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National Livestock Methane Program

National Needs and Gaps Analysis

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Executive summary

This report is an agreed output for MLA as manager of the National Livestock Methane Program (NLMP). The program had the twin objectives of researching new ways to mitigate methane production and at the same time increase or maintain productivity of ruminant systems in Australia. This Needs and Gaps report has looked at research to date and recommends opportunities for future research investment using criteria for a range of on farm practices.

The methods used for the Needs and Gaps analysis involved:

i) evaluation of the research outcomes from relevant projects within and outside NLMP for reducing methane emissions form ruminants;
ii) identifying the likely magnitude of methane emissions reduction from different methane mitigation practices;
iii) identifying the likely effect of each practice on animal productivity;
iv) considering the barriers to adoption or commercialisation for each practice and its likely costs of implementation;
v) evaluating the technical risk, chances of the research succeeding and possibilities of a 'quantum leap' in the development of a new and productive technology;
vi) assessing the time required for additional research to be implemented on-farm and its likely cost; and
vii) assessing the capability of the practice for underpinning Emissions Reduction Fund (ERF) methodologies for implementation by producers.

Marginal Abatement Cost Curve (MACC) analyses were undertaken to assist the assessment of individual practices. The MACC analyses predicted potential animal and national emissions reductions based on scaling up predictions for animals on a standard farm within a region to the national level based on stock numbers. These analyses were based on assumptions, some well supported by literature, but others with little research backing. The MACC analyses also predicted the likely effects of adopting specific practices on farm profitability using a range of prices for carbon credits.
The methane mitigation practices considered were grouped under the NLMP themes, although no research was undertaken within the program on 3-nitrooxypropanol (NOP) or biochar and vaccine research was restricted to identifying potential peptide sequences that could be used a vaccine antigens. These non-NLMP practices were included because they are potential areas for additional investment and may be effective ways for profitably reducing methane emissions from ruminants. The following potential methane mitigation practices were evaluated:

- Genetics: Selection of beef cattle, sheep and dairy cows for low methane emissions
- Feed Supplements
  - Grape marc offered to sheep during feed-gap periods or feedlot cattle and dairy cows replacing ingredients with similar metabolisible energy content
  - Red algae as a supplement at around 2% of the diet for all animal classes
  - Nitrate as a feed supplement to partially replace urea in all animal classes
  - Wheat feeding at 9 kg/d to dairy cows grazing pasture
  - Plant bioactive compounds, 'C' and 'L', from Australian acacia and melaleuca species, as a supplement to all animal classes
  - 3-nitrooxypropanol (NOP), a commercial DSM supplement to all animal classes
  - Biochar as supplement at around 1% of the diet for all animal classes
- Forages
  - Leucaena plantations for the northern coastal beef region

    - Other legumes, mainly temperate legumes with high productivity and antimethanogenic properties for ruminants in southern Australia
    - Australian native shrubs as a component of the forage mix in south west Australia for grazing during the autumn feed-gap period
- Rumen function
    - Energy capture from methane not emitted due to implementation of an anti-methanogenic practice and more efficient capture of energy from fermentation
    - Vaccination against methanogens
- Adoption of best management practices (BMP's)
- Non methane inhibiting, but potential areas for adoption
    - NIR calibrations for forage digestibility and composition
    - Use of database from southern Australian forages from ELLE project
    - Use if the intra-ruminal device (IRD) for measuring methane emissions from animals

The MACC analyses showed clearly that the greatest economic benefits come from those methane mitigation practices which increase animal productivity and have low costs for implementation. The price paid for carbon credits has an impact on profitability, but the effect is generally smaller than the impact of a practice on animal productivity. Adoption of current best management practice (BMP) options tended to show greater expected financial returns than the majority of the direct mitigation practices. BMP options have relatively low methane mitigation potential, but result in improvements in animal productivity and have relatively low costs for implementation.

Supplementing diets with red algae or NOP, feeding 9 kg wheat daily to dairy cows at pasture, or introducing Leuceana plantations reduced methane emissions from individual animals more than the other scenarios and had positive effects on animal productivity. Breeding animals for low methane emissions resulted in low reductions in methane emissions and will be difficult to implement across industries, except for the dairy industry. Nitrate supplements when replacing urea result in small reductions in methane emissions with little effect on productivity, whereas manipulating the rumen to capture more energy from methane saved would have a large impact on animal productivity.

From the Needs and Gaps analysis, *The priorities for investment into methane mitigation projects based on the capacity to reduce methane, the potential productivity gain, barriers to implementation and time and cost of research were as follows:*

High priority

- Develop a dose response curve for leucaena to allow development of an ERF methodology
- Red marine macro-algae evaluation in different classes of ruminants and development of a commercial growing, harvesting and drying process, plus a method of supplementing to grazing livestock
- Manipulation of rumen function and biochemical pathways to allow markedly enhanced capture of energy from digestion and reduced methane emissions
- Evaluate two selected plant bioactive compounds in sheep for reducing methane emissions and quantify effects on productivity. If positive results, pursue commercialisation plan

Medium-High

- Evaluate characteristics of biochar and effects in vitro; undertake a dose-response experiment with ruminants to identify changes in rumen function, methane emissions and animal productivity

- Evaluate Archaea surface peptides for development of vaccines; evaluate effective routes for vaccination against rumen Archaea; collaborate with New Zealand team

Medium

- Evaluate NOP under Australian forage conditions
- Genetic selection for low methane emissions in dairy cows
- Determine specifications of wheat for reducing methane when fed at high rates to dairy cows on pasture; determine dose response or other information needed for development of an ERF methodology and explore for feedlot application Evaluate legumes, specifically lucerne and red clover, with superior agronomic characteristics for methane mitigation properties in vitro and subsequently in animals if positive
- Plant breeding programs for i) biserrula to improve productivity and palatability and reduce photosensitivity in animals and ii) chicory for improving persistence and ability to fill identified feed gaps

Low

- Grape marc - most information already obtained and national mitigation potential is low
- Nitrates - much information already obtained, but could examine effective ways for reducing methaemoglobin concentrations, but national mitigation potential is low
- Best Management Practices - Not research but adoption and extension needed

Several areas were identified for investment in the short term irrespective of the rank given for longer-term research because the research is likely to lead either to improved application of several methane mitigation practices or provide evidence needed to show more detailed research is warranted.

- Wheat feeding to dairy cows at pasture: specifying the quality of wheat needed for the methane mitigation effects; developing best management practices for introducing wheat feeding at high rates; drafting an ERF methodology,
- Leucaena: dose response curve for methane mitigation and verification of faecal NIR for predicting proportion of leucaena in the diet; final draft of an ERF methodology,
- Nitrate: evaluation of the effectiveness of providing with nitrates antioxidants and N-acetylcysteine to reduce the rise in methaemoglobin,
- Bioactives: evaluate compounds C, L and G, extracted from Australian native plants for anitmethanogenic effects in sheep,
- Grape marc-tannins: apply the new tannin analytical techniques to other animal feed ingredients known to impact on animal productivity across domestic animal species;
- Shrubs: incorporate shrubs effectively into plant physiological simulation models for effective use in whole farm economic analyses.

Table of Contents

1 Background

This report is one part of the agreed outputs for MLA to deliver to the Commonwealth Department of Agriculture in its role as manager of the National Livestock Methane Program (NLMP). The program had the twin objectives of researching new ways to mitigate methane production and at the same time increase or maintain productivity of ruminant systems in Australia. The purpose of this study is to analyse the research done to date and recommend opportunities for future research investment using results from a study into Marginal Abatement Cost Curve analysis of potential mitigation options and combining this with results from NLMP and best estimates of likely technical success from future research and adoption.

Approximately 16% of Australia's greenhouse gas (GHG) equivalent (CO_{2e}) emissions come from agriculture and around 65% of those emissions are derived from livestock digestion, primarily as methane from the stomach (rumen) of cattle, sheep and goats (Wiedemann et al. 2013). Cattle are responsible for about 70% of methane emission from ruminants in Australia.

There is increasing pressure on ruminant industries from international organisations, governments and large corporate customers to reduce GHG emissions. In recent years, there has been increasing calls for reducing the number of cattle in the world and some have suggested governments apply a tax on red meat. Many international companies in the food retail and preparation businesses are limiting purchases of products to farms that undergo sustainability audits, including for GHG emissions. Although an international process has not yet been put in place to legally limit GHG emissions from individual countries, many sub-national governments have introduced a price on carbon emissions. The total worldwide carbon market was estimated at US$360 billion in 2013 and over 3 billion people are expected to be covered by these carbon pricing systems by 2020. Thus, there are increasing government push and corporate industry pull incentives around the world to reduce GHG emissions from ruminants.

Methane from ruminants represents a loss to the animal of from 2-12% of digested energy and, if reduced, would decrease national greenhouse gas emissions and may, in some cases, increase energy available to livestock for productivity. The Australian government, along with several R&D Corporations, Universities, CSIRO and commercial companies, has invested substantial funds in research to understand the factors controlling methane emissions from ruminants and how they may be reduced. These funds were deployed initially through the Reducing Emissions from Livestock Research Program (RELRP) program, then extended to the National Livestock Methane Program (NLMP) in Filling the Research Gap Round One initiative, further through Filling the Research Gap Two program (FtRG2) and through the adoption and extension programs of Action on the Ground and the Extension and Outreach.

This report primarily examines outcomes from the NLMP research, but considers in its recommendations outcomes from related programs. The NLMP projects had five themes covering methods for measuring methane emissions from ruminants, the role of genetic selection for reduced methane emission, the use of various feed additives, the potential role of anti-methanogenic forages and how methane emissions may be reduced from a more detailed understanding of rumen function.

The sixteen projects within NLMP were:

1. Measuring methane in the rumen under different production systems as a predictor of methane emissions;
2. Development of gas selective membranes (for intra ruminal capsules);
3. Evaluation and optimisation of Greenfeed Emission Monitoring units for measuring methane emissions from sheep and cattle;
4. Genetic technologies to reduce methane emissions from Australian beef cattle;
5. Understanding methane reducing tannins in enteric fermentation using grape marc as a model tannin source;
6. Development of algae based functional foods for reducing enteric methane emissions from cattle;
7. Supplementation with tea saponins and statins to reduce methane emissions from ruminants;
8. Strategic science to develop dietary nitrate and defaunation as mitigation methodologies for grazing ruminants;
9. Practical and sustainable considerations for the mitigation of methane emissions in the northern Australian beef herd using nitrate supplements;
10. Enteric methane mitigation practices through manipulation of feeding systems for ruminant production in southern Australia;
11. Impacts of leucaena plantations on greenhouse gas emissions and carbon sequestration in northern Australian cattle production systems;
12. Best choice shrub and inter-row species for reducing emissions and emissions intensity;
13. The mechanism of antimethanogenic effects of bioactive plants and products on methane production in the rumen;
14. Efficient Livestock and Low Emissions from southern grazing systems;
15. Culture independent metagenomic approaches for understanding the functional metabolic potential of methanogen communities in ruminant livestock; and
16. Comparative analyses of rumen microbiomes to mitigate methane and improve feed utilization.

This report examines the progress made within broad project areas from NLMP research as well as other potential methods not studied within NLMP, but showing potential for reducing methane emissions from ruminants. Research areas considered promising for future investment are ranked in order of priority at the end of the analysis.

2 Methodology

The overall economic benefit from a methane mitigation practice depends on: i) methane emission reduction; ii) potential animal productivity gains; iii) cost of implementation; iv) extent of adoption and v) carbon credits earned. However, when assessing the priorities for future research, other factors must be considered including: i) technical risk of the research and its chances of success; ii) cost of the research; iii) complexity, compatibility with current farming practice and other factors influencing likelihood of adoption; iv) a successful commercialisation pathway; and v) capability of underpinning Emissions Reduction Fund (ERF) methodologies for implementation.

The methods used for the Needs and Gaps analysis involved: i) evaluation of the research outcomes from NLMP projects and other potential methods for reducing methane emissions form ruminants identified from the literature; ii) consideration of the outcomes from the Marginal Abatement Cost Curve (MACC) analyses of these methods and the veracity of the assumptions used in these analyses; iii) the potential of a practice for adoption or commercialisation and its likely costs of implementation; and iv) the opportunity for the research to succeed and lead to a 'quantum leap' in knowledge for the development of a new and productive technology.

Each potential mitigation practice was assessed and prioritised on the basis of the likely methane mitigation potential on a farm and national basis in relation to likely animal productivity gains and cost of implementation. Potential practices directly targeting methane mitigation were also compared with the adoption of several management practice changes, identified in this report as 'Best Management Practices' (BMPs), where better adoption of current knowledge may lead to reduced methane emissions while improving animal productivity. Comparisons between methane mitigation practices were made primarily using a Marginal Abatement Cost Curve (MACC) analysis (Cotter *et al*. 2015) for individual representative livestock properties in different regions of Australia and aggregated for similar properties across the country to obtain likely national figures for possible greenhouse gas abatement, when different levels of adoption were assumed.

3 Status of methane mitigation research

Research progress is briefly outlined for potential methane mitigation practices from within and also outside NLMP projects. Assumptions made for application in the MACC analyses are outlined and future research needs considered prior to analysis of the gaps in knowledge and likely future research needs. The status of research is discussed first in relation to the NLMP themes and then other potential practices for reducing methane emissions that have been reported.

3.1 Genetics

There was only one project (01200.044; B.CCH.6310) within NLMP investigating the impact of breeding beef cattle for reduced methane emissions. However, similar research is being undertaken in FtRG2 projects for sheep (B.CCH.7310; B.CCH.7320; B.CCH.7620) and dairy cattle (Ben Hayes; pers com), in New Zealand and other places around the world (Garnsworthy *et al.* 2012).

3.1.1 Status of research

Genetic variability is inherent between livestock animals and is known to apply to methane emissions. The variation in methane emissions is manifested through a lower feed intake and consequently methane emissions for the same growth rate (known as low residual feed intake, RFI or net feed intake NFI) and/or a lower methane production for the same feed intake (known as residual methane production, RMP). Research within NLMP and related sheep projects, funded under FtRG2 program, have demonstrated that methane emission has a moderate heritability of about 0.2 in both cattle and sheep. Animals with lower methane emissions appear to have smaller rumens and faster rate of passage of digesta through the foregut (B.CCH.7310). There is some evidence that the microbial population and particularly the population of methanogens differs between the high and low methane emitting animals (01200.059;B.CCH.6620). Although the heritability for methane emissions is moderate, the genetic variation between animals appears is small relative to other traits such as growth rate or milk production.

Methane emissions have been measured in respiration chambers for approximately 1000 beef cattle with known genetic background. This research is expensive and limits the number of animals on which methane emissions can be measured. Methane measurements have also been undertaken using the Greenfeed Emission Monitoring (GEM) system in the Tullimba research feedlot. Measurement of methane emissions with the GEM system is less expensive than respiration chambers, but is still too expensive for routine assessment of methane emissions of individual animals. Hence, for information on methane emissions to be incorporated into genetic evaluation schemes, it will be necessary to use indirect approaches. The only practical way will be to use genomic selection which relies on the relationships between phenotypic and genotypic variation among related individuals. Research relating phenotypic (individual animal) methane emissions to DNA genomic sequences is being conducted. Although more measurements are needed, there is potential to assess, with reasonable reliability, the methane emission potential of individual animals from genomic information. However, there is doubt this will be worthwhile financially for producers.

3.1.2 Methane reduction potential

An analysis by Fennessy *et al*. (2015; B.CCH.6133 report) indicates that the potential for genetic improvement through direct selection on methane traits is limited compared with that already being achieved through the associated changes due to selection for improved productivity. Estimates for the annual decrease in methane emissions in southern beef cattle through selection for improved productivity and direct selection for reduced methane range from around 0.2% to 0.4% per year depending on the selection pressure applied to different traits. The gain from genetic selection is cumulative resulting in an accumulated reduction in methane emissions of from 4-8% over 20 years. However, the impact of genetic selection for reduced methane emissions depends on the selection pressure placed on lowering methane emissions compared with selection for other traits like growth and reproduction. The impact on national methane emissions depends also on extent to which the genes are passed through the national herds.

3.1.3 Ease and cost of implementation

The beef sire selection tool used by most southern cattle breed societies (BREEDPLAN) has been modified within project 01200.047; B.CCH.6310 to incorporate both RFI and RMP. This upgrade of BREEDPLAN would make it a simple task to include a breeding value for RFI and RMP in selection indices designed to maximise profit from selection of breeding stock by individual farm enterprises. The selection pressure applied for a reduction in methane emissions through varying the weightings for each trait in the selection index, will be influenced by the price for carbon credits provided an Emissions Reduction Fund (ERF) methodology has been approved. However, the analysis by Fennessy *et al*. (2015) showed that placing high selection pressure on lowering methane emissions reduced gains in other traits influencing productivity. The practice was only of financial benefit to a producer when the price for carbon credits was very high. Currently, a methodology is being scoped within the NLMP project for using the updated BREEDPLAN to reduce methane emissions.

Although with the updated BREEDPLAN the selection of bulls for low methane will be simply included in the current practice used by commercial producers and will have negligible additional cost, selection of low methane emitting sires by seed-stock breeders to produce these commercial bulls will be expensive. Selection for low methane emission bulls requires a reliable method for measuring methane from individual animals. Currently, respiration chambers, GEM or similar units or the SF_6 methodology have been used to measure methane emissions from beef cattle, sheep and dairy cattle. Each of these methods requires expensive equipment and intensive animal handling and is unlikely to be undertaken by individual seed-stock breeders. Methane emission measurements from individual seed-stock animals or their progeny are most likely to be undertaken by breed societies through their nucleus herd/flock programs. However, the number of animals covered by these schemes is likely to remain small, particularly in the beef and sheep industries. Consequently, for genetic selection for low methane emitting animals to become wide spread in ruminant industries, except the dairy industry, where few bulls dominate genetic improvement and their genes are spread widely through artificial insemination, genomic selection will be required. Relationships need to be established between phenotypic methane emissions and gene sequences. An estimate of greater than 5000 observations would be required to establish reliable relationships for predicting methane emissions from genomic records and these relationships need to be determined for each breed type.

Currently approximately 1000 records for methane emissions and genomic data have been obtained for the Australian Angus breed. Further records could be collected through the Beef Information Nucleus (BIN) herds at the Tullimba research feedlot as part of the Angus breed society sire evaluation program. Little information is available for other southern beef breeds or for *Bos indicus* cattle and major programs would need to be instigated to obtain the information needed for these breeds. At least six years are likely to be needed before a methane emissions trait could be effectively used by the Australian Angus breeders. A longer time would be required for other beef breeds, northern cattle and sheep.

Dairy cattle represent a different case. The Holstein-Friesian dominates internationally, genomic selection is established, and relatively few bulls dominate genetic improvement and their genes are spread widely through artificial insemination. Hence the trait could be introduced into the dairy industry in a much shorter time because measurement of the required animals would be simpler and the rapid dissemination of genes through artificial insemination.

3.1.4 MACC analysis assumptions and predictions

For the MACC analyses, it was assumed that there would be an 8% improvement in RFI (reduced intake for same growth rate) and an 8% reduction in residual methane production over 20 years for southern beef and feedlot cattle. For northern beef cattle and for sheep, no change in RFI was assumed and an 8% reduction in methane over 20 years was used in the MACC analyses. A 16% reduction in methane emissions was assumed for dairy cattle over the same time period. These assumptions were made prior to the Fennessy *et al*. (2015) analyses and appear to be on the high side of probability. No increase in productivity was assumed due to the reduced energy lost as methane. The latter assumption may be over conservative because an increase in rate of digesta passage is likely to improve nutrient supply to the animal except on extremely low quality diets.

