CROWN FUNDED:

MINISTRY OF AGRICULTURE AND FORESTRY

Agricultural Inventory Research Programme 2004/2005

Introduction

Funded and coordinated by the Ministry of Agriculture and Forestry with the support of the Ministry for the Environment, Climate Change Office, and conducted by research institutes including Massey and Lincoln University, AgResearch, Landcare Research, Crop and Food, NIWA, and Dexcel. The total expenditure in 2004/5 was $500,000 inclusive of GST.

Nitrous Oxide Research Programmes

1) Hill country nitrous oxide emissions- extending the range of information and identifying key processes.

2) Soil compaction effects on the nitrous oxide emission factor EF3.

3) Documentation of nitrous oxide inventory methodology used for determining the national inventory emissions.

4) An initial investigation into indirect nitrous oxide fluxes from an agricultural stream.

5) Determination of water filled pore space measurement as a means of determining nitrous oxide emissions from New Zealand soils.

6) Review of research into nitrous oxide emission factor EF3 – emission rate from deposition of dung and urine onto pasture.

7) Paddock scale comparison and validation of nitrous oxide emission prediction methods.

8) Nitrous oxide research programme data archiving

9) Independent review of the nitrous oxide research programme - 2002 to 2005.

Methane Research Programme

1) Validating the SF6 technique using calorimetry. This forms part of NZ/ Australia climate change partnership programme.

2) Methane emissions from growing lambs – is the emission factor for lambs different?

3) Does afforestation of pasture increase methane uptake?

4) Use of satellite imagery to assess the time changes in mean nitrogen concentrations and digestibility values for incorporation into the national inventory.

5) Independent review of the methane research programme - 2002 to 2005.

NATIONAL INSTITUTE FOR WATER AND ATMOSPHERE (NIWA)

Drivers of variability in non-CO2 greenhouse gases

This project aims to quantify changes in the distribution and isotopic composition of methane and nitrous oxide in the background atmosphere of NZ, the Southern Ocean and Antarctica. The work includes international collaborations and seeks to understand past and current changes in the atmosphere and relate these to emissions and removal processes. High precision measurements of methane and nitrous oxide are made at Baring Head (near Wellington), in Antarctica, and on trans-Pacific ships to determine the gas concentrations and their variability. Gas samples extracted from polar ice cores are used to infer greenhouse gas concentrations over the last 2000 years, a period of major relevance to our current climate, to provide comparisons with current changes and constrain our current estimates of greenhouse gas sources and sinks. This data is used in related research developing models to understand and predict future atmospheric changes.

The data are made available by FTP and are submitted to international databases such as the NOAA Global View Cooperative Data Centre in the USA, Global Atmospheric Watch programme, and the World Meteorological Organisation data storage centre in Japan.

Funding: **FRST $831k**
Timeframe Current contract expires June 2006, and will be extended to September 2007 by FRST.

Verification of agricultural greenhouse gas emissions

This project aims to develop techniques and methodologies for accurate greenhouse gas emission estimates that can confirm the efficacy of claimed ruminant emission mitigation methods. The research will develop new instruments for ruminant methane measurements based on emerging solid-state technologies. The work will use related research results on alternative tracers to SF6 carried out by NIWA as part of the AgResearch led *Global Processes in Terrestrial ecosystems* FRST programme.

Funding: **FRST $200k** (FRST/AgResearch $80k)
Timeframe: Current contract expires June 2006, and will be extended to September 2007

Regional greenhouse gas emissions – methane (and nitrous oxide)

This project aims to improve information on NZ emissions of agricultural greenhouse gases on a range of spatial scales, from farm to landscape scales, providing a basis for better quantifying and managing greenhouse gas inventories. The research will apply a range of techniques including transects and vertical profile measurements of the gases, combined with models to estimate fluxes from the 'footprint' area.

(Related NIWA research is funded from the Land Care *Greenhouse gas emissions from the terrestrial biosphere* FRST programme. This research is developing methods to evaluate the smaller N2O

fluxes, using micrometeorology techniques and has developed a method that uses CO2 fluxes as a tracer to derive the N2O fluxes.)

This research links directly to and is coordinated with the research of the MethaNet (and N2ONet) groups.

Funding: FRST $163k (FRST/LandCare $90k)
Timeframe Current contract expires June 2006, and will be extended to September 2007

LANDCARE RESEARCH

Work closely with other CRI's to underpin and strengthen New Zealand's ability to mitigate net emissions, and enable New Zealand to meet its obligations under the United Nations Framework Convention for Climate Change (UNFCCC) and the Kyoto Protocol.

Research projects themes

- Ways of reducing greenhouse gas emissions from land-based activities,
- New technologies to measure and monitor greenhouse gas emissions to internationally accepted levels of accuracy.

Reducing greenhouse gas emissions from the terrestrial biosphere
Determine how physical and biological processes on land regulate the production of New Zealand's major greenhouse gases-carbon dioxide (CO2), methane (CH4) and nitrous oxide (N2O),

- Identify and quantify New Zealand's terrestrial carbon sources and sinks
- Develop measurement and monitoring techniques to reduce uncertainty in New Zealand's greenhouse gas inventories.