The cost of using superior bulls was assumed to be zero for commercial producers because it would be done through existing breeding plan software for cattle and sheep. The cost of selecting sires by seed-stock breeders was not considered in the MACC analyses, but would be large at several thousand dollars per bull and needs to be taken into account when deciding research priorities.

The MACC analyses assuming the effects of genetic selection after 20 years, showed little change in farm profitability for all regions and a small reduction in methane emissions. Assuming a 10% adoption across all animal sectors in Australia, the total reduction in methane emissions from beef cattle, sheep and dairy cows was predicted to be approximately 500,000 carbon dioxide equivalents annually (Table 6). This represents less than 1% of total enteric greenhouse gas emissions from ruminants in Australia of 55.6 m tonnes annually (Wiedemann *et al*. 2013). However, with fulfilment of current and future research projects, selection of cattle within the Angus herd, feedlots, sheep and dairy cows only would be feasible. When these alone are considered, the influence of breeding for reducing methane emissions would account for around 25% of that assumed for the whole Australian herd - equivalent to less than 0.2% of the total enteric greenhouse gas emitted from ruminants in Australia annually.

3.1.5 Additional research needed

The above assessment indicates that the national saving in enteric greenhouse gas emissions that could be derived from genetic selection is low even when applied across all ruminant sectors in the country. The cost of implementation of genetic improvement for reducing methane emissions on individual properties is likely to be low, but the cost to seed-stock breeders of selecting low emitting animals will be high. Current research within Australia applies only to the breed of Angus cattle (the Hereford breed society is concentrating on grass fed animals), sheep and dairy cows.

Considerable funds have already been invested in measurement of methane emissions from a limited number of Angus cattle and sheep. There is a potential to develop relationships between the methane emissions of individual animals and genomic sequence variation which could in the future be used to select low methane emitting sires and dames within studs, but this would have to be repeated for each breed. It may be reasonable to argue that further funds could be invested to make the genomic selection of low methane emissions from specific Angus lines reliable. If achieved, genomic selection of low emissions sires could be of benefit to individual breeders and their stock end-users for market access benefits.

There appears to be little benefit from selecting *Bos indicus* cattle for low methane, when other practices seem likely to be more effective for reducing methane emissions from northern cattle. The high cost to measure methane emissions, the slow genetic progress, negative impact on other productivity traits and small impact on national greenhouse gas emissions does not make attractive further investment into breeding for methane emissions for the Australian sheep industries.

3.1.6 Priority for further investment

Priority for further investment in using a genetic approach for reducing methane emissions is regarded as low, except perhaps for completing genomic-methane emissions relationships for Angus breed cattle. However, for producers using genetic means to improve RFI where less feed is consumed for the same performance, there would be a financial gain if the saving in methane emissions from this practice was quantified and an ERF methodology approved. Funds could be invested to determine the relationship between lowering RFI and methane emissions to allow development of an ERF methodology. Priority for further investment in using a genetic approach for reducing methane in dairy cows is regarded as medium because of the potential to transfer selected genes more rapidly through the industry.

3.2 Supplements

3.2.1 Grape marc

3.2.1.1 Status of research

Grape marc consists of the skins, seeds, stalks and stems remaining after grapes have been pressed to make wine. It can be dried and made into pellets, ensiled or remain fresh before being used as an animal feed or as a fertiliser. Grape marc contains condensed tannins with a range of compositions, high concentrations of oils and tartaric acid (NLMP project 01200.007; B.CCH.6410). All these compounds have the potential to reduce methane emissions in ruminants (Moate *et al*. 2014c). The project developed novel analytical methods for identifying different types of tannins within grape marc. These assays are a major improvement of previous tannin assays because they quantify a wide range of different phenolic compounds and will be valuable for determining the tannin profile and possible modes of action in other animal feed ingredients. An evaluation of the relative effects of the oil content or the tannin content and composition of grape marc samples was made using *in vitro* fermentation experiments. Grape marc samples high in oil reduced total fermentation with little independent effect on methane emissions. However, tannins, particularly extractable tannin with smaller polymer chains and with a lower cis/trans ratio were found to be the most effective for reducing methane production with little effect on total fermentation. Grape marc from white grapes tends to be the least processed and have higher concentrations of extractable tannin of the desired composition than red grape marc, stems or seeds.

Grape marc can have a relatively high fibre and low metabolisable energy content because of its high stalk and stem content. Three experiments within NLMP have been conducted feeding grape marc to dairy cows and sheep and one experiment was conducted within the Action on the Ground (AOG) program where grape marc was fed to beef cattle under feedlot conditions.

The effectiveness of grape marc for reducing methane production without a negative impact on animal productivity appears to depend on the relative energy content of the control diet compared with the grape marc sample included in the diet. Dried grape marc provided greater benefit than ensiled grape marc in one experiment with dairy cows, but this may have been related to the higher fibre and lignin content of the ensiled product (Moate *et al.* 2014c). Brahman cattle appear to better maintain productivity when fed grape marc than Angus cattle which may reflect their superior ability to digest high fibre diets.

In the first dairy cow experiment conducted in project 01200.017; B.CCH.6460, either dried/pelleted or ensiled grape marc replaced approximately 5 kg of 13 kg of lucerne hay per day in a diet providing 4 kg/day concentrate. The cows were in the late lactation phase of production. The fibre (neutral detergent fibre, NDF, and acid detergent fibre, ADF) content of the lucerne hay and dried/pelleted grape marc were similar and lower than for the ensiled grape marc. Milk yield from cows offered the control diet or the diet containing dried/pelleted grape marc was not significantly different, but methane emissions expressed as g/day, g/kg DMI or g/kg milk were 20-25% lower for the grape marc diet. Methane emissions expressed in g/day were also significantly lower for the cows consuming the ensiled grape marc product, but methane emissions expressed as g/kg milk were similar to the control cows.

In the second dairy cow experiment, either red or white ensiled grape marc replaced approximately 4.5 kg of freshly cut pasture in a daily diet containing 5 kg maize. The ADF content of the grape marc was about 35% higher than for the pasture. Although methane emissions expressed as g/day were reduced by approximately 14% for the cows consuming grape marc, milk yield was depressed and there were no differences between treatments in methane emissions expressed as g/kg milk. This result reflects the lower energy content of grape marc compared with the fresh pasture.

In the sheep experiment in project B.CCH.6460, either crimped or ensiled grape marc was used to replace an oaten hay of similar metabolisable energy content in diets offered to the animals in sufficient quantity to maintain live weight. Although intake was similar and there was a trend for reduced methane emissions in the sheep offered the diets containing grape marc, variation in methane emissions measured by face mask was too great for these differences to be significant. However, based on the trend in results, a 30% inclusion of grape marc when the energy content of the diet components was similar, suggests that methane emissions could be reduced by 10% without affecting animal performance.

In the AOG project with feedlot cattle, either 10% or 20% of the diet was grape marc which replaced the 10% maize silage and some barley. Inclusion of 20% grape marc in the diets of Angus cattle in a feedlot did not significantly reduce feed intake, but reduced methane emissions expressed as g/d by around 10%. However, growth rate was reduced by 30% in the cattle consuming 20% grape marc in the diet and methane emissions expressed as g/kg live weight gain increased by 25%. Adding 20% grape marc to the diet for Brahman cattle did not alter growth rate and reduced methane production expressed as g/d by approximately 25%. However, there was a reduction in feed intake. Methane production expressed as g/kg DMI was reduced by only around 5%. Grape marc is not a likely practical option for northern cattle because of the distance from grape growing regions.

The results from experiments with grape marc suggest that it has a place for feeding mainly as a replacement to low quality diets. However, three MACC analyses were undertaken: i) dairy cows in late lactation where energy content of the grape marc and forage it replaces are similar; ii) sheep fed low quality diets near maintenance; and iii) feedlot cattle where methane emissions and performance are both reduced.

3.2.1.2 Methane reduction potential

Results from the experiments outlined above suggest that methane emissions may be reduced by approximately 20% when grape marc replaces a forage of similar metabolisable energy (ME) content for dairy cows without a negative effect on milk yield. Similarly, if grape mark is offered to sheep during the summer-autumn feed-gap period in southern Australia, it can maintain animal weight and reduce methane emissions by around 10%. Using grape marc in feedlot diets does not appear practical because of reduced intake of available energy and reduced performance. However, if used, the results indicate methane emissions could be reduced by around 10%.

3.2.1.3 Ease and cost of implementation

Marc sourced directly from the winery has no cost except for loading and transport. Processed marc costs around $12/t when steam distilled, $40-$50/t when crimped (roller mill to crush seed) and$100/t when dried. On-farm use is best made into bunker silage for longer-term storage.

3.2.1.4 MACC analysis assumptions and predictions

Grape marc was assumed to be fed for three months of the year to non-pastoral zone sheep during the summer feed-gap period with a reduction of 10% in methane emissions, to dairy cows with a 20% reduction in methane emissions and no change in milk yield and to feedlot cattle with a 10% reduction in methane emissions and a 25% reduction in growth rate. It has been assumed that crimped grape marc was fed to the animals at an on farm cost of $73.33 per tonne, including loading and transportation for sheep, but no cost was applied to dairy or feedlot cattle because it was assumed to replace other ingredients.

The MACC analyses predict that with 10% adoption across the southern sheep regions, there would be a reduction in greenhouse gas CO_{2e} of approximately 100,000 tonnes annually (Table 6). If feeding grape marc was also adopted by 10% of the dairy and feedlot industries, there would be a further reduction in greenhouse gas CO_{2e} of around 45,000 tonnes. Feeding of grape marc to sheep during the feed-gap period appears to be marginally profitable, but would depend on the distance transported and the opportunity for ensiling. Similarly, the MACC simulations suggest that grape marc is profitable when fed to dairy cows as a replacement for a feed ingredient with similar ME content. However, widespread use of grape marc in feedlot industries is most unlikely because of its low ME content and probable reduction in animal productivity.

3.2.1.5 Additional research needed

Research from recent projects has demonstrated the value and limitations from feeding grape marc in different ruminant industries. This research indicates that there is limited opportunity for animal producers to feed grape marc profitably with the best options for sheep in southern Australia during the summer-autumn feed-gap period. However, care is needed in relation to possible risks from chemical residues.

No further research appears to be needed for evaluating the role of grape marc for reducing methane emissions from ruminants.

However, application of the new tannin assays to understanding the impact of other tannin containing feed ingredients on animal performance could be valuable.

3.2.1.6 Priority for further investment

Priority for future investment is considered low, expect for application of the novel tannin assays across animal nutrition.

3.3 Macro-algae

3.3.1 Status of research

NLPM project 01200.035; B.CCH.6510 showed that the red marine alga, *Asparagopsis taxiformis*, when collected by wild-harvest in the filamentous tetrasporophyte phase, air-dried and ground, reduced methane emissions *in vitro* by up to 99% without depressing substrate digestibility or volatile fatty acid production when included at up to 2% of total substrate organic matter. Subsequently, an experiment funded outside NLMP with rumen cannulated tropical cattle fed Flinders grass hay showed an average 14% reduction in methane emissions per unit of feed intake over a period from 21-29 days after 2% OM intake of *Asparagopsis* was administered daily into the rumen. There was no evidence of microbial adaption to the alga over the 29 day period. Feed intake was not depressed and in fact rose by 6% (approaching significance) compared with the controls during the last methane measurement period in respiration chambers.

A more extensive experiment funded outside NLMP has recently been conducted with adult wether sheep. The sheep were fed a typical export shipping pellet with 0, 0.5, 1.0, 2.0 or 3.0 % of organic matter provided as dried, ground *Asparagopsis* mixed with crushed lupins. The intake of red alga was approximately 0, 13, 26, 58 and 80 g/d, respectively for the 5 treatments. The sheep were fed at 1.2 times maintenance energy intake and methane emissions were measured in chambers on days 30, 51 and 72 after introduction to the algal supplement. There was no indication of adaption of the microbes to the algae over the 72 day period and feed intake was not significantly affected. Methane emissions were reduced linearly (R^2 = 0.82) as the amount of alga in the diet was increased (Figure 1). When 3.0% of organic matter was included as algae, there was a reduction of 84% in methane emissions per unit of feed intake. At the last methane measurement period, there was a 0.86% fall in methane emissions for every gram of algae included in the diet.

The mechanism for reduction of methane is presumed for some algae. The red algae used in the sheep experiment contained 0.22 mg/g DM of halogenated metabolites. Halogenated methane analogues, such as bromochloromethane (BCM), inhibit methane production by reacting with reduced vitaminB12 which inhibits the cobamide-dependent enzyme methyl-coenzyme (CoM) reductase step in methanogenesis. *Asparagopsis* produces more than

100 low molecular weight metabolites containing bromine and chlorine that have antimicrobial activity. Bromoform is a secondary metabolite produced by *Asparagopsis* and inhibits methanogenesis by also reacting with a vitamin B12 cofactor, CoM reductase, in a similar way to 3-nitrooxypropanol (NOP).

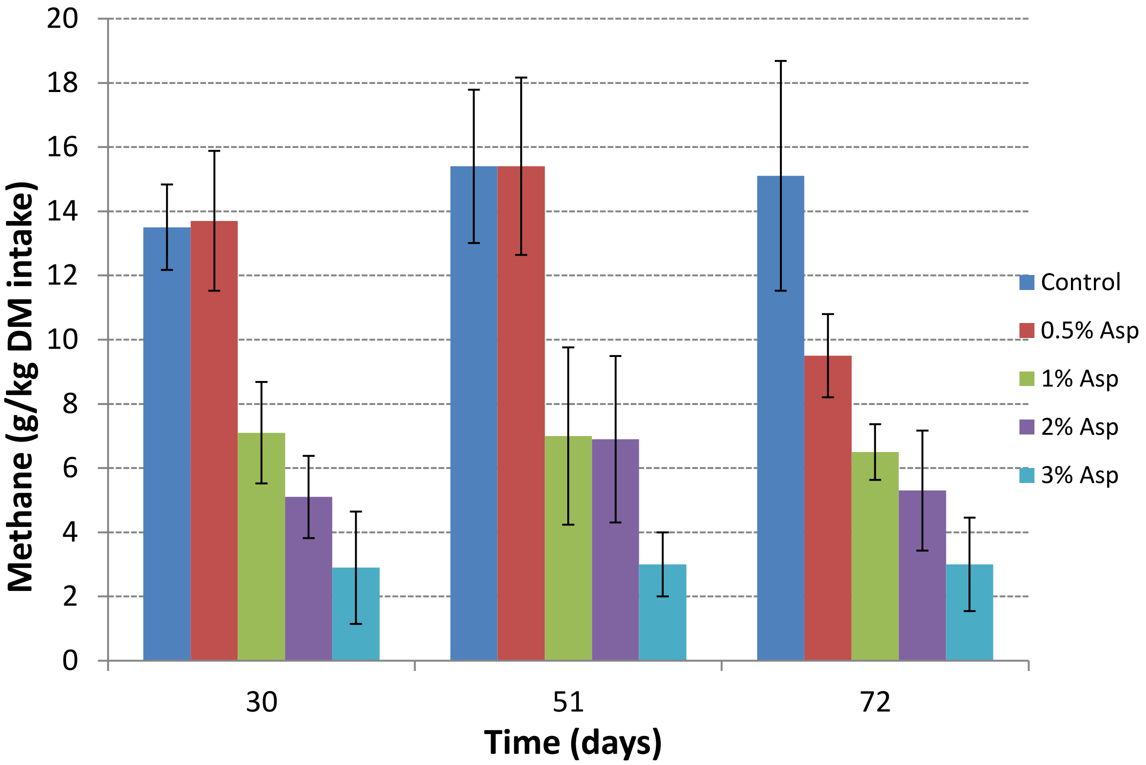


Figure 1. Mean (± sem) methane emissions (g/kg DM intake) measured at three intervals throughout the experimental period for sheep fed a pelleted diet and supplemented with (0-3% OM basis)*Asparagopsis* (Asp.) on a daily basis.

A provisional patent relating to the use of algae for reducing methane emissions (Method for reducing total gas production and/or methane production in a ruminant animal) was lodged on 21 January 2014 and updated to an international patent, PCT/AU2015/000030, on 21 January 2015. The information quoted above comes from that patent application.

Several studies have used BCM to inhibit methane emissions in ruminants (McCrabb *et al*. 1997; Tomkins and Hunter 2004; Mitsumori *et al*. 2012). These studies show that BCM can reduce methane production in cattle and goats by more than 90%. Although high doses of BCM (0.6 g/100 kg live weight) were shown to reduce feed intake, doses that depressed methane production by around 60% had no significant effect on intake. Similarly, several experiments (Goel *et al*. 2009; Mitsumori *et al*. 2012) showed no effect of BCM on digestibility or efficiency of microbial growth. A recent experiment by McSweeney in the FtRG2 project B.CCH.7610, where methane emissions were depressed and hydrogen concentration in the rumen increased with chloroform, showed a marked increase in hydrogen trapped in VFA and in microbial tissue. McCrabb *et al*. (1997) showed that BCM significantly increased the efficiency of feed use in tropical cattle.

Although BCM at concentrations sufficient to have marked effects on methane emissions does not reduce feed intake of cattle and increases productivity, no experiments have yet been undertaken with ruminants offered diets containing low concentrations of the red algae *ad libitum*. Such an experiment is essential to determine whether there will be negative effects of the algae on feed intake of highly productive animals.

3.3.2 Methane reduction potential

If the results from the BCM and chloroform studies are assumed to be similar to those obtained with *Asparagopsis*, a reduction in methane emission of 50-60% would seem practical without any negative effect on feed intake. The resulting increase in hydrogen concentration within the rumen would be expected to increase the proportion of propionate produced, increase microbial growth and increase animal productivity. Support for this possibility comes from an earlier experiment (Tomkins *et al*. 2009) with BCM included in a diet offered to feedlot cattle at the rate of 0.3 g/100 kg live weight. Cattle receiving the BCM showed an increase in growth of 100 g/d which approached significance (P=0.07).

3.3.3 Ease and cost of implementation

Currently the cost of wild-harvesting *Asparagopsis* is approximately $200/kg. However, Ridley Agriproducts are working with James Cook University (JCU) to establish a marine macroalgae production system. Considerable research has been undertaken at JCU to identify the ideal growing temperature and conditions for the *Asparagopsis* species. They have identified the effects of genetic strains, sea temperature and nutrient availability on the concentrations and types of halogenated metabolites produced. This information can be exploited to select the most appropriate genetic stock, identify the best location for production and the ideal nutrient requirements. With appropriate algae selection and growing conditions, high methane mitigation is likely to be achieved with 2% or less *Asparagopsis* included in the diets of ruminants.

JCU personnel have demonstrated that *Asparagopsis* can be cultured on ropes similar to the culture of mussels, a process that can be readily commercialised (Figure 2). The research to date suggests that *Asparagopsis* can be grown in association with other aquaculture enterprises in southern Australia such as salmon or tuna farming and would be a valuable means of reducing pollution from these industries. If rope culture systems become viable and the algae is used to improve the sea environment, prices below $1.50/kg are feasible.

Figure 2. Red algae being produced commercially using rope culture techniques

3.3.4 MACC analysis assumptions and predictions

Several MACC analyses were conducted for the red algae. It was assumed that methane emissions could be reduced by either 30% or 60%, with 0, 20, 40 or 80% of the energy saved from reducing methane emissions being used for productivity. The method used to convert the saved energy into changes in growth rate was based on the National Greenhouse Gas Inventory and is explained by Cotter *et al*. (2015) and outlined in the Rumen-metabolic pathway section below. It was assumed that the algae scenario could be applied to all production circumstances, because the effective dose rate for cattle is likely to be less than 50-100 g/d and could be provided in lick or block form to grazing animals. The cost of providing red algae was assumed to be $1.50/kg and applied at the rate of 100 g/head per day.

When a 60% reduction in methane emissions and 10% adoption across the Australian ruminant industries is assumed, the MACC analyses predicted methane output would be reduced by over 3 million tonnes CO_{2e} (Table 6). This represents around 10% of total Australian emissions from ruminant animals. At a price of $1.50/kg for algae and no price on carbon credits, the practice would be profitable for the beef industry if 40% of the energy saved from the reduced methane emissions was used for productivity. However, if the price of carbon credits were $14/tonne, profitability would be increased by approximately $21/head/year, indicating the benefit from an approved ERF methodology for algae. Calculations presented in the Rumen-metabolic pathway section below suggest that 40% retention of the saved methane energy may be close to reality.

3.3.5 Additional research needed

The results obtained for red algae suggests there is a good potential for it to have a major role in reducing methane emissions across the Australian ruminant industries. However, research with animals to date is limited. The following projects need to be undertaken to provide additional information confirming its potential value across industries.

- Demonstrate that at algal intakes needed to substantially reduce methane emissions, feed intake is not depressed and productivity is maintained or increased. Experiments are needed for feedlot and dairy animals.

- Demonstrate that algae can be fed to rangeland animals through lick-blocks or other methods to prove it could be a valuable method for reducing methane emissions from breeding herds and flocks which are responsible for the majority of enteric greenhouse gas losses in Australia, including measuring variation in intake between animals and seasons.

- Undertake further evaluation of the potential impact of compounds or metabolites from the algae on animal and food safety.

- Continue research specifying factors and conditions that affect the concentration of halogenated metabolites produced by the red algae, and their stability and biological effectiveness under conditions typical of commercial use.

- Undertake studies for proof of concept for large scale production of the algae under commercial conditions and its value for reducing aquaculture pollution.

3.3.6 Priority for further investment

Priority for future investment is considered high. However, there need to be decision points during the conduct of the research to ensure that the use of algae can be effective for methane emissions reduction, will enhance productivity, will not have deleterious effects on animal or human wellbeing and can be profitable with relevant prices for carbon credits when appropriate ERF methodologies are available.

3.4 Nitrate

3.4.1 Status of research

Adding nitrates as a supplement to the diets of sheep and cattle has been examined in two NLMP projects, 01200.031; B.CCH.6440 and 01200.048; B.CCH.6450. Non-protein nitrogen sources are fed to ruminants to increase microbial growth, feed digestibility, feed intake and productivity when crude protein concentration in the diet is less than about 60g/kg dry matter (Minson 1990). Typically, urea has been used as the non-protein nitrogen source in dairy and feedlot diets and in lick-blocks available to sheep and cattle grazing dry, low quality pastures. However, if the non-protein nitrogen is provided from nitrates, hydrogen is used in the conversion of nitrate to nitrite and then to ammonia. These nitrate reduction reactions have a lower free energy change than reactions utilising hydrogen for methane production within the rumen and therefore have a competitive advantage. Consequently, adding nitrate to diets reduces methane emissions, while providing non-protein nitrogen for microbial growth (Leng 2008; van Zijderveld *et al.* 2010). However, if the concentration of nitrite in the rumen rises and nitrite is absorbed into the blood, nitrite poisoning created by excess methaemoglobin in the blood can occur. Methaemoglobin reduces the oxygen carrying capacity of the blood and can result in animal death.

There have been numerous experiments and reviews of the effect of nitrate feeding on methane emissions from ruminants (van Zijderveld *et al*. 2011; Lee and Beauchemin, 2014; Nolan *et al*. 2015). The general consensus is that adding nitrate to the diet of ruminants linearly reduces methane production (to a maximum of approx. 50%) as the amount eaten increases, with little further reduction in methane emission as nitrate intake continues to increase (Lee and Beauchemin, 2014; Cohn *et al*. 2014). Theoretically, 1 g of nitrate reduces methane production by 258 mg. However, complete efficiency of hydrogen uptake by nitrate is not observed, with an average efficiency of hydrogen uptake being around 90% (van Zijderveld*et al*. 2011; Nolan *et al*. 2010; Li *et al*. 2012; Li *et al.* 2013; Callaghan 2014). A rounded estimate is that 10 g nitrate/kg DMI can reduce methane emissions by up to 10%. However, feeding more than 7 g nitrate/kg DMI is not recommended for grazing cattle in the approved ERF methodology because of the risk of nitrite poisoning (Commonwealth 2014).

A review of the literature by Lee and Beauchemin (2014) suggests that across many experiments feed intake and growth rate of cattle are not negatively affected by nitrate feeding and will increase if the rumen microbes respond to non-protein nitrogen. However, nitrate would normally be fed to ruminants as a replacement for urea when providing non-protein nitrogen to animals. There is wide variation across experiments in the effects of nitrate supplementation on feed intake and animal performance when it is fed in the place of urea. Recent studies suggest that cattle fed diets containing nitrate under total mixed ration conditions have a reduced feed intake of 7-15% compared with diets containing isonitrogenous amounts of urea (Hulshof *et al.* 2012; Hegarty *et al.* 2013; Velazco *et al*. 2014). However, experiments where nitrate has been provided at isonitrogenous rates and compared with urea in cattle fed low quality tropical forage suggest that intake of nitrate and urea supplemented animals is similar (Callaghan 2014). Similarly, there is little evidence nitrate supplementation reduces intake or productivity of lactating dairy cows when compared with urea supplementation (van Zijderveld*etal*. 2010, 2011). Adding nitrate to feeds or lick-blocks appears to change the feeding behaviour of cattle, resulting in smaller and more frequent meals when total mixed rations are fed and lower intake of lick-blocks under dry-season tropical pasture conditions (Velazco *et al*. 2014; Callaghan 2014).

Experiments with sheep indicate similar responses to cattle when nitrates are included in either total mixed rations or supplements with lower quality forage diets (Nolan *et al*. 2010; Li *et al*. 2013; de Raphelis-Soissan *et al*. 2014). However, there appears to be a consistent increase in wool growth from 12-37% when nitrates are fed to sheep (Li *et al*. 2013; de

Raphelis-Soissan*et al*. 2014). The increase in wool growth is thought to be caused by nitric oxide formed from nitrite causing dilation of blood vessels and increasing blood flow to the skin.

The literature suggests that nitrate can at least partially replace urea in circumstances where ruminant animals respond to the addition of non-protein nitrogen sources. Nitrate has been included in lick-blocks with urea and fed to cattle under rangeland conditions when the crude protein content of the pasture is less than 6% dry matter. Research from NLMP project 01200.031; B.CCH.6440 suggests that the maximum intake of nitrate from lick-blocks under rangeland conditions is around 20 g/animal/day. Extending the same results to sheep would suggest that maximum intake of nitrate by sheep grazing dry pasture in southern Australia would be around 3.4 g/animal/day or approximately 4 g/kg DMI. However, for feedlot cattle offered mixed grain/forage rations the upper limit to nitrate inclusion appears to be around 10 g/kg DM (Hulshof *et al.* 2012 and Velazco*et al*. 2014). Safe nitrate inclusion rate appears to be higher again for dairy cows offered mixed rations at 20 g/kg DM.

3.4.2 Methane reduction potential

Callaghan (2014) showed that feeding 50 g nitrate/day to tropical cattle eating a low protein pasture reduced methane production by 11.6 g/day, which was a reduction of 16% compared with urea supplemented cattle and represented an efficiency of hydrogen uptake by nitrate of 89%. Using this relationship, if maximum intake of nitrate is assumed to be 20 g/d, methane emissions are calculated to be reduced by approximately 6.5%. Evidence from project 01200.031; B.CCH.6440 suggests that when non-protein nitrogen supply is adequate for rumen microbial metabolism of cattle grazing low protein tropical forages, feed intake of cattle consuming up to 50 g/d nitrate is not affected.

Similar assumptions can be made for sheep grazing low quality forage with 3.4 g/day of nitrate reducing methane emissions by 6.5%. The results from Hulshof *et al.* (2012) and Velazco*et al*. (2014) for feedlot cattle suggest that an intake of 10 g/kg would reduce methane emissions by around 15% per unit of dry matter intake. Evidence from several studies (Hulshof *et al.* 2012; Hegarty *et al.* 2013; Velazco *et al*. 2014) also suggests that feed intake of feedlot cattle offered nitrate supplements is reduced by approximately 10%. On the basis of the experiment by Velazco *et al*. (2014) it is assumed that live weight gain for the control animals is 2.0 kg/day and the growth rate of the nitrate supplemented animals would be that resulting from a 10% reduction in feed intake. Methane reduction in dairy cows based on the experiments of van Zijderveld *etal*. (2010, 2011) appears to be around 15% when nitrate intake is 20 g/kg with feed intake and milk yield not being changed.

3.4.3 Ease and cost of implementation

Nitrate is readily included in diets or lick-blocks as either calcium nitrate or ammonium nitrate as a full or partial replacement for urea. Integrity of lick-blocks is sometimes reduced because a greater proportion of the block is from the nitrate compound than from urea. The cost of nitrate compounds is a little higher than the cost of an equivalent amount of nitrogen from urea.

The major concern with feeding nitrate for reducing methane emissions is the risk of nitrite poisoning through increases of methaemoglobin in the blood. An increase in methaemoglobin concentration is particularly dangerous when animals are likely to be subjected to exercise (Callaghan NLMP project 01200.031; B.CCH.6440).

3.4.4 MACC analysis assumptions and predictions

MACC analyses were undertaken for: i) cattle in northern Australia for the period of the year when pasture crude protein content has declined to less than 6% dry matter with a reduction in methane of 6.5%; ii) sheep in southern Australia for periods of the year when pasture crude protein content is less than 6% dry matter with a reduction in methane of 6.5%; iii) feedlot cattle where methane emission were reduced by 10% and feed intake and growth rate reduced by 10%; iv) dairy cows where methane emissions were reduced by 20%, but intake and milk yield were unaffected.

The MACC analyses predicted that with a 10% adoption across the animal industries, feeding nitrate would reduce national greenhouse gas CO_{2e} by only around 360,000 tonnes annually (Table 6). This represents a reduction of approximately 0.6% of the total greenhouse gas emissions in enteric methane from ruminants in Australia.

3.4.5 Additional research needed

A considerable amount of research has been conducted investigating the effects of nitrate feeding on methane emissions mitigation, animal productivity and animal health. A significant proportion of the research has been considered during the development and approval of an ERF methodology for providing nitrate supplements to grazing cattle.

There appears to be little need for additional research into the efficacy of nitrate feeding. However, the risk of nitrite poisoning remains a major concern for producers wishing to use nitrate to reduce greenhouse gas emissions. These concerns means that the amount of nitrate offered to animals will be conservative and generally low reducing the impact on methane mitigation.

NLMP management commissioned a review of the literature based on understanding the physiology and biochemistry behind the formation of methaemoglobin in the blood of animals fed nitrate. This review resulted in several possible ways to reduce the formation of methaemoglobin or increase the rate of conversion of methaemoglobin back to haemoglobin (Nolan *et al*. 2015).

A case can be made based on the literature review to conduct experiments examining whether the provision of riboflavin and/or other antioxidants such as vitamin E, ascorbic acid, selenite and curcumin, in combination with N-acetylcysteine, would reduce the risk of nitrite poisoning when nitrates are provided to ruminants.

3.4.6 Priority for further investment

The priority for investment is considered low because of the small impact on methane emissions, the considerable amount of research already undertaken around the world and the high risk of nitrite poisoning.

The exception is that because nitrate feeding to grazing cattle is already an approved ERF methodology, a small number of experiments could be undertaken soon to test the hypothesis that dietary antioxidants in combination with N-acetylcysteine may reduce the risk of nitrite poisoning. Such experiments do not appear to have been published.

3.5 Wheat feeding to dairy cows

3.5.1 Status of research

Two experiments have been conducted within project 01200.017; B.CCH.6460 to show that when crushed wheat grain is fed at a rate of approximately 9 kg in two daily feeds with either freshly cut ryegrass pasture or chopped lucerne hay, methane production per kg DMI was reduced by 30% to greater than 50%, respectively, compared to the pasture alone or a diet providing the same amount of crushed maize grain (Moate *et al.* 2012, 2014a, b). Milk yield was significantly higher by 21% in the experiment comparing fresh pasture with pasture plus wheat. However, there were no significant differences in milk yield when wheat was compared with maize. The minimum amount of wheat fed daily to cause a substantial depression in methane emissions from dairy cows has not been evaluated.

The composition or energy value of the wheat samples used in these experiments were not determined. The last experiment in the project compared 9 kg/d of crushed wheat with 9 kg/d of crushed maize fed with a longer cut lucerne hay. The wheat sample used in the last experiment was of extremely poor quality, with pinched grains, low starch content and many grains not crushed during processing. Milk yield was lower for the cows consuming crushed maize than for those consuming wheat. However, there were no differences in methane emissions between the two treatments. These results suggest that normal, high starch content wheat with a rapid rate of fermentation in the rumen is needed to substantially lower methane emissions when fed twice daily at rates of approximately 9 kg/d to dairy cows. There is a need to determine the specifications of wheat samples and processing conditions needed to reduce methane emissions when feeding high quantities of wheat to dairy cows at pasture. The cause of the depression in methane emissions when wheat is fed at high rates to dairy cows has not been determined. However, it is known that low rumen pH which is likely under these circumstances results in an increase in rumen hydrogen concentration and a move in rumen metabolism from high methane-producing acetic acid pathways to lower methane-producing propionic acid dominant pathways (Janssen 2010).

3.5.2 Methane reduction potential

Two experiments show a reduction of from 30% to 50% in methane emissions from lactating dairy cows fed 9 kg wheat per day spread over two equal feeds. The reduction in methane occurred when wheat replaced fresh pasture and when wheat substituted for maize grain.

3.5.3 Ease and cost of implementation

Feeding 9 kg wheat to dairy cows daily is not a common practice, particularly for pasture based systems where the best response in terms of milk yield was observed. However, providing wheat of the correct specification to cows consuming total mixed rations would be a simpler practice because it would replace other cereal grains and ingredients. Feeding high amounts of highly digestible wheat could lead to a substantial risk of rumen acidosis unless managed carefully.

3.5.4 MACC analysis assumptions and predictions

The MACC analyses assumed 9 kg of wheat was fed daily to dairy cows consuming pasture which resulted in a depression in methane emissions of 40%. The cost of wheat was assumed to be \$250/t compared with \$92/t for pasture or silage. Milk yield was assumed to be increased by 20% with wheat feeding.

The MACC analyses predict that feeding high amounts of wheat to dairy cows would reduce greenhouse gas CO_{2e} by just over 50,000 tonnes annually if adopted by 10% of farms (Table

6). This represents approximately 5% of the estimated 470,000 estimated to be emitted from Australian dairy cows. With the above assumptions, the feeding of wheat to dairy cows was predicted to be profitable even when there was no price for carbon credits.

3.5.5 Additional research needed

Additional research is needed:

- To clarify the specifications of the wheat needed to reduce methane emissions
- To determine the amount of wheat that needs to be fed and whether a dose response curve for methane mitigation can be derived
- To identify interactions between length and type of fodder and effects of rate of passage of digesta from the rumen on the methane mitigation response of wheat
- To develop a best management practice protocol for feeding high amounts of wheat dairy cows to reduce the risk of acidosis and ensure methane emissions are reduced
- To scope an ERF methodology for feeding wheat to dairy cows to enable payment for carbon credits obtained through the feeding of wheat to dairy cows
- To determine whether crushed wheat can replace maize or sorghum in feedlot rations and reduce methane emissions

The technical risk for the proposed research is low and cost would be relatively modest.

3.5.6 Priority for further investment

The priority for further investment is ranked as medium. There is potentially a substantial methane mitigation outcome, but the risks for acidosis and animal health problems are present. There is an opportunity immediate for research to specify the characteristics of wheat needed to achieve the anitmethanogenic effect because the practice can be adopted now and the returns to producers are potentially high.

3.6 Plant bioactive compounds

3.6.1 Status of research

Research within NLMP project 01200.021; B.CCH.6530 has shown that several plant species, specifically the Tar Bush shrub, *Eremophila glabra* and the legume pasture plant, Biserrula, reduce methane emissions from *in vitro* fermentation cultures (batch and longer term Rusitec) and from sheep compared with control diets (Banik *et al*. 2013; Li 2013; Li *et al*. 2014; Table 1). When *E. glabra* was included at 15%, 25% and 40% with oaten chaff and lupins for 33 days in a Rusitec fermentation system, methane emissions were reduced linearly with dose to be 45% less than the controls (Li *et al.* 2014). Other bioactive compounds, called C, L and G have been extracted from native Australian melaleuca and leptospermum plants and shown to substantially reduce methane emissions *in vitro*, but have not yet been tested in animals. The compound L, when included in a batch culture fermentation assay reduced methane emissions substantially with a 97% reduction occurring when added at the rate of 250 µL/g dry matter incubated. Subsequent studies over 10 days using the Rusitec long-term *in vitro* fermentation assay showed approximately 85% reduction in methane emissions when C was included at a rate of 25 µL/g dry matter incubated or L included at a rate of 50 µL/g dry matter incubated.

An estimate of the likely reduction in methane emissions when C or L are provided as a supplement to animals was obtained by comparing the reduction in methane emissions when the bioactive plants Eremophila and Biserrrula were assayed *in vitro* (batch and Rusitec) with the reduction when fed to sheep. The results of this comparison are presented in Table 1.

Table 1. Comparison of methane reduction from *in vitro* assays and *in vivo* feeding to sheep

Treatment	Dose	% inhibition *in vitro*	Testing type	% inhibition *in vivo*	Scale of effect# (*in vitro/in vivo*)	Authors*
Bioactive plants						
E. glabra	15 % E. glabra/ 85% oaten chaff	37.0	rusitec	14.7	2.5	Li et al.
Biserrula	100% biserrula	13.2	rusitec	20.0	0.7	Banik/Hutton
Biserrula	50% biserrula/50% subclover	45.4	rusitec	*10.0*	4.5	Banik/Hutton
Biserrula	50% biserrula/50% subclover	51.0	batch	*10.0*	5.1	Banik/Hutton
Biserrula	100% biserrula	*80.0*	batch	20.0	4.0	Banik/Hutton
ESEF$					3.4	
C and L						
C	25 µL/g DMi	86	rusitec	*25.3*	3.4	Garcia et al.
L	25 µL/g DMi	59	rusitec	*17.2*	3.4	Garcia et al.
L	50 µL/g DMi	85	rusitec	*25.1*	3.4	Garcia et al.

#Scale of effect = % methane inhibition *in vitro*/% methane inhibition in *vivo*; *manuscripts in preparation, $Average Scale of effect for all treatments.

3.6.2 Methane reduction potential

The average scale of effect for the experiments with both *in vitro* and *in vivo* results was used to estimate the likely reduction in methane emissions when compounds C or L are included as supplements for ruminants. The comparison suggests that both compounds C and L when included at the rate of 25 ml/kg DM and 50 ml/kg DM, respectively, would reduce methane emissions by approximately 25%. Although a dose response analysis has not been conducted with the compound G, it appears to have similar methane inhibiting effects as C.

3.6.3 Ease and cost of implementation

The most promising bioactive compounds extracted from plants based on *in vitro* studies are the compounds C, L and G. The *in vitro* studies suggest that they could be included in diets at concentrations from 25 to 50 g/kg feed. If so these compounds could be readily fed to all ruminant types in Australia either as supplements or in lick-blocks. The cost of the compounds is difficult to estimate. However, it is probable both can be manufactured. If so, the cost will depend on likely scale of demand for the compounds.