Emissions estimation, and reduction of uncertainty in inventories:

- Measurement and modelling of CH_4 and N_2O at paddock scales,

Mitigation processes and strategies:

- Testing novel mitigation strategies
- Understanding of greenhouse gas emissions to enhance policy development and on-ground action to mitigate emissions.
- Use of intact soil cores to assess the effect of soil water content on nitrous oxide emissions from a poorly drained pasture soil
- Is soil a useful sink for methane?
- Modelling nitrous oxide emissions from New Zealand grazed pastures
- Nitrous oxide inventory: estimating a nitrous oxide emission factor for animal urine

- Methane production from agriculture
- Reducing nitrous oxide flux from animal wastes
- Can we increase soil carbon to offset methane and nitrous oxide emissions from agriculture?
- Modelling nitrous oxide emissions from New Zealand's grazed pastures
- Refining the uncertainty in nitrous oxide emissions from New Zealand agricultural soils
- NzOnet contract research under contract to MAF, through the national research network NzOnet, we undertake collaborative research with other CRIs on methodology for abatement of agricultural greenhouse gas emissions.

II INDUSTRY FUNDED

DAIRY INSIGHT

2006

A Cross-Sector Approach to Nitrification Inhibitors $114,320

2003/2004:
Life-Cycle Analysis
Contractor: AgResearch Ltd

Determining the environmental performance of the New Zealand dairy industry compared with overseas industries by:

- Measuring the environmental performance of the New Zealand dairy industry using resource use indicators (land, energy, water) and pollutant indicators (greenhouse gases, eutrophication, acidification)
- Comparing the performance of the New Zealand Dairy Industry with other countries where comparable data has been produced (Australia, Denmark, France)

Funding: $56,250

Reducing Economic and Climate Change Impacts on Farm Energy Use
Contractor: Massey University

Reduction of energy consumption, direct costs and greenhouse gas emissions by:

- Developing a method for measuring total on-farm greenhouse gas emissions, energy consumption and costs to ascertain if farms are good candidates for energy efficiency improvement;

- Identifying the opportunities for development of new energy-efficient technology for on-farm applications

- Disseminating a set of user-friendly energy efficiency recommendations to farm advisors, consultants and farmers that can be implemented at low cost

- Providing detailed end-use breakdown of electricity and time-of-use profiles that will enable alternative energy supply and end-use options to be analysed (e.g. solar water heating installations, time of use tariffs, etc.)

Funding $80,000

PASTORAL GENOMICS *(PG)*

Current Year

PG is involved in a protoplast fusion programme off shore that is intended to introgress the condensed tannin biosynthesis pathway from Rabbit's Foot Clover into White Clover. This is approximately 12 months from proof of function.

In addition AgResearch is looking at the utility of high anthocyanin lines of White Clover for a cisgenic® based up regulation of the condensed tannin biosynthesis pathway in white clover leaf.

The high sugar grasses have shown real progress

Previously Identified.

The projects that have a potential role in methane mitigation are summarised as follows:

- Gene discovery through the analysis of mutated genes in the model plant *Arabidopsis*. Gene(s) identified will be analysed in both clover and ryegrass for their effect on CT biosynthesis. We are currently in our fourth year of this research which is being carried out under contract with AgResearch.

- Gene discovery through the analysis of high Condensed tannins (CT) cotton fibres to identify the key genes involved in CT biosynthesis. This research has resulted in the identification of a gene that increases one aspect of CT biosynthesis, and another that increases DMACA staining (specific to CT's) in rice leaves. This work has been running for four years at ViaLactia and will be extended to ryegrass in the coming months.

- Using a non-GM technology ViaLactia is assessing the possibility of combining the genomes of ryegrass and a CT containing plant. This work is currently in its second year at Crop & Food.

- Using a similar non-GM technology as programme 3 PG is assessing the efficacy of combining the genomes of white clover and another clover that contains CT's in the leaf. This work is being carried out off shore and has been running for approx. 6 months.

- Under contract from PG AgResearch has an active gene discovery programme for genes involved in clover CT biosynthesis, and currently has several gene candidates in functional genomics. This programme has been running for approximately two years.

- PG also has a small programme in the area of high soluble carbohydrates in ryegrass. Through ViaLactia's relationship with IGER in the UK, PG has access to markers for marker assisted selection, linked to high soluble carbohydrate traits in ryegrass. A license for these tools for ryegrass breeding are currently available to NZ seed companies.

- In the past ViaLactia has identified that plants with high levels of stored fumarate can have a significant effect on methane production from model rumen systems. This is not an area of active research for PG at this time.

- Through ViaLactia PG has identified a ryegrass gene that increases the total digestibility of ryegrass in rumen fluid. Whilst it is not certain, it is possible that this would have a beneficial effect on methane production by raising the overall ME that the cow can derive from that ryegrass.