3.6.4 MACC analysis assumptions and predictions

For the MACC analyses, a methane reduction of 25% was assumed and the practice was applied across all ruminant production systems. No energy from the saved methane was assumed to be used for animal productivity. Feed intake was assumed not to be affected by supplementation with the plant bioactive compounds. The cost of providing the bioactive compounds was assumed to be $0.50/day for cattle and $0.10/day for sheep.

The MACC analyses predicted that with a 25% reduction in methane emissions and 10% adoption across all Australian ruminant industries, greenhouse gas emissions CO_{2e} would be reduced by approximately 1.37 million tonnes annually (Table 6). This is a reduction of approximately 2.5% of total ruminant enteric greenhouse gas emissions. However, the MACC predictions are based on assumptions that have not been verified in animals. In addition, at the prices used for the bioactive compounds in the MACC analyses, their use as supplements was not profitable for any industry even when the price for carbon credits was set at $50/tonne.

3.6.5 Additional research needed

The longer term *in vitro* assays with compounds C and L, and the ability of G to virtually eliminate methane production form pure cultures of mathanogens, are sufficiently encouraging to warrant animal dose response experiments. Initial experiments should be conducted with sheep in respiration chambers to determine a dose response to both compounds over a period of approximately 3 months. If these are promising, effects of the compounds on feed intake and productivity will be needed as well as experiments with cattle.

If these initial experiments are still promising, consideration will need to be given to the commercial viability of using the bioactive compounds as a practice for reducing methane emissions. If the compounds seem to be commercially viable, experiments evaluating their applicability to all ruminant production systems may be required.

3.6.6 Priority for further investment

The priority for further investment is ranked high for the initial dose response evaluation of C, L and G in sheep. Further investment will be influenced by the results from the initial animal experiment and the potential opportunity for commercialisation of the products.

3.7 3-nitrooxypropanol (NOP)

NOP was not studied within NLMP, but will be used in an experiment within the FtRG2 project B.CCH.7610.

3.7.1 Status of research

3-Nitrooxypropanol(NOP) has been shown to reduce methane emissions in sheep and dairy cows. NOP and the ethyl variant, ethyl-3-nitrooxypropanol, are compounds synthesised by the animal feed supplement company DSM in Switzerland. The compounds appear to bind to the active site of the enzyme methyl-coenzyme (CoM) reductase which catalyses the last step in the reduction of CO_2 to CH_4 by the hydrogenotrophic methanogenic archaea. The compounds are highly volatile, with a short survival time in feed or the rumen unless imbedded in other compounds that reduce the volatility. DSM is currently working to reduce the volatility for practical feeding of the compounds. The company is also undertaking toxicology evaluation.

In vitro and *in vivo* experiments have been conducted (Patent No. US 2014/014 7529 A1 - May 29, 2014; Haisan *et al*. 2014; Reynolds *et al*. 2014; Martinez-Fernández *et al*. 2014). The longest experiment has been for 30 days. All experiments with animals have shown a significant reduction in methane emission and methane yield (methane/feed intake). However, the range in methane depression has been from 4-29% over five experiments. The mean reduction in methane/kg feed intake is close to 15%. The methane reduction potential was maintained from 14 to 30 days in one experiment. Some of the variation in methane reduction can be attributed to the method of feeding. For most experiments reported, the compounds were placed directly in the rumen once or twice daily or once daily wrapped in tissue paper. In another experiment, the compound was mixed with ground barley, molasses and canola oil and put into a total mixed ration.

On the basis of the experiments reported to date, a reduction in methane emissions in animals of 15% appears to be a reasonable assumption. However, the methane reduction obtained in several *in vitro* studies has been as high as 95%. The discrepancy between the types of experiments could result from the high volatility of the compound or a high rate of degradation within the rumen, associated with the pulse method of feeding in the animal experiments. Experiments are required where the dose of NOP mimics commercial feeding regimes to determine its potential for reducing methane emissions. There may be a need to place NOP within slow-release rumen capsules. With effective release of the NOP compound in the rumen throughout the day, the company believes methane emissions reduction of around 40% is likely.

There is no evidence NOP reduces feed intake. However, in one experiment (Reynolds *et al*. 2014) digestibility of organic matter tended to decline at the highest dose of 2.5 g/d. Another experiment showed an increase in body weight gain in lactating dairy cows with NOP, but this did not appear or was not measured in the other experiments. There was no effect of NOP on milk production, but milk protein content increased. An increase in energy available to the animal would be expected because hydrogen concentrations in the rumen are known to rise. The likely impact of trapping some of the energy saved from methane mitigation is discussed in Rumen metabolic pathway section below. NOP is likely to be available for all production situations because of the low dose rate required. An effective dose for cattle appears to be around 2 g/d and this amount could readily be provided in lick-blocks or other supplements for grazing animals.

3.7.2 Methane reduction potential

Based on the experiments conducted with animals to date, a reduction in methane emissions of 15% following supplementation with NOP is suggested. However, likely improvements in the method for reducing volatility and for feeding NOP suggest a 30% reduction in methane emissions could be achieved. The DSM company believes methane emissions savings from ruminants could be as high as 40%.

3.7.3 Ease and cost of implementation

NOP should be easily fed as a supplement to all ruminant classes because such a small amount of around 2g/d is required for cattle. However, if the volatility issues cannot be overcome, the compound may need to be incorporated into a slow-release intra-ruminal device that would need to be given to stock in a manner similar to drenching.

DSM has not yet divulged a price for NOP, but it is most likely to be based around expected impacts on high productivity dairy cows.

3.7.4 MACC analysis assumptions and predictions

MACC simulations were conducted for all ruminant classes assuming both a 15% and a 30% reduction in methane emissions. NOP was assumed not to affect feed intake or productivity. However, similar to other compounds that reduce methane emissions, it is probable that a proportion of the energy from saved methane would be used for productivity. The cost of NOP was assumed to be $0.25/day for cattle and $0.05/day for sheep.

Around 1.65 million tonnes of greenhouse gas equivalents were predicted to be saved across Australia when NOP reduced methane emissions by 30% and the process was adopted by 10% of ruminant industries (Table 6). A reduction in emissions of this order would represent a 3.5% decline in ruminant greenhouse gas emissions in Australia.

However, at the prices assumed for the cost of NOP, none of the scenarios predicted it would be profitable if energy from the methane saved was not used for animal productivity.

3.7.5 Additional research needed

The DSM company is likely to continue research into reducing volatility and improving feeding protocols for NOP. However, the company research is unlikely to evaluate the applicability of NOP for rangeland cattle and sheep. Since the majority of enteric greenhouse gasses emitted in Australia are from northern beef properties, evaluation of the potential and practicality of supplementing these animals with NOP would be valuable research for Australia, if DSM is willing to licence the product for these applications and if delivery constraints can be solved. If application to grazing ruminants appears feasible, dose response relationships will be needed for the development of ERF methodologies.

3.7.6 Priority for further investment

The priority for further investment is ranked medium for the initial evaluation of the effectiveness of NOP for reducing methane emissions in cattle and sheep fed typical Australian dry season and wet season pastures. Further investment would depend on the outcome from these experiments and may require the development of dose response curves for methane mitigation. These curves could then be used to develop appropriate ERF methodologies for different ruminant systems.

3.8 Biochar

3.8.1 Status of research

The effectiveness of biochar as a means for reducing methane emissions from ruminants was not studied within NLMP, but several *in vitro* fermentation assays with rumen fluid (Leng*et al.* 2012a, 2013; Hansen *et al*. 2012)and one experiment with cattle (Leng*et al.* 2012b) suggest there may be an opportunity for further research. The experiment with young cattle found that feeding 0.6% biochar increased growth rate by 25% and reduced methane emissions by 22%without affecting feed intake. The cattle were fed an unusual diet of dried cassava root chips and cassava foliage and the growth rate of the cattle was low at around 140 g/day. Nevertheless, the impact of biochar in this cattle experiment was larger than observed for the *in vitro* experiments, where the depression in methane emissions ranged from around 10-17%. Leng *et al*. (2013) showed that the reduction in methane emissions *in vitro* also varied with the type of biochar.

Biochar has a large surface area to weight ratio and is extremely porous. Leng*et al.* (2012b) postulates that this porous structure stimulates microbial colonisation and biofilm formation, which enhances microbial growth and increases VFA and protein supply to the animal. Methanogens are found on the outer surface of biofilms and are thought to remove H_2, which stimulates the digestion of cellulose and other feed compounds by maintaining a low hydrogen tension. The additional microbial growth and incorporation of H_2 into microbes may be one reason for the decrease in methane production and increase in growth rate. Biochar has also been shown to increase the ratio of methanotrophs to methanogens in rice paddy soils. If the same occurs on biochar in the rumen, the increase in methanotrophic, methane oxidizing organisms, would also reduce methane release and increase microbial growth. Because the original research was conducted with Asian cattle fed unconventional diets, its applicability to common forage, feedlot or dairy cow diets is unknown. Evidence from Leng *et al.* (2012b) suggests growth rate could be increased due to improved microbial growth.

3.8.2 Methane reduction potential

On the basis of the one cattle experiment and the *in vitro* experiments, it is assumed that biochar can reduce methane emissions by 15% for all ruminant classes. However, more experiments are required to verify these results.

3.8.3 Ease and cost of implementation

If biochar is effective in reducing methane emissions when supplemented at a rate of less than 1% of the diet, it should be easily provided to all classes of ruminant animals by incorporation into diets or blocks. The current price for biochar is around $400/tonne when supplied in large quantities. However, the source of biochar may influence its effectiveness as a methane mitigation agent in ruminant diets. Variation in biochar characteristics and selection of samples with the necessary specifications may alter the price of biochar suitable for feeding to ruminants for methane mitigation.

3.8.4 MACC analysis assumptions and predictions

The MACC simulations assumed a 15% reduction in methane emissions, and a 15% increase in growth rate or productivity. The cost of biochar when added at 0.6% of the diets was assumed to be 2cents/day for cattle and 0.5 cents/day for sheep.

When applied to all ruminant industries with a 10% adoption, the MACC analyses predicted greenhouse gas emissions from the Australian industries would be reduced by approximately 825,000 tonnes CO_{2e} annually (Table 6). In addition, because of the low cost and presumed productivity increase, the practice was predicted to be profitable for all ruminant industries simulated even when there was no price for carbon credits.

Great caution needs to be applied when interpreting the MACC analyses for biochar because the assumptions of a 15% increase in productivity and a low cost dominate the financial outcomes. These assumptions were made on the basis of a single experiment using uncommon cattle and diets. Nevertheless, the example demonstrates the importance on financial outcomes of improving productivity with a low cost treatment when reducing methane emissions.

3.8.5 Additional research needed

Research is needed to verify the results from the single animal experiment conducted. The research should first identify the impact of biochar characteristics on microbial populations and growth potential. This research may best be undertaken using *in vitro* rumen fermentation cultures. Once the specifications have been clarified for effective methane emissions mitigation, biochar should be provided in a dose response experiment to identify its effectiveness for reducing methane emissions and for stimulating productivity. These initial animal experiments may be best undertaken with sheep. If successful, further research with forage fed and feedlot cattle and dairy cows may be warranted. The research should strive to determine the reasons methane emissions are decreased and animal productivity increased.

The technical risk for research into biochar is initially high, but if the results obtained by Leng *et al*. (2012b) are verified, the technical risk diminishes, but the cost of the research will remain considerable.

3.8.6 Priority for further investment

The priority for further investment is ranked medium-high for the initial evaluation of the effectiveness of biochar based on the one experiment with cattle. However, it may best be considered as a component of the Rumen-metabolic pathway research project because of the potential of biochar to modify rumen function.

If biochar characteristics required for reducing methane emissions can be specified and biochar is effective when evaluated in sheep, further experimentation would be warranted.

3.8.7 Other supplements tested

Several additional supplements were examined within NLMP projects including almond hulls, citrus pulp, tea seed saponins, fermented red rice statins and wheat treated with a compound called bioprotect to reduce fermentation rate. None of these supplements showed substantial and prolonged methane mitigation potential or they had adverse effects on animal health. Further research with these supplements is considered unwarranted.

3.9 Forages

3.9.1 Leucaena

3.9.1.1 Status of research

Leucaena can be grown in the northern coastal environment and has the potential to be harvested then dried and made available for inclusion in mixed rations for feedlot cattle or other ruminants. Results from NLMP project 01200.035; B.CCH.6510, where cattle grazed either irrigated leucaena plantations or non-irrigated plantations planted in rows with Rhodes grass or naturalised pasture, respectively, showed a substantial increase in growth rate and a reduction in methane emitted compared with cattle grazing pasture alone. At Belmont, with irrigated leucaena and pasture, mean growth rate over the cattle growing period from 325 to approximately 600 kg liveweight, was 0.87 and 0.67 kg/day, respectively, for the leucaena based and Rhodes grass only pastures. The growth rate was approximately 23% faster for the cattle consuming leucaena (Figure 3). Average methane output (g/kg live weight gain) was 28% less for the leucaena group than for the Rhodes grass group (Figure 4). The mean digestibility of dry matter in the plant material eaten by the leucaena grazing cattle was estimated to be 61.5% and, for the Rhodes grass grazing cattle, 58.5%. Feed intake was estimated using a marker to be 8.7 kg/d for the cattle consuming Rhodes grass only and 7.6 kg/d for cattle consuming leucaena and Rhodes grass.

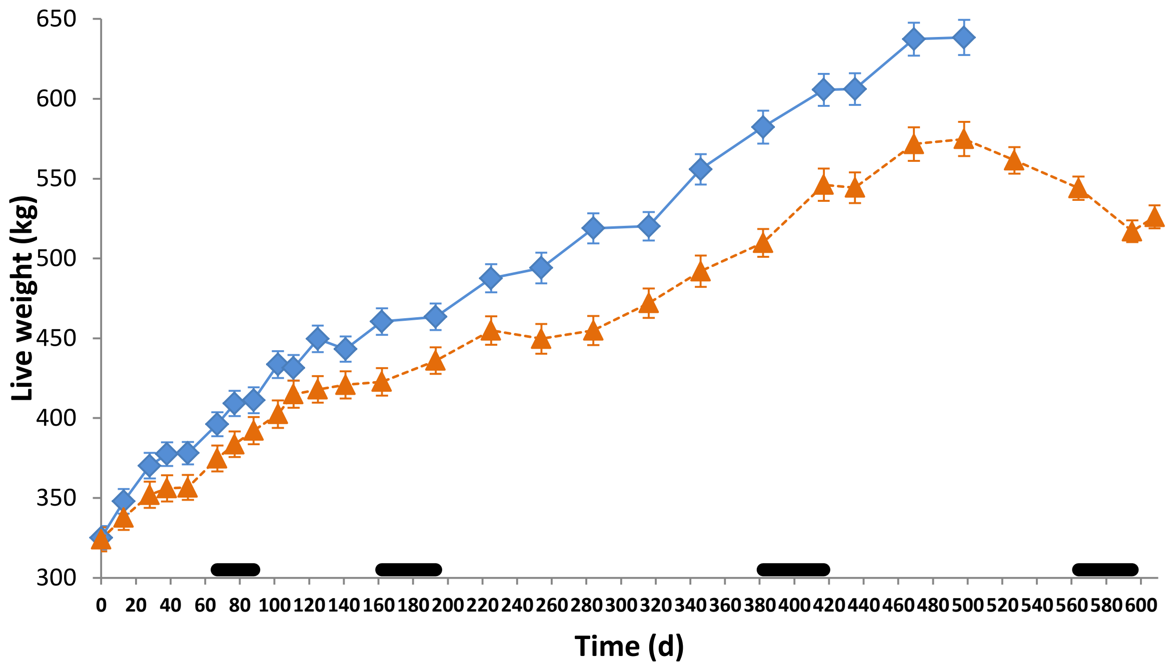


Figure 3. Average live weight of cattle grazing either irrigated leucaena-Rhodes grass pasture (◇) or irrigated Rhodes grass pasture (△). Dark lower bars indicate methane measurement periods.

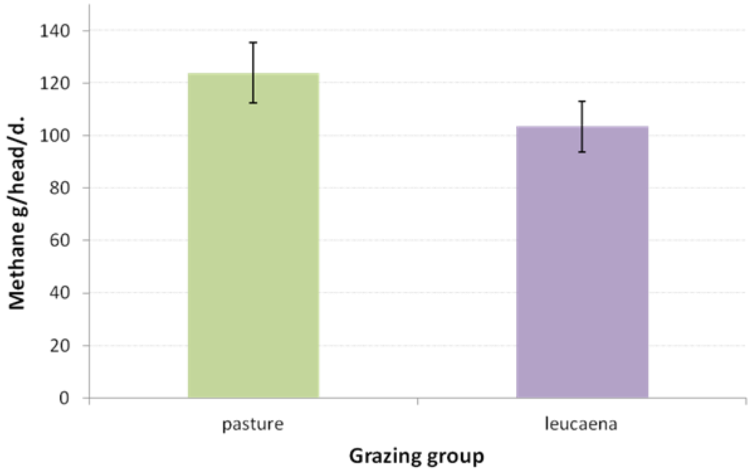


Figure 4. Methane emissions from cattle grazing Rhodes grass pasture only or Rhodes grass pasture with leucaena plantations.

Several leucaena growers in Queensland are considering harvesting leaf material from leucaena plantations, drying and pelleting the product for feeding to livestock, including feedlot cattle. Leucaena appears not to have been added to feedlot diets as a replacement for traditional protein sources and fibre. However, the harvesting and drying of the plant leaves and small stems may provide an alternative to silage or cotton seed. Freshly harvested leucaena has been fed at 22% and 44% with Rhodes grass and methane output measured from cattle in respiration chambers (Kennedy and Charmley, 2012). The experiment showed that methane emissions declined from 19.4 g/kg DMI to 17.8 g/kg DMI. Using these results and extrapolating to zero leucaena in the diet, methane emissions for any leucaena proportion in the diet could be calculated from the following equation derived from the Kennedy and Charmley (2012) experiment:

Methane emissions (g/kg DMI) = 21 - 0.07273 * leucaena % in diet

Feedlot nutrition consultant, Rob Lawrence, used the composition of the leucaena material collected by Kennedy and Charmley (2012) and shown in Table 2 to formulate a feedlot rations including leucaena.

Table 2. Composition of harvested leucaena based on Kennedy and Charmley (2012)

OM g/kg DM	NDF g/kg DM	ADF g/kg DM	ADL g/kg DM	N g/kg DM	NDF-N g/kg DM	C g/kg DM	GE MJ/kg DM	OMD %	RUP %
921	336	230	89	37.8	2.2	447	19.7	66	73

Feedlot ration calculations were based on tempered wheat (best reflects energy density of a number of grain processing methods with some moisture required). All rations include whole cottonseed as the cheapest form of protein and effective fibre, but also as a high energy source because of its lipid content. Roughage sources include corn silage and straw because leucaena leaves lack effective fibre. Leucaena NDF and ADF values are lower than whole cottonseed when consisting of mainly leaf and small particle size means effective fibre is limited to an assumed value of 5%. Crude protein was assumed to be 23.6% (3.78% Nitrogen x 6.25) and other nutrients were used within energy equations to reflect a similar gross energy value of 19.7 MJ/kg DM. These assumptions provided a digestible energy (DE) value of 14.7MJ/kg, a metabolisable energy (ME) value of 11.8MJ/kg, a net energy for growth (NEg) of 1.24Mcal/kg and a net energy for maintenance (NEm) of 1.88Mcal/kg. The net energy values were used in the feedlot cattle performance prediction.

Performance comparison based on a four ration finishing cattle for export (100DOF (days on feed), 340kg HSCW) scenario, based on the assumption that leucaena does not affect feed intake and an amount consumed of 11.4 kg/head/day. In the following Table 3, leucaena was balanced against silage and grain.

Table 3. Predicted effects of increasing leucaena inclusion in a feedlot diet on diet composition and animal performance.

	Leucaena Inclusion %			
	0	10	15	20
DM%	73.6	77.1	78.9	79.3
CP%	13.7	15.0	15.5	16.1
NEgMcal/kg	1.41	1.38	1.37	1.35
eNDF%	9.2	8.8	9.0	9.2
Daily gain kg/hd	2.0	1.94	1.91	1.88
FCE	5.72	5.89	5.96	6.08
DOF	100	103	104	106

The analysis suggests that growth rate would fall by 6% (2.0 to 1.88 kg/day) and days on feed (DOF) would be extended by 6 days if there were no positive effects of Leuceana on feed intake because the net energy content of the diet fell from 1.41 to 1.35 Mcal/kg with the inclusion of 20% Leuceana.

A 20% inclusion of leucaena leaf and small stem in a feedlot diet based on the results from Kennedy and Charmley (2012) is predicted to reduce methane emissions by 7% at the same feed intake. The effect of drying leucaena on its ability to reduce methane emissions from ruminants is unknown. An experiment adding dried leucaena to a feedlot diet is required to evaluate the accuracy of the above assumptions, particularly that feed intake will be reduced when the energy density of the diet is lowered with the inclusion of leucaena.

3.9.1.2 Methane reduction potential

The leucaena grazing experiments suggest that methane emissions are reduced by around 28% and growth rate increased by 23%. (A more recent reanalysis of the Open path laser measured methane results from project 01200.035; B.CCH.6510, suggest that methane emissions may be closer to 20% reduction, rather than the initial value of 28%.) Results from Kennedy and Charmley (2012) indicate that 20% inclusion of leucaena in feedlot cattle diets would reduce methane emissions by 7%. There is also a carbon sequestration in soil resulting from leucaena plantations. Estimates as high as 37.4 t C/year have been made for this sequestration (Conrad, 2014), but these high values have been queried by others (pers com).

3.9.1.3 Ease and cost of implementation

The leucaena plants grazed at both sites in the NLMP project were established stands at the full production rate. Normally full grazing cannot commence until 18 months to 2 years after establishment. The cost of establishing leucaena is $250-$350/ha if undertaken by the farmer and $450/ha when established under contract. The agronomy for leucaena planting and establishment is well understood.

3.9.1.4 MACC analysis assumptions and predictions

Leucaena applications are applied only to Northern Coastal Beef and Feedlots industry sectors. Methane emissions were assumed to decrease by 28% for cattle grazing leucaena planted pastures and growth rate increased by 23 %. For feedlot cattle, methane emissions were assumed to decrease by 7%, but growth rate was also depressed by 6% when 20% leucaena was added to the diets.

When applied to the northern coastal region with a 10% adoption, the MACC analyses predicted greenhouse gas emissions from the region’s cattle would be reduced by approximately 112,000 tonnes annually (Table 6). This is equivalent to approximately 7.5% of greenhouse emissions from cattle in the region. Use of leucaena proved to be profitable over a twenty year period without a payment for carbon credits, because of the increase in animal productivity.

Feeding leucaena to feedlot cattle with the assumptions made for the MACC analysis, resulted in a reduction of about 10,000 tonnes annually of greenhouse gas CO_{2e} with a 10% adoption rate (Table 6). However, because it was assumed energy intake and growth rate would be depressed by the inclusion of leucaena in feedlot diets, the practice was not profitable.

3.9.1.5 Additional research needed

Although there is good evidence that feeding leucaena increases productivity and reduces methane emissions from cattle (Kennedy and Charmley 2012; project 01200.035; B.CCH.6510), there has been insufficient research to provide an accurate algorithm for predicting the response in methane emissions reduction and performance improvement for different proportions of leucaena in the diet of cattle. This relationship is needed for development of an ERF methodology for producers to claim carbon credits when adopting a leucaena feeding system. The leucaena dose response experiments could also be used to verify the near infrared (NIR) calibrations that have been developed for predicting the proportion of leucaena in the diet of an animal based on a scan of faeces (Coates and Dixon 2007).

Research is also needed to demonstrate whether the methane mitigation properties of leucaena leaves remain when they are dried. In addition, further research including dried leucaena in feedlot diets is needed to determine whether the assumption from the feedlot nutrition model used in the analyses described where feed intake was decreased are indeed accurate, and the likely commercial viability of this as a feed component.

The cost of the proposed research would be modest.

3.9.1.6 Priority for further investment

The priority for further investment is ranked high for the leucaena dose response experiment because this will allow development of an ERF methodology and provide additional revenue for those producers adopting leucaena feeding practices. Methane emissions from cattle would likely be measured using respiration chambers. The experiments would also be used to verify NIR calibrations for predicting the proportion to leucaena in the diets of animals for application of the ERF methodology. The technical risk of such an experiment is low.

Further research into the possible role for leucaena in feedlot diets has medium priority as it depends on farmers with leucaena plantations establishing a harvesting and drying process that would produce sufficient feed needed by feedlots.

3.10 Temperate legumes

3.10.1 Status of research

Legumes are important components of pastures for stock across Australia. They provide nitrogen through rhizobial bacteria for associated plants in the pasture mix, frequently have higher digestibility and protein content than associated grasses and some can provide biomass growth at times when other plants have senesced, for example deep rooted lucerne. Cattle and sheep grazing legumes often produce less methane than those grazing grasses (Archimede *et al*. 2011), which may reflect lower concentrations of indigestible fibre and relatively higher digestibility of legume forage compared to grass herbage. The lower fibre content can result in increased rate of passage of digesta, which is known to reduce methane emissions per unit of energy digested (Janssen 2010). Furthermore, some legume species have high biomass production, and with their greater digestibility and higher intake than grasses lead to faster turnoff of stock. This results in less fodder energy being used for maintenance and therefore lowers methane emission per unit of livestock product (methane intensity).

The NLMP project 01200.042; B.CCH.6540, FtRG 2 project, B.CCH.7510, and the earlier RELRP project, B.CCH.1067, have measured biomass growth throughout the year as well as digestibility and methane emission potential determined by laboratory assays for over 150 accessions of southern Australian grasses, legumes and herbs. Several temperate legumes showing different capacity for biomass production and/or methane mitigation potential have been fed to sheep within these projects either at pasture or in pens with hand-cut fresh or dried forage and methane emissions have been measured.

The laboratory *in vitro* assays showed consistently that the annual legume *Biserrulapelecinus* (Biserrula) and the perennial legumes *Dorycniumhirsutum* (Hairy Canary clover) and *Trifolium pratense* (red clover) consistently produced less methane than other plant species examined. Neither Biserrula nor red clover appeared to reduce microbial activity *in vitro* and Biserrula decreased the ratio of acetate:propionate within the fermentation suggesting more efficient use of digested energy by animals. Biserrula produced about 80-85% less methane per unit of dry matter incubated than the other annual legumes assayed, whereas red clover produced around 40% less methane than the most commonly used perennial legume, lucerne. Although Canary clover reduced methane emissions by approximately 45% compared with other perennial legumes assayed, it reduced microbial activity and produced less total gas and VFA than average. Canary clover also had a low biomass yield and poor digestibility and is unlikely to be a practical option for reducing methane emissions on farms. On the contrary, Biserrula has a biomass yield and digestibility near average for the annual legumes, whereas red clover biomass yield and digestibility were higher than average for the perennial legumes examined.

Methane emissions from red clover were not measured in animals in experiments associated with NLMP experiments, but Kasuya and Takahashi (2010) observed that cows consuming red clover silage had lower methane emissions than cows offered Timothy hay silage. However, three experiments have been conducted in the Australian projects where Biserrula has been fed to sheep and methane emissions compared with other grasses and legumes.

In one experiment (project B.CCH.7510), sheep in pens were offered *ad libitum* freshly cut monocultures of annual ryegrass (*Loliumperenne* cv. Robust), bladder clover (*Trifolium spumosum*); subterranean clover (*Trifolium subterraneum*), French serradella (*Ornothopus sativas*) or biserrula (*Biserrula pelecinus*). The *in vitro* fermentation assays suggested methane emissions would be high for the bladder clover, medium for subterranean clover and serradella and low for biserrula. Methane emissions measured in respiration chambers were about half for the sheep fed biserrula than for the sheep fed the other legume species. The methane emissions from sheep offered ryegrass were intermediate between the legume extremes. A second experiment in the same project involved grazing sheep on monocultures of vegetative stage annual ryegrass; subterranean clover; a choice between plots of ryegrass or subterrnaena clover; serradella; or biserrula. Methane emissions were measured using portable accumulation chambers. Methane emissions from the sheep were approximately 10% less for those animals grazing biserrula and ryegrass pastures than those in the other treatments. The third experiment (project 01200.042; B.CCH.6540) involved feeding biserrula (B) or serradella(S) hay to sheep in pens and measuring methane emissions in respiration chambers. There were five treatments: 100% S; 75% S - 25% B; 50% S - 50% B; 25% S - 75% B or 100% B. Digestibility of organic matter and intake of metabolisable energy were significantly greater for sheep fed biserrula than for the sheep fed serradella. There was a small linear depression in methane emissions per unit of metabolisable energy with increasing proportions of biserrula in the diet. The response in methane emissions reduction due to biserula was less than those experiments where fresh vegetative biserrula was fed to sheep. Similarly, *in vitro* studies in project B.CCH.6540 have shown a smaller impact on methane emissions reduction with dried biserrula than with fresh or freeze dried samples.

Lucerne accessions examined in project B.CCH.6540 generally had the highest biomass yields for all the perennial legume species examined, although *in vitro* digestibility was around average and methane emissions were higher than average. An earlier project (B.CCH.1067) investigating the effect of lucerne cultivars and individual plants on methane emissions measured *in vitro* found large differences between individual plants. A few plants from the Aurora accession were found to have high total gas production, suggesting high energy availability, but low methane emissions. These results suggest that lucerne may be used for improving animal productivity through its high biomass production thereby reducing methane emissions intensity, but plants may be available for breeding to also reduce methane emissions from future cultivars

3.10.2 Methane reduction potential

The experimental results outlined above suggest that a methane mitigation potential of 10% would be reasonable for swards of biserrula when mixed with other species such as subterranean clover. There is evidence of wide variability between individual luncerne plants in digestibility, but variation in other characteristics such as methane emitting capacity has not been investigated. Including subterranean clover in the pasture mix would boost biomass production, animal performance and reduce methane emissions intensity. Depending on the legume chosen and the time of year when it is producing green herbage, it is possible the inclusion of legumes could improve animal productivity by up to 30%.

3.10.3 Ease and cost of implementation

Legumes are frequently included in pasture mixes and adding cultivars with lower methane potential should not increase costs.

3.10.4 MACC analysis assumptions and predictions

The MACC analyses for other legumes (not reported by Cotter *et al*. 2015) were applied across all sectors of the ruminant industries assuming a 10% reduction in methane emissions and a 10% improvement in productivity.

With these assumptions and a 10% adoption rate, the analyses suggest that approximately 1.2 m tonnes of greenhouse gas CO_{2e} would be saved nationally (Table 6). However, considerable effort is needed to find suitable legume species for northern Australia in areas where leucaena cannot be grown.

3.10.5 Additional research needed

Investment is required to take further to fruition results from the project identifying red clover and biserrula as potential methods for reducing methane emissions while improving animal productivity. Experiments with animals are needed to evaluate the use of red-clover as an option for reducing methane emissions. An agronomic assessment is then needed to determine the regions where introducing red clover may be an appropriate farm practice. Experiments investigating the impact of red clover consumption by ruminants on methane emissions and productivity could be undertaken and the identity of specific phenolic compounds determined using the assays identified in NLMP project 01200.007; B.CCH.6410.

A breeding program is needed for biserrula to improve its growth performance, improve its early growth palatability and remove its propensity to cause photosensitivity in sheep, while maintaining its ability to reduce methane emissions.

A breeding program investigating the possibility of selecting lucerne plants or accessions with low methane emissions potential would assist in reducing greenhouse gas production through both improved animal performance and lower emissions per unit of feed intake.

Chicory, although not a legume, fills a similar role as lucerne and has a real potential as a valuable feed supply, particularly over winter and summer feed-gap periods and could assist farmers in southern Australia adapt to anticipated changes in climate. Investment is required for a substantial plant breeding program to exploit the variability that has been identified within this project and allow the development of persistent cultivars with growth characteristics suitable for filling identified annual feed gaps and maintaining high nutritional quality.

3.10.6 Priority for further investment

The priority for further investment is ranked medium based on the potential to increase animal productivity while reducing methane emission intensity.

3.11 Australian native shrubs

3.11.1 Status of research

Introducing rows of Australian native shrubs with inter-row pasture species which show antimethanogenic properties has been shown to be an effective method for reducing methane emissions from sheep in south-west Australia, while increasing productivity. The autumn feed-gap period with low availability of poor quality, senesced pasture is a major limitation to sheep productivity in the region. Traditionally, sheep are offered supplementary grain during this period of the year, which is expensive to supply and labour-intensive to feed. Many Australian native shrubs grow well in this region and provide relatively high quality feed, with high protein content, particularly when consumed with senesced pasture (Revell*et al*. 2013).

The NLMP project 01200.020; B.CCH.6520 has demonstrated that strip-grazing sheep on native shrubs with preserved inter-row pasture species for 6-8 weeks in autumn substantially increased the growth rate and condition of sheep and eliminated to need for supplementary feeding. There were two shrub treatments. One involved a selection of shrubs that had been shown through laboratory fermentation assays to produce lower rates of methane emissions than the other group of shrubs that generally produced more biomass. The inter-row pasture species used was a mixture of Biserrula and subterranean clover. The Biserrula was chosen because it produced lower methane emissions than many other plant species when evaluated with the laboratory fermentation assay.

Methane emissions expressed as g/MJ of metabolisable energy intake were shown to be 26% lower for the sheep grazing the shrub treatments than for sheep grazing pasture alone and receiving a grain supplement. Although the methane output was approximately 5% lower for the antimethanogenic shrubs, the differences were not significant. Previous research (Mayberry *et al*. 2009) found that the more productive *Atriplex* saltbush species produced more methane per unit of organic matter than when sheep were given a more conventional diet.

The2014 experiment also showed that average growth rate for sheep grazing the shrub plots was 142 g/day compared with 69 g/day for the pasture plus grain treatments. When averaged over the year, the use of shrubs during an eight week autumn period would substantially increase the weight and body condition of sheep. Although the shrubs were largely denuded of leaves during grazing, their biomass readily regenerated during the remainder of the year to develop a sustainable once-yearly shrub grazing system. A further advantage from the shrub grazing system will occur when the grazing period can be extended past the autumn-break rainfall event and new season pasture is allowed to become established before being grazed. This practice would greatly improve total pasture growth rate and the availability of feed over the winter period.

Simulation modelling has predicted the introduction of shrubs with inter-row pasture species increases whole farm profitability by an average of 24% when occupying an optimal 10% of the farm area (Monjardino*et al*. 2010, 2012). The greatest impact occurred when the shrub resource and pasture was grazed during the autumn feed-gap period through reducing costs of supplementary feeding, deferment of the use of other feed resources and allowing higher stocking rate and improved animal performance.

3.11.2 Methane reduction potential

Results from the NLMP project 01200.020; B.CCH.6520, 2014 experiment showed that average methane emissions per unit of metabolisable energy consumed were 26% lower for sheep grazing the shrub treatments than for sheep grazing pasture alone. When a reduction in methane emissions of this magnitude is assumed to occur over an eight week period of the year, annual methane emissions (intensity) would be reduced by approximately 4% annually for farmers adopting the autumn shrub grazing systems.

3.11.3 Ease and cost of implementation

Estimates from the Future Farming Industries, Enrich program, suggest that 500 to 1500 shrubs are required per hectare with each plant costing around $0.30. Weed and vermin control is generally necessary during the establishment period. Estimates of total cost for establishment range from $250 to $450/ha depending on whether the planting is done by farm staff or by contractors (Emms and Revell 2014).

Depending on the time of establishment and rainfall during late winter-spring, a short period of four weeks grazing would normally be expected in the first autumn following establishment. Grazing for 6-8weeks should then be possible in subsequent years through to a life expectancy of a plantation of approximately 15 years.

3.11.4 MACC analysis assumptions and predictions

MACC analyses were not conducted for the shrub-inter-row pasture scenario. However, by assuming 4% annual methane mitigation and applying results from other marginal abatement cost curve (MACC) analyses, considering the number of sheep in areas where the procedure would be applicable and assuming a 10% adoption of the practice, it is estimated that approximately 12,300 tonnes of greenhouse gas CO_{2e} would be saved annually across Australia.

3.11.5 Additional research needed

Little further research is needed to prove the benefits from growing rows of Australian native shrubs with inter-row pastures for grazing during the autumn feed-gap period to reduce methane emission intensity, improve animal productivity and farm profitability. However, funds are required to ensure adoption of the practice across appropriate regions of south west Australia.

Application of whole farm simulation models appears to be the most appropriate method for assessing the benefits for individual farms and determining the site and area for planting. Further investment is needed to more effectively represent the physiology of shrub growth within the current models used by farm advisors. The models also need modification to allow for reductions in methane emissions when this is known to occur through the consumption of specific plant species.

Other areas of research that could be investigated in relation to native shrubs include: quantifying below ground carbon sequestration; water and nutrient utilisation; the effect of second grazing on productivity and grazing antimethanogenic species in the inter-rows in Spring.

3.11.6 Priority for further investment

The priority for further investment is ranked low in relation to additional research to prove the value of shrubs to farming systems in south-west Australia.

However, along with the effective use of data generated from other NLMP projects, application of results to individual farms would be enhanced with an upgrade of whole farm models to better represent the growth characteristics of native Australian shrubs.

3.12 Rumen-metabolic pathways

Research in two projects with NLMP (01200.038; B.CCH.6610: 01200.059; B.CCH.6620) and one within FtRG2 (B.CCH.7610) have advanced greatly understanding of the organisms responsible for methane formation and the metabolic pathways involved in nutrient digestion and methane synthesis. The research has identified a new methylotropic methanogenic *Archaea* species with an as yet undiscovered biochemical pathway that produces methane from preformed plant methyl groups. The efficiency of this pathway for methane production is four times greater than the normal methanogen pathways with less loss of hydrogen. Research into rumen function has been instrumental in identifying the relative contributions of individual metabolic pathways within the rumen and identifying possible methods for manipulating these pathways. In addition, the research has identified a range of surface proteins that are responsible for metal transition into *Archaea* but which are not found in rumen bacteria or other organisms within the rumen. These surface proteins are ideal candidates for epitopes that could be used as antigens for developing vaccines against rumen methanogenic organisms. Figure 5 illustrates an overview of the organisms and pathways responsible for hydrogen production and utilisation as well as the synthesis of methane.

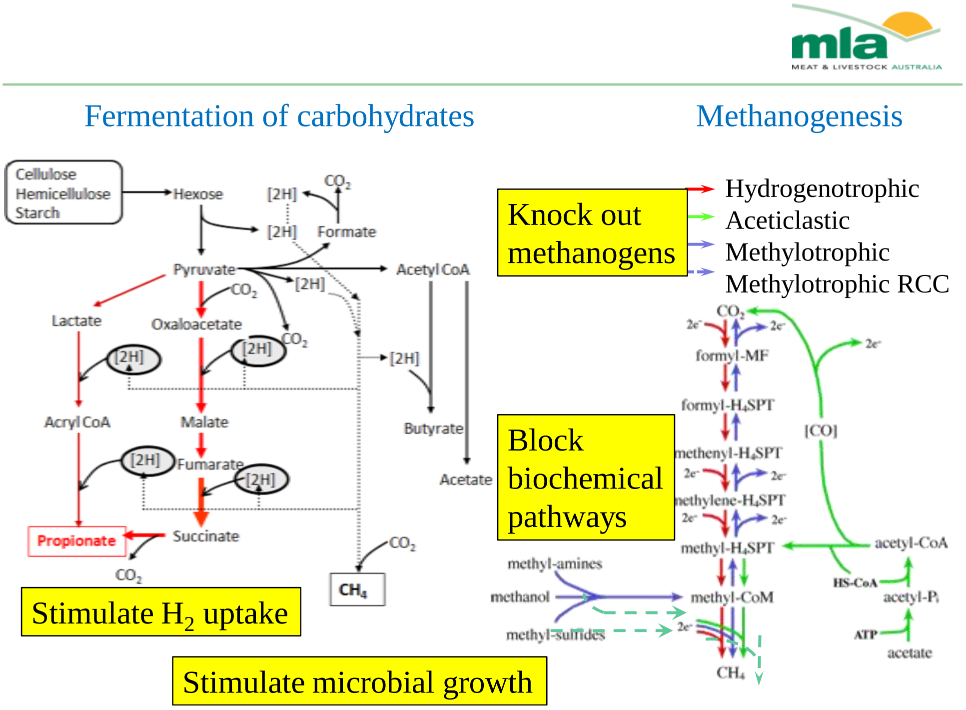


Figure 5. Outline of the organisms and biochemical pathways involved in the fermentation of dietary substrates (left side) and for the formation of methane. Opportunities for manipulating methane production are outlined in yellow.

3.12.1 Energy capture from saved methane

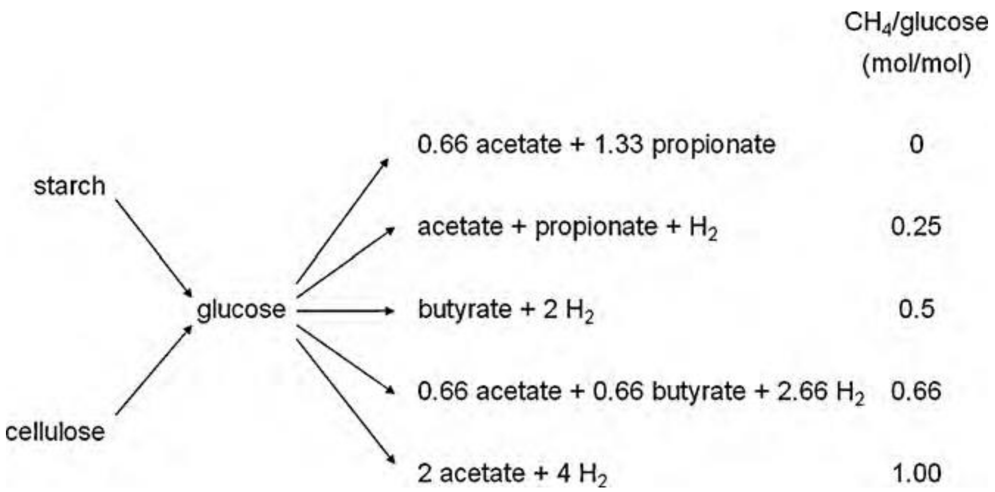
3.12.1.1 Status of research

Although a previous analysis by McSweeney and McCrabb (2002) of experiments in which methane had been inhibited suggested that the efficiency of feed use was increased by approximately 10%, there appears not to be an accepted value in the literature for the amount of energy saved through methane mitigation that is used for animal productive functions. Hence, a calculation has been undertaken within the MLA NLMP coordination project (01200.075) to estimate from first principles an estimate of the amount of methane energy saved by inhibiting methane emissions that could be retained for animal productive purposes. Estimates of potential impacts on live weight gain and marginal profitability have also been made assuming different proportions of energy are captured for animal productive purposes within MACC analyses for vaccination and algae scenarios. These estimates from the MACC analyses provide an initial indication of the potential for saved methane energy to contribute to the financial viability of various methane mitigation practices and highlight the importance of developing practices that increase the proportion of feed energy that can be used for productivity.

3.12.1.2 Methane reduction potential

The calculations were based on knowledge of control by rumen hydrogen concentration of the relative rates of the five pathways available within the rumen for the conversion of glucose from either starch or cellulose fermentation by rumen microbes to volatile fatty acids (Janssen 2010). The assumptions, set out below, suggest that around 35-40% of the energy not lost in methane could be used by the animal.

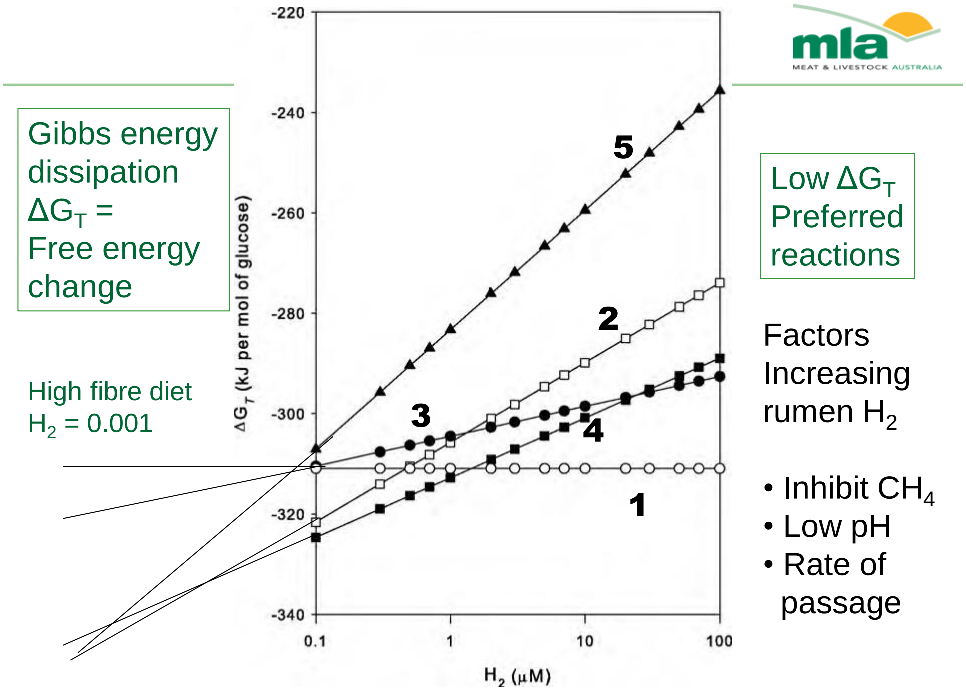
The primary carbohydrates fermented in the rumen by micro-organisms are starch and cellulose. Both these compounds consist of chains of glucose molecules linked either by 1-4 α bonds in the case of starch or 1-4 β bonds for cellulose. Hence, fermentation of either starch or cellulose produces glucose as the primary substrate for micro-organisms to use within the rumen of animals. Glucose can be degraded by five competing pathways to produce volatile fatty acids (VFA). These pathways produce different amounts of methane and have different efficiencies of energy conversion from glucose to VFA as follows:



The reactions are numbered 1-5 from the highest to lowest efficiency. The bottom pathway (5) produces the most methane and has an efficiency of conversion of glucose energy to VFA energy of 62% compared with the top pathway (1) which produces no methane and has a efficiency of conversion of energy of 93%. Clearly, the more energy that passes through the top pathway, the lower the methane production and the higher the efficiency of energy use by the animal. Understanding the importance of these competing pathways for converting dietary energy into substrates that can be used by the animal, provides a great opportunity to investigate ways for manipulating rumen metabolism to ensure priority for the high energy yielding pathways.

Glucose energy capture (%)	
93	1
86	2
78	3
72	4
62	5

Two major factors drive the competition between these competing biochemical pathways; i) Gibbs energy dissipation or free energy change with lower free energy pathways being preferred; and ii) the relative Michaelis Menten kinetics of the reactions, particularly the relative km values. Janssen (2010) shows that the Gibbs energy dissipation of the five reactions change with hydrogen concentration in the rumen. The hydrogen concentration in the rumen increases with certain methane inhibition practices, such as algae, chloroform, bromochloromethane (BCM), NOP; low pH due to grain feeding; and high rate of passage of digesta. The relative competitiveness of the five reactions change as rumen H_2 concentration changes as illustrated below where the free energy change of reaction 1 is not affected by hydrogen concentration, but it is markedly reduced in pathway 5:



Pathways 4 and 5, which produce large amounts of methane at low efficiency of dietary energy conversion dominate when hydrogen concentration is low as with a high fibre diet, but the energetically more efficient pathways that produce less methane tend to be dominant at high rumen hydrogen concentrations.

Accounting for the effects of rumen hydrogen concentration on the relative activity of the five pathways based in Gibbs free energy change alone proved to be insufficient to predict the changes observed in methane emissions and VFA ratios when algae was fed to sheep (Tomkins unpublished) or BCM fed to goats (Mitsumori *et al*. 2012). There appears to be no information in the literature on the relative km values for these five reactions. Consequently, the relative rates of these reactions were further altered on a trial and error basis until the approximate reductions in methane and changes in VFA patterns observed when algae were fed to sheep and BCM fed to goats were predicted. When this occurred, the relative rates of the above five reactions were such that approximately 40% of the energy in glucose was retained in VFA.

This approach is still an over-simplification because the effect of microbial growth as a hydrogen sink and as a supply of energy and protein to the animal was not taken into account. However, the calculations do provide a logical way for suggesting the possible amount of energy saved from methane mitigation practices that may be used for animal productive purposes. Further mechanistic rumen simulation modelling is required to better account for the changes in these reaction rates and the contribution microbial growth may make to the nutrients available to animals when various methane mitigation practices are adopted.

Research within the FtRG2 project, B.CCH.7610, has shown that when methane emissions are reduced by intra-ruminal chloroform, hydrogen concentration in the rumen increases and hydrogen is captured in VFA and in additional microbial growth. Nevertheless, there was still hydrogen from saved methane emissions unaccounted for by these processes. This experiment showed that much of the additional hydrogen could be captured if a hydrogen accepting nutrient, phloroglucanol, was fed to the animals. Phloroglucanol accepts hydrogen in the synthesis of acetate, thereby providing more energy-containing substrates to the animal. There are also organisms in the rumen known as acetogens, which are normally at low concentrations. The acetogens can utilise carbon dioxide and hydrogen to also synthesise acetic acid. Identification of ways to increase the activity of acetogens within the rumen should allow capture of excess hydrogen for animal productive purposes. Furthermore, it is known that the rumen also contains low concentrations of a group of organisms called methanotrophs. These organisms breakdown methane and utilise the energy for their growth. Again, little is known about these organisms and the conditions within the rumen that alter their opportunity for growth.

In summary, several opportunities for manipulating rumen function to greatly improve the capture of energy from fermented dietary ingredients, to reduce the loss of energy through methane and capture that energy for use by animals have been identified. However, additional research is required to evaluate the hypotheses and develop practical methods for capturing additional energy under production systems.

3.12.1.3 Ease and cost of implementation

Although several opportunities for capturing more energy from the rumen fermentation process and energy from methane saved through mitigation practices have been identified, none are at a stage where they could be applied within the animal industries. The cost for implementing future possible methods for manipulating rumen function is unknown at this time.

3.12.1.4 MACC analysis assumptions and predictions

MACC analyses were used to demonstrate the critical importance of capturing the energy from methane not released due to various mitigation practices on animal productivity and enterprise profitability. These analyses predicted the effects of 0, 20, 40 or 80% of the energy saved from reducing methane emissions being used for productive purposes. The National Greenhouse Gas Inventory calculations for cattle were used to convert the saved energy into live weight gain (Cotter *et al*. 2015). The MACC analyses were conducted for antimethanogen vaccination and for red algae supplementation as mitigation methods with 30% and 60% reduction in methane emissions, respectively. The effect on predicted live weight gain and profit per head of capturing different proportions of energy from the 60% saving in methane emissions when red algae is added to the diet for the northern coastal beef scenario is illustrated in Table 4. The price of algae was assumed to be $1.50/kg and a carbon credit price of $14/tonne was used for this analysis.

Table 4. Predicted effect of capturing increasing proportions of the energy in methane from the 60% reduction in methane emissions resulting from the inclusion of algae in diets for the northern coastal beef scenario in the MACC analysis with algae costing $1.50/kg and the price of carbon at $14/tonne.

Methane energy captured (%)	Productivity gain (Live weigh, g/day)	Profit ($/head)
0	0	- 41.17
20	22	2.97
40	44	35.24
80	89	111.65

This MACC analysis demonstrates the significance of improving animal productivity on enterprise profit and highlights the potential gain for producers if methods can be found to manipulate rumen function to capture more energy for animal productive functions.

3.12.1.5 Additional research needed

Additional research is required to test many hypotheses about ways for capturing additional energy within the rumen for animal production purposes. The research will increase the fundamental understanding of ways to manipulate the microbes and desired biochemical pathways within the rumen. This process would be greatly enhanced if concepts developed during the process were evaluated quantitatively through computer simulation modelling.

The research needed would be relatively long term, first testing hypotheses and then developing practical ways to achieve the improvements in animal performance on farm.

3.12.1.6 Priority for further investment

The priority for further investment is ranked high for improving the understanding of rumen function and manipulating biochemical pathways to capture the most energy possible from fermentation of nutrients within the rumen. The research will be expensive and long-term, the technical risk is high but the potential rewards are high. Fundamental research of the type proposed often leads to the development of entirely new technologies. Past history from all industries shows that all major advancements in productivity come from new technologies developed from fundamental scientific research.

3.13 Methane vaccination

3.13.1 Status of research

Vaccination against rumen *Archaea* offers farmers potentially a low-cost option to reduce methane emissions in sheep and cattle. If successful, this option is particularly attractive because, under a best case scenario, it would require only one or two treatments in young animals for a lifetime effect. Research within the NLMP project 01200.038; B.CCH.6610 is directed partially towards identifying cell surface proteins that are unique to methanogens and do not occur on other microbes within the rumen. These specific surface proteins could be ideal for a vaccine target. Similar research is being undertaken in an associated project (B.CCH.2056) which is identifying possible peptide motifs from *Archaea* phages. However, no research within NLMP has been directed towards the development of vaccines.

Results from previous research suggest there is potential to reduce methane emissions by vaccination against a mixture of methanogenic organisms within the rumen. A7.7% reduction in methane emissions has been reported following use of a crude vaccine which did not include all *Archaea* genetic lines(Wright *et al*. 2004b). More recently, Wedlock *et al*. (2013) suggested that a 20% reduction in methane emissions is highly probable when the 'entire genetic repertoire' of *Archaea* is examined to identify motifs common to all Archaea but not to rumen bacteria. Research is currently being conducted within New Zealand to identify possible antigens and develop a vaccine.

Wright *et al*. (2004b)measured feed intake in vaccinated and non-vaccinated sheep for 5 days prior to methane measurements in chambers and found no effect of vaccination on feed intake. It is considered unlikely that vaccination against rumen methanogenic organisms will have a negative effect on intake. However, it would be expected that hydrogen concentrations within the rumen would rise as a result of vaccination and that a proportion of the energy saved from reducing methane emissions would be captured for animal productive purposes.

3.13.2 Methane reduction potential

Results from previous research and current projections of possible New Zealand research outcomes suggest that an effective vaccine should reduce methane emissions by around 15%.

3.13.3 Ease and cost of implementation

Many domestic animals already receive vaccinations for disease prevention and adding antigens for methane mitigation should be simple and cheap.

3.13.4 MACC analysis assumptions and predictions

Two MACC simulations were conducted assuming either 10% or 20% reduction in methane emissions. The simulations were across all ruminant production systems. The cost of the vaccinations was assumed to be $4.50 for cattle and $2.00 for sheep. The MACC analyses, assuming a 10% adoption of the practice estimated that approximately 1.32 million tonnes of greenhouse gas CO_{2e} would be saved annually across Australia, representing approximately 2.4% of Australia's emission from ruminant livestock.

3.13.5 Additional research needed

The New Zealand research group (Wedlock *et al*., 2013) is attempting to identify antigens by bioinformatics analysis of genomes from the most rumen-abundant methanogens and first test these against cultured methanogens. If successful, the antigens will be combined with selected adjuvants for testing in animals. Progress with the research is being held closely, but a decision point about identifying antigens and adjuvants is proposed for June 2015. The NLMP project (01200.038; B.CCH.6610) and associated project have identified by different and similar means to the New Zealand team a range of *Archaea* bacteriophage peptides and surface peptides unique to *Archaea*. These peptides may potentially be valuable antigens for developing vaccines and need be first tested with cultured organisms. MLA instigated a review (B.CCH.2088) to identify an approach to vaccine development that may be successful in providing high concentrations of IgA antibodies in the rumen. The recommended approach is based on using synthesised virus-like particles incorporating the target antigens. These particles lack viral genome, but have high immunogenic competence similar to intact viruses. The review also recommended the site of immunisation be through the sub-lingual salivary gland to enhance the production of secretory-IgA antibodies into the rumen.

The most appropriate way forward would be to collaborate with the New Zealand team. However, discussions with the group have not resulted in the desire for them to collaborate. The route being taken by the New Zealand team is more conventional, so there may be an argument that a competitive approach will lead to the evaluation of alternative practices to broaden the chances of success.

3.13.6 Priority for further investment

The priority for further investment is ranked medium-high because of the technical risk of developing a vaccine that produces sufficient intra-ruminal IgA antibodies and the large investment required. Nevertheless, if a vaccine could be successfully developed, the ease of implementation is high and the cost relatively low.

4 Best Management Practices

4.1 Status of research

A great deal of research has been conducted in the past that has allowed development of on-farm practices to improve reproductive performance and efficiency of feed utilisation. The focus of any management practice aimed at reducing methane emissions is to reduce the amount of feed that is used for maintaining the animal, thus allowing more for productive purposes. The major contributor to Australian enteric greenhouse gas emissions are breeding animals. Improving reproductive performance, reducing the proportion of reproducing animals in a herd or flock and increasing growth rate of animals for sale will reduce the total amount of feed eaten by the herd that is used for animal maintenance. Although total methane emissions may increase if improving the efficiency of feed use results in a larger animal carrying capacity, methane intensity or the amount emitted per unit of saleable product will be reduced.

The factors limiting uptake by producers of management practices that are known to increase the efficiency of feed use for productive functions are a complex combination of practice-specific and producer-specific characteristics, including relative advantage, complexity, compatibility with current farming practices and risk.

4.2 Methane reduction potential

The methane mitigation potential from adopting best management practices to increase the proportion of feed used to produce saleable products will generally be relatively small as shown by the MACC analyses for beef cattle and sheep at less than 5% of total methane intensity, but there are exceptions such as the 14.8% reduction predicted for supplementation of phosphorus to cattle in northern Australia.

4.3 Ease and cost of implementation

Some best management practices may be simple to implement and others difficult. The cost of change within an individual enterprise can be significant in producer time, operational and financial changes including changes to the farming system. However, some changes may not involve additional time or financial inputs, other than closer management of stock and resources.

4.4 MACC analysis assumptions and predictions

Simulation of best management practices within MACC was done through changes in stock numbers, breeds, size of reproducing animals and timing of management practices such as mating and weaning or through the provision of supplements at strategic times. The methane savings were calculated using the National Greenhouse Gas Inventory in relation to herd or flock structure and animal productivity expressed as growth rate, milk of wool yield.

The MACC analyses for increasing adoption of Best Management Practices within the beef industry were predicted to save approximately 290,000 tonnes of greenhouse gas CO_{2e} if adopted by 10% of the industry (Table 6). This represents only about 0.5% of total greenhouse gas emissions from Australian ruminant livestock annually. However, the practice proved to be highly profitable because they generally result in large increase in sale of animals.

4.5 Additional research needed

No additional research is recommended because the approach is focussed on adopting practices known to increase enterprise feed conversion efficiency .

4.6 Priority for further investment

The priority for further research investment is ranked low, because best management practices revolve around adopting practices based on current knowledge.

5 Other NLMP project outcomes

Several NLMP projects have produced information or technologies that have arisen during the course of the research and although not directly related to methane mitigation, have the potential to be used to improve the efficiency of animal production or can be applied for use in humans. Three of these research spinoffs are described.

5.1 NIR calibrations of forage quality

5.1.1 Status of research

In NLMP project 01200.42; B.CCH.6540, over 1000 samples from the different forage species and maturities were analysed in the laboratory for estimated dry matter digestibility in sheep, total nitrogen, neutral detergent fibre, acid detergent fibre and organic matter content. Dried and ground material from these samples were scanned using near infra-red spectroscopy (NIR) technology to develop prediction calibrations. The calibrations, established using results from all forage species and growth stages and referred to as 'global' calibrations, were extremely robust and regarded by NIR specialists to be either very good or excellent for predicting values of unknown samples. Despite being global calibrations, the predicted values for different plant groups, such as annual legumes or perennial grasses. were extremely accurately, with R^2 values of regressions between measured and predicted values being above 0.95 for all forage classes except perennial grasses. The calibrations were also shown to be robust and accurate for predicting dry matter digestibility at different times in the season for individual plant species.

5.1.2 Additional research needed

The NIR calibrations developed in the project are robust, but the forages used were from a relatively restricted environment. Forages from many other southern Australian environments are needed for validation and extension of the calibrations. In addition, the calibrations have been developed for dried and ground forage samples. The need to prepare the samples before scanning substantially reduces the practicability of widespread use for predicting forage quality on farm. Recent developments in the application of NIR technology on farms has resulted in handheld NIR instruments that can be used in the field. Data generated from these instruments can be relayed directly through telegraphic or satellite systems to a 'master' instrument anywhere in the world and predicted results returned in real-time. Such instruments would provide landholders with immediate information needed to make management decisions about pasture and stock management. However, for such a system to be applicable on farm, the NIR scans must be made on forage in the field. Funds are needed to build on the NIR calibrations developed in this project and adapt them for use real-time in the field with fresh forage.

5.1.3 Priority for further investment

The priority for further investment is ranked high, but it is important to identify a commercial partner to ensure that there is a company who can help with development of the handheld instrument and the web-based real-time scan and prediction transfer.

Preliminary discussions have been held with a commercial company in UK who is interested in investing in the area. An R&D proposal is in progress for developing a joint program with several commercial organisations including pasture seed companies and consultant groups, research organisations and the UK company.

5.2 Exploitation of ELLE database

5.2.1 Status of research

The ELLE project (01200.042; B.CCH.6540) has quantified the seasonal growth patterns, digestibility and nutrient composition of 154 accessions from 109 species of annual and perennial grasses, legumes and herbs that can be grown in southern Australia. Many of the plant samples collected have been used to assess their likely methane emission potential through the use of laboratory fermentation assays. The research has resulted in development of the most extensive database yet created of information on Australian forage plants. The database can be used by landholders and their advisors to identify species or accessions best suited to increase forage supply and quality at different times of the year for specific regions. The database is also extremely valuable for plant breeders to identify the range in trait variability for potential new plant breeding programs. Even greater use of the project data could be made if it were used to provide the basis for predicting growth and composition of individual forage species within whole farm simulation models.

5.2.2 Additional research needed

For the forage growth and quality information collected in project (01200.042; B.CCH.6540) to have greatest impact on farm decision making, it should be integrated into a whole farm prediction model(s). The project provides excellent data for inclusion in existing plant physiological models through development of algorithms that allow the prediction throughout the year of the growth, composition and nutritional value for animals of all of the plant species and cultivars examined. A robust model for each plant species can be achieved because the first year plants were grown without water stress and under high fertility conditions to provide information needed to predict optimum plant performance. Results from the subsequent years with 16 species can then be used to test the model in terms of the accuracy of prediction for actual productivity and composition of these plants under different environments. Given that this prediction methodology proves successful, the models can then be used by agronomists and others to identify the best candidate species (perhaps cultivars/varieties) of plants for a specific niche (e.g. to fill a needs gap within an environment to meet the needs of individual land managers). In this respect, the information collected for a wide range of the plants will enable each to be considered for particular niches, the most important of which in southern Australia are winter and the late summer-early winter gaps. The size of the investment would be considerable and would require close integration of model developers with plant physiologists.

5.2.3 Priority for further investment

The priority for further investment is ranked high because incorporating information on selected individual plant species from the ELLE database into whole farm simulation models will make the process of identifying the best forages for specific regions and farms more precise.

5.3 Intra-ruminal device for measuring methane emissions

5.3.1 Status of research

The project, in combination with project 01200.006; B.CCH.6220, aimed to develop an intra-ruminal device that can be administered to an animal by mouth and measures remotely methane and other gases produced from the rumen of animals, unimpeded in any environment. All other methods for measuring methane emissions from animals are either inappropriate in production agriculture or require disruption to normal animal routines. If successful, the intra-ruminal device would play an important role in evaluating the effectiveness of potential methane inhibiting practices and verifying ERF methodologies. The principle for the device is based on encapsulating miniature gas sensors within a hollow polymer case that has a permeable membrane to allow diffusion of gases produced in the rumen. The concentration of the gases (methane, carbon dioxide and hydrogen) are measured by sensors within the capsule and the data are either stored for later downloading or transmitted from the animal electronically using G3-G4 communications technology.

The project involved a great deal of technical expertise and innovation to allow the device to operate within the rumen of an animal and to transmit information to external monitors, including:

- Development of specific membranes, using nano-particles of silver and graphene, that are permeable to selected gases of interest, while excluding other potentially corrosive gases like hydrogen sulphide, and reduce the formation of microbial colonies which interfere with gas permeability over time,
- Selection and adaptation of commercial gas monitors for miniaturisation and operation within the capsule,
- Inclusion of temperature and pressure monitors within the capsule because both variables affected gas measurements,
- Development of sophisticated electronic circuit boards and software to coordinate operations, optimise timing of gas and other measurements, and for the storage and transmission of data,
- Design of the capsule and its seals to persist within the harsh environment of the rumen for long periods of time, with the inclusion of pressure release valves,
- Extending the life of the power source to approximately one month, first by identifying and programming gas measurements in relation to temperature and pressure changes to provide optimal data with minimal power use and, secondly, by identifying a new source of lithium-ion batteries with greater power storage capacity,
- Development of an electronic ear-tag for receiving data from within the rumen and facilitating transmission to a remote computer system,
- Miniaturisation of the devise to be suitable for oral application to sheep,

A number of prototypes were developed during the project, with each prototype being superior to the previous one. Experiments confirmed that intra-ruminal device measurements closely followed the concentrations of gases measured in the rumen headspace of animals. The device also revealed a different pattern of methane and hydrogen gas concentrations in the rumen associated with specific methane mitigation practices such as administration of chloroform or alterations to the composition of the diet or amount fed. The current prototype successfully stores data from the sensors within the capsule, but the transmission of data to a remote recorder appears to be limited to a range of around 3 km.

Several limitations remain with the current capsule prototype for determining methane emissions from sheep or cattle. The concentration of an individual gas within the rumen depends on the relative rate of production of all gases. Methane emission rate is known to increase after sheep are fed a meal, but the concentration of methane within the rumen was shown to fall. The measured depression in methane concentration occurred because the rate of production of carbon dioxide was relatively greater than the rate of production of methane. Although, for individual animals there were associations between methane concentration within the rumen and methane production measured in respiration chambers, there was large between animal variation and little overall correlation. These observations confirm that the intra-ruminal device alone cannot be used to quantitatively predict methane (or hydrogen) production rate from animals. Measurement of a marker gas released at a constant rate in the rumen will be needed to estimate accurately methane production from individual animals. Provision of a marker gas will require further research to identify the best gas, ways for its constant release and inclusion of a monitor specific for that gas.

Another limitation to the current prototype is that, although the sensors and system can operate for up to one month in air, the conditions within the rumen are such that the sensors appear to be failing after about 10 days. The reason for this failure is suspected to be related to moisture and/or volatile compounds permeating the membranes and affecting the sensors. Further research would be needed to fully understand the reasons for sensor failure and identification of a remedy.

Despite these limitations, the device has several valuable applications. It is a simple and accurate way to measure hydrogen concentration in the rumen of animals and hydrogen concentration is known to have a major influence on specific biochemical pathways during feed digestion affecting energy capture for animals and methane production. Through monitoring temperature and pressure within the rumen, it could be used as a simple way of measuring grazing behaviour, eating and ruminating patterns for research. The device has high longevity in air and could be used to measure cheaply gas concentrations in respiration chambers, portable accumulation chambers. However, the most likely application of the principles developed within this and its associated project, 01200.047; B.CCH.6220, is for monitoring changes in gas concentrations along the gastrointestinal tract of humans. The shorter passage time of digesta of less than two days means that issues relating to longevity of the device are not of concern.

5.3.2 Additional research needed

Further research is required if the device is to be used to measure gas production rates from animals, rather than concentrations of gases within the rumen. The research involves: i) inclusion of a sensor to measure the concentrations of a marker gas released at a constant rate within the rumen; and ii) enhancement of the longevity of the gas sensors when immersed in the rumen through the introduction of scrubbers or other means to remove moisture and potential volatile compounds. However, before this research is undertaken, decisions are needed about a potential commercialisation pathway given potential animal welfare concerns relating to the lithium-ion batteries that power the device remaining in animals for life and capsule recovery and disposal at abattoirs.

These issues are not of concern when the device is used for monitoring human health and further research, in collaboration with a commercialising partner, would be desirable to evaluate the value of the device in clinical medicine.

5.3.3 Priority for further investment

The priority for further investment is ranked low because of the relatively high technical risk in being able to introduce a marker gas monitoring system and the negative impacts of the rumen environment on gas sensors. There are also potential animal welfare concerns with lithium-ion batteries remaining in the rumen for the lifetime of the animal and capsule recovery and disposal at abattoirs.

6 Needs and gaps assessment

The status of current research, methane mitigation potential, likely productivity improvement, barriers to and cost of implementation, and possible financial returns for producers have been considered for several potential mitigation practices. The predicted outcomes from the MACC analyses for various practices for a northern coastal beef enterprise with assumed carbon credit prices of $0, $14 or $50 per tonne are shown in Table 5 and illustrated in Figure 6. Predictions for other ruminant enterprises show similar trends (Cotter *et al*. 2015). Aggregation of predicted greenhouse gas CO_{2e} savings across all Australian ruminant industries is shown in Table 6 for the potential mitigation practices examined when 10% adoption is assumed for the appropriate practices for different sectors.

Table 5. Investment and emissions predictions from the MACC analysis of a northern coastal beef property (Cotter *et al*. 2015)

	Production Efficiency	Phosphorus Supplementation	Genetics*	Vaccination 10% reduction in methane	Vaccination 20% reduction in methane	Leucaena	Algae 30% reduction in methane emissions	Algae 60% reduction in methane emissions	Plant bioactives	Nitrates	NOP 15% reduction in methane emissions	NOP 30% reduction in methane emissions	Biochar
Project Life (years)	1	1	1	1	1	20	1	1	1	1	1	1	1
Emissions Savings (tonnes C02e)	81.7	117.6	89.8	116.7	233.4	327.0	350.0	700.1	292.0	76.0	175.0	350.0	175.0
Marginal Profit / Loss at a Carbon Price of $0	$86,640	$33,852	$0	-$3,249	-$3,249	$2,770	-$39,530	-$39,530	-$131,765	-$32,851	-$65,883	-$65,883	$22,629
Carbon Credits at a Carbon Price of $14	$1,143	$947	$1,258	$1,634	$3,268	$4,578	$4,900	$9,801	$4,088	$1,064	$2,450	$4,900	$2,450
Carbon Credits at a Carbon Price of $50	$4,084	$3,382	$4,492	$5,835	$11,670	$16,350	$17,500	$35,005	$14,600	$3,800	$8,750	$17,500	$8,750
Marginal Profit / Loss at a Carbon Price of $14	$87,783	$34,799	$1,258	-$1,615	$19	$7,348	-$34,630	-$29,728	-$127,677	-$31,787	-$63,433	-$60,983	$25,079
Marginal Profit / Loss at a Carbon Price of $50	$90,724	$37,234	$4,492	$2,586	$8,421	$19,120	-$22,030	-$4,525	-$117,165	-$29,051	-$57,133	-$48,383	$31,379
*Simulation for 1 year after 20 years of accumulation of genetic gain. Same for all except dairy													

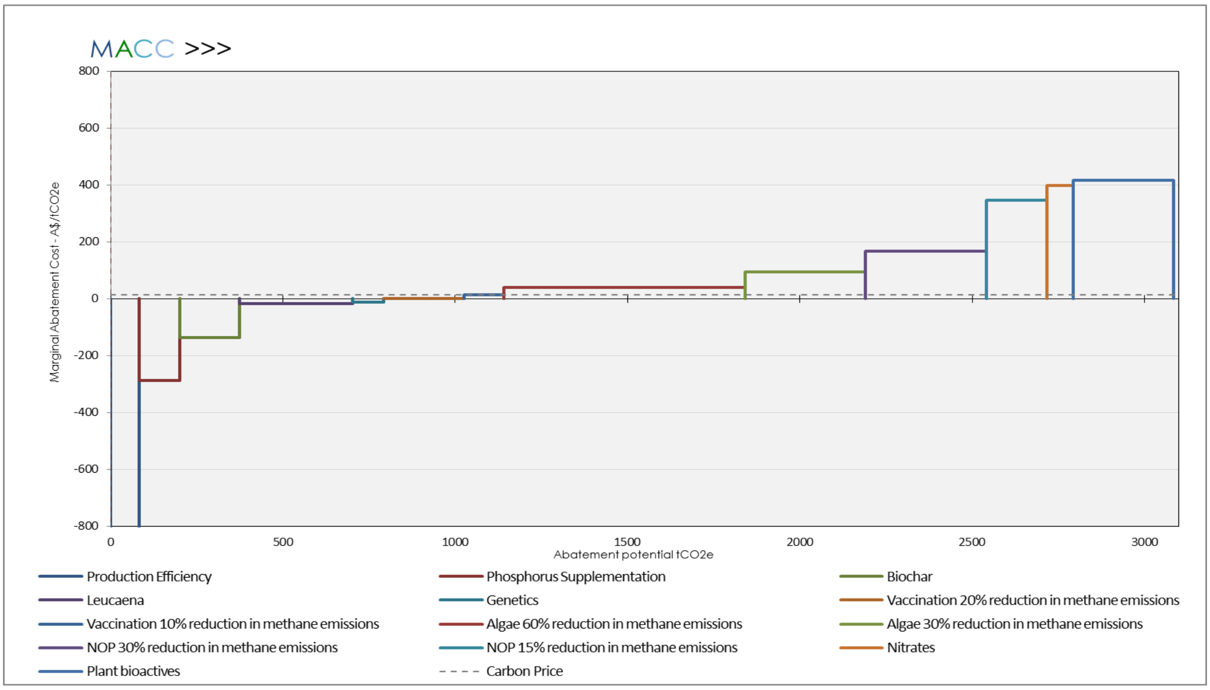


Figure 6. Illustration of the results in Table 5 showing the marginal abatement cost (negative values are profit) on the Y axis and the amount of methane mitigated on the X axis for each potential methane mitigation practice considered in the MACC analyses (Cotter *et al*. 2015).

The MACC analyses showed clearly that the greatest economic benefits come from those methane mitigation practices which increase animal productivity and have low costs for implementation. The price paid for carbon credits has an impact on profitability, but the effect is generally smaller than the impact of a practice on animal productivity. However, the predictions from the MACC analyses depend greatly on the assumptions made and the conclusions are influenced by veracity of these assumptions. Many of the assumptions have strong experimental support, but others are based on limited information and require additional research.

Table 6. Potential national emissions savings (CO_2 equivalent tonnes) from MACC analyses assuming 10% adoption. Values for other legumes, shrubs and energy capture were based on relativity to other MACC analyses with assumed similar responses.

Practice Option	Beef	Sheep	Dairy	Feedlot Beef	Total	% of National Total
Production efficiency	286,675				286,675	2.21
Phosphorus Supplementation	415,768				415,768	3.21
Genetics Beef/sheep	366,326	85,275		10,428	487,407	3.56
Genetics dairy			25,378			0.20
Wheat feeding			51,273		51,273	0.40
Grape marc		100,048	31,722	13,543	145,313	1.12
Vaccination 20% reduction in methane emissions	1,041,216	221,201	31,733	27,085	1,321,235	10.19
Leucaena	103,178			9,480	112,658	0.87
Other legumes	830,066	306.178	120,398		1,256,642	9.69
Native shrubs		12,310			12,310	0.09
Algae 60% reduction in methane emissions	2,456,753	663,565	95,166	81,256	3,296,740	25.42
Plant bioactives	1,023,764	276,653	39,652	33,857	1,373,926	10.59
Nitrates	266,121	65,012	23,791	8,803	363,727	2.80
NOP 30% reduction in methane emissions	1,228,522	332,013	47,583	40,628	1,648,746	12.71
Biochar	614,243	166,026	23,792	20,314	824,375	6.36
Energy capture	1,023,764	276,653	39,652	33,857	1,373,926	10.59
National Total					**12,970,721**	

With the assumptions made, BMP options tended to show greater expected financial returns than the majority of the direct mitigation practices. These options have relatively low methane mitigation potential, but result in improvements in animal productivity and have relatively low costs for implementation. The analyses indicated that genetic selection, biochar, leucaena plantations for northern coastal beef, grape marc when offered to sheep for maintenance feeding and for dairy cows when replacing an ingredient of equivalent energy content, and vaccinations are also potentially profitable. These direct methane mitigation practices showing potential profitability generally had low costs for implementation and/or resulted in assumed substantial gains in productivity. Many of the most effective practices for reducing methane were assumed to have high costs of implementation such as NOP, feeding algae or plant bioactive compounds and were not predicted to be profitable unless either energy from mitigated methane was used for productive purposes or the price of carbon was high.

6.1 Prioritising future research investment

When assessing priorities for future investment in alterative methane mitigation practices, the following issues have been considered:

- i) the methane mitigation potential at the animal level
- ii) the methane mitigation estimates at the national level;
- iii) the effect of the practice on animal productivity;
- iv) the barriers to and cost of implementation; and
- v) the technical and commercial risk and cost of the additional research needed
- vi) the likely time to first practical implementation.

Several outcomes from the NLMP projects were identified that have potential opportunities to improve productivity for producers without a direct effect on methane emissions including the development of NIR calibrations for assessing the nutritional quality of forages, and use of the database created within project 01200.042; B.CCH.6540 for inclusion in simulation models for improving pasture species selection and farm productivity.

The NLMP management team has scored each potential methane mitigation practice and other NLMP outcomes for the criteria outlined above (where relevant) to develop a relative priority for future investment (Table 7).The scores were based on information provided in the text for each scenario. The values used for methane mitigation potential are described when discussing each scenario. The values for productivity gains are either outlined when discussion each scenario or based on estimates of methane mitigation with 40% of the energy saved being used for productive purposes in proportion to figures given in Table 4. Other scores are subjective and based on the discussion about implementation, technical risks in achieving research objectives, costs of additional research and likely time to first application of a technology on farm. These assessments were used to place a relative priority on areas for future research. The major factor(s) limiting implementation of each practice or successful research outcome(s) is outlined for each scenario in Table 8.

Irrespective of the rank score given in Table 7, there are several areas of research that if undertaken soon is likely to be of significant benefit to specific industries. These include:

- Wheat feeding to dairy cows at pasture: specifying the quality of wheat needed for the methane mitigation effects; developing best management practices for introducing wheat feeding at high rates; drafting an ERF methodology,
- Leucaena: dose response curve for methane mitigation and verification of faecal NIR for predicting proportion of leucaena in the diet; final draft of an ERF methodology,
- Nitrate: evaluation of the effectiveness of providing with nitrates antioxidants and N-acetylcysteine to reduce the rise in methaemoglobin,
- Bioactives: evaluate compounds C, L and G, extracted from Australian native plants for antimethanogenic effects in sheep,
- Grape marc-tannins: apply the new tannin analytical techniques to other animal feed ingredients known to impact on animal productivity across domestic animal species;
- Shrubs: incorporate shrubs effectively into plant physiological simulation models for effective use in whole farm economic analyses.

Table 7. Assessment of future research priorities. Methane mitigation potential is derived from the analysis of each scenario above and other criteria are scored on a relative basis either from 0-50 (0 low-50 high) or as a rank from low to high.

Scenario	Animal methane mitigation potential (%)	National methane mitigation potential[a] (% total)	Productivity gain (%)	Barrier/cost to implement[e] (Relative score)	Technical risk/cost research[e] (Relative score)	Investment priority (Relative value: subjective NLMP managers)
Genetics B/S[i]	6	3.5	0.8	20	20	Low
Genetics dairy	12	0.2	2	15	15	Medium
Grape marc	10	1.1	0	10	5	Low
Algae	60	25.4	8[b]	15	25	High
Nitrate	6	2.8	0	50	10	Low
Wheat feeding	40	0.4	20	30	5	Medium
Plant bioactives	25	10.6	3.5[b]	15	30	High
NOP	35	12.7	4[b]	15	10	Medium
Biochar	15	6.4	15	15	30	M-H
Leucaena	28	0.9	20	20	5	High
Other legumes	10	9.7[c]	10	10	20	Medium
Native shrubs	4	0.1	5	15	5	Low
Energy capture	25	10.6[d]	18[f]	35	40	High
Vaccination	15	10.2	2[b]	5	50	M-H
BMP[g]	3	5.2	20	20	10	Low
NIR forages	NA	NA	NA	5	5	High
ELLE database	NA	NA	NA	10	20	M-H
IRD[h]	NA	NA	NA	40	40	Low

[a]Calculated from Table 6, with energy capture added across all regions; [b]Assumed a proportion of energy from reduced methane emission used for productivity; [c]Assume applied to greater area than other scenarios; [d]Based on similar results for plant bioactives and added to Table 6 before calculation; [e]Partial explanation of scores in Table 8; [f]Assumed 80% methane mitigation energy saved based on table 4; [g]Best Management Practices; [h]Intra-ruminal device for measuring methane emissions, [i]Beef and Sheep.

Table 8. Key factor limiting implementation of practice or successful research outcomes

Scenario	Key limiting factors
Genetics	Difficulty and cost of measuring methane emission from sires in studs; lack of direct benefit for breeders and commercial producers
Grape marc	Cost of transport and need to ensile grape marc on farm
Algae	Likely cost of commercial production and difficulty in supplying at a cost to users of <$1.50/kg
Nitrate	Reduced productivity and high risk of nitrite poisoning
Wheat feeding	Risk of rumen acidosis affecting animal health and specification of wheat quality
Plant bioactives	Uncertainty about the cost of the products and risks associated with research outcomes
NOP	Uncertainty about the cost effectiveness for grazing ruminants because the price will be set for dairy cows
Biochar	Uncertainty about the specifications of biochar, its availability and efficacy in normal ruminant systems
Leucaena	High cost of establishment and influences of drought
Other legumes	Limited methane mitigation potential; requires targeting species with high tannin contents
Native shrubs	Limited methane mitigation potential, shrub establishment costs and controlled feeding systems
Energy capture	Theoretical potential very high, but products and implementation procedures depend on successful research
Vaccination	Readily implemented, but technically difficult and expensive research
BMP	Little research needed, implementation costs often high and adoption low
NIR forages	Requires financial input from commercialising company and additional research
ELLE database	Requires significant funds for incorporating data into plant models and use in on-farm strategic models; uncertainty of benefits
IRD	Technical risk of failure high under commercial conditions, potential animal welfare issues

Bubble graphs are presented in Figures 7-9 summarising information from Table 7. The size of the bubble is a reflection of the estimate of likely costs of research.

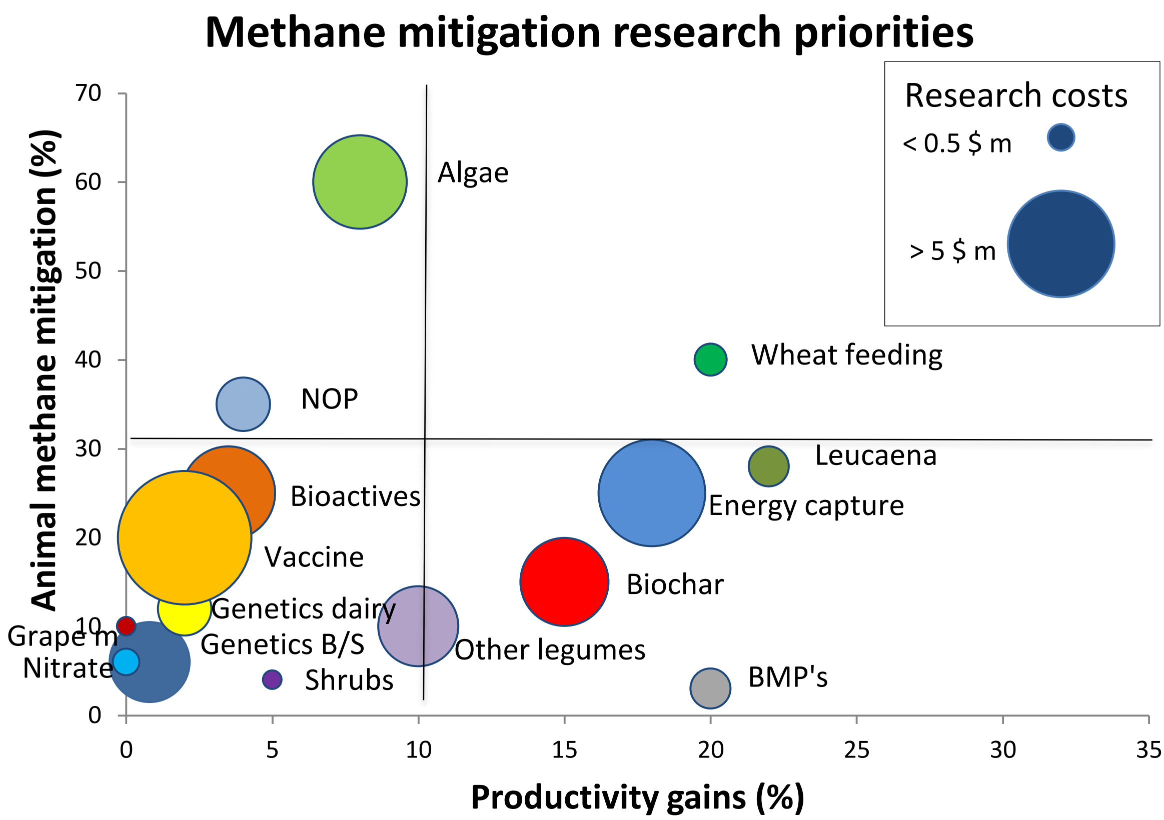


Figure 7. Relationship between the methane mitigation potential in individual animals and estimated productivity gain for a range of methane mitigation practices examined. The size of the bubble-dot represents a relative estimate of the likely cost and risk of further research required. The highest priority practices would be in the upper right segment. The lines are the objectives MLA set for achievement. Genetics B/S is for beef and sheep.

The results in Figure 7 show, based on the assumptions used, that algae, feeding 9 kg wheat daily to dairy cows at pasture, supplementing with NOP or introducing Leuceana plantations reduces methane emissions from individual animals more than the other scenarios. Supplementing animals with nitrates when replacing urea results in a small reduction in methane emissions with no effect on productivity. BMP's generally result in large productivity gains but with limited reduction in methane emissions. If the rumen could be manipulated to capture more energy, the effects on productivity could be large while the changes in fermentation pathways within the rumen would reduce the production of methane. However, more research is required to develop sufficient understanding of ways to manipulate rumen function that could lead to the development of a product for use on farms.

Figure 8 shows a similar relationship to Figure 7 but the percentage national methane mitigation from all practices is shown on the Y axis. This figure shows that whereas wheat feeding to dairy cows and leucaena plantations were highly effective for reducing methane from individual animals, on a national basis the methane mitigation potential of these practices is small because of the limited number of animals for which the treatments are effective. However, algae, NOP, plant bioactives, energy capture and methane vaccination remain nationally effective methods for reducing methane emissions because they are presumed to be applicable to most ruminant production systems. However, a number of these practices require a substantial amount of additional research before effective practical methods could be developed or to reduce the cost to levels that make widespread application profitable.

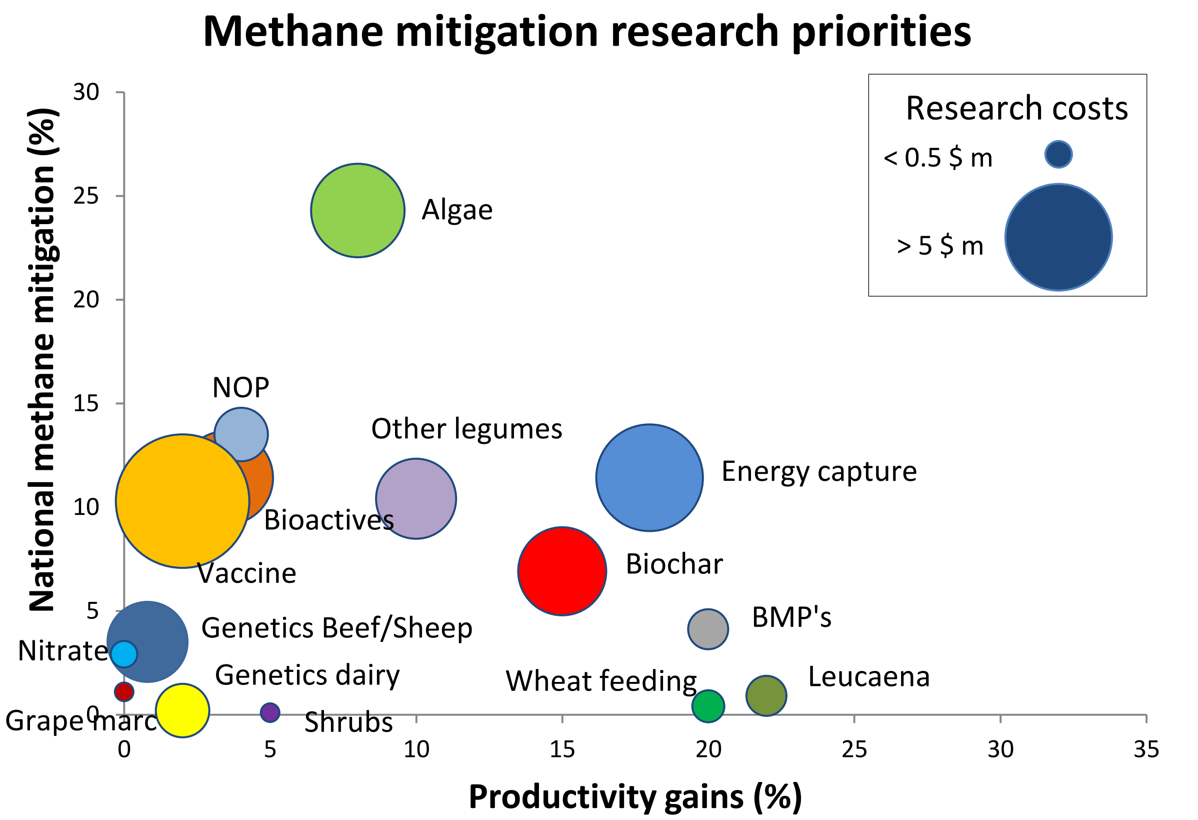


Figure 8. Relationship between the methane mitigation potential for all ruminant industries in Australia, expressed as a percentage of the potential total mitigation from all practices considered (12.97 m tonnes annually), and likely productivity gain for a range of methane mitigation practices. The size of the bubble-dot represents a relative estimate of the likely cost and risk of further research required. The highest priority practices would be in the upper right segment.

Figure 9 shows the relationship between the methane mitigation potential for individual animals and the magnitude of the likely barriers to implementation. The most desirable practices would lie in the upper left sector and include algae, NOP and wheat feeding to dairy cows at pasture. However, the latter does have risks associated with ruminal acidosis and the need to specify the quality of the wheat being fed. The analysis based on barriers to implementation makes vaccination a more attractive option, but a great deal more research is required until an effective product is developed.

6.2 Methane mitigation research outcomes, time to implementation and research needs

An estimate has been made of the likely time to first implementation on farm after considering the additional research needed for the potential methane mitigation practices examined. There are several practices with sufficiently well advanced knowledge to allow immediate implementation on farms. These include leucaena plantations in the northern coastal beef region, grape marc as a supplement for low quality feed during the southern summer-autumn feed-gap period, substitution of nitrate for urea in diets or lick-blocks, introduction of other legumes and/or Australian native shrubs and implementation of best management practices. Leucaena stands out from these practices because it substantially reduces methane emissions and increases animal productivity. Although these practices are ready for adoption, their methane mitigation effect on either individual animals (Figure 10) or nationally (Figure 11) is relatively low. No additional research is considered necessary for grape marc or BMPs. Research could be undertaken with nitrates to test hypotheses relating to reducing the formation of methaemoglobin or increasing its reconversion to haemoglobin. However, the mitigation potential of nitrates is low compared with other practices, risks to animal health are high and additional research is judged to be of low priority. Although sufficient is known about the agronomy and feeding of leucaena for it to be adopted, additional research is required to determine dose response curves with methane emissions and animal productivity to allow development of an ERF methodology. The methodology is required before producers can be paid for reducing greenhouse gas emissions.

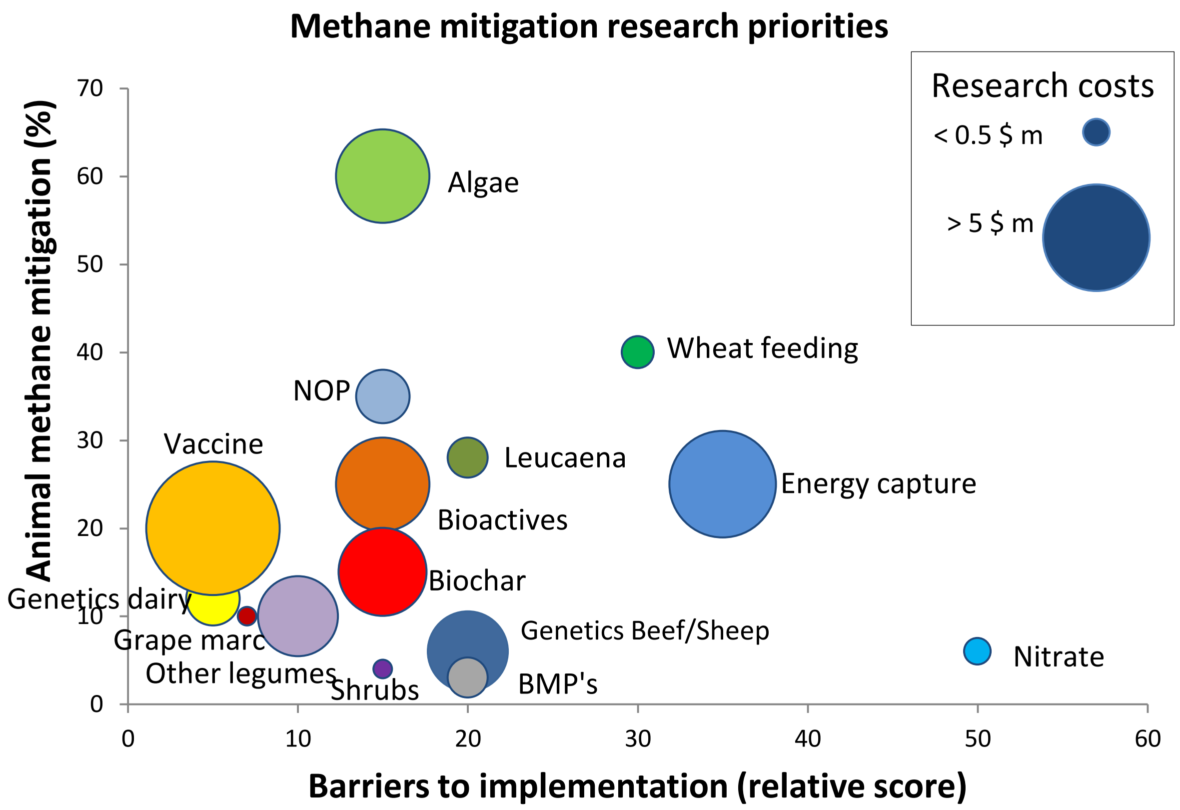


Figure 9. Relationship between the methane mitigation potential in individual animals and likely barriers to implementation for a range of methane mitigation practices. The size of the bubble-dot represents a relative estimate of the likely cost and risk of further research required. The highest priority practices are in the upper left segment.

Other practices such as feeding large quantities of wheat to dairy cows grazing pasture and NOP require a modest amount of additional research. This research should be achievable within approximately 2 years. A clearer definition of the quality of wheat needed for effective methane mitigation and determination of appropriate methods of feeding wheat to minimise the risk of acidosis are required before wheat feeding can be safely implemented. Further investment on wheat feeding is likely to be determined by the dairy industry funding groups, because the methane mitigation potential nationally is small (Figure 11).

Research is needed to evaluate the effectiveness of NOP within Australia for grazing ruminants. Other issues relating to NOP volatility and methods for effective feeding are expected to be solved by the commercialising company. Final adoption of NOP by Australian producers will depend primarily on the price, but also on practical feeding methods.

Although methane production Breeding Values can now be included in the beef cattle BREEDPLAN software and can be used with $index software to select the most profitable sires, the high cost and difficulty in measuring methane emissions on a large number of sires or their progeny means that the practice is unlikely to be implemented in the near future. A further six years at least would be needed to first measure methane emissions on large numbers of animals and then develop algorithms based on genomics for predicting methane emissions from individual animals for selection in stud operations using DNA analysis only. Furthermore, information has been collected to date almost exclusively for the Australian Angus breed and not for the other two major beef cattle classes in Australia, Herefords and northern cross-bred cattle. Despite the impact of genetic selection being cumulative over time, the small annual reduction in methane emissions based on a breeding plan that includes low methane selection, suggests selective breeding will have only a small impact on national greenhouse gas emission. For these reasons, further investment in genetic selection for reducing national methane emissions is regarded as low. These constraints are not so likely in the dairy industry and further investment for genetics in this industry is ranked medium.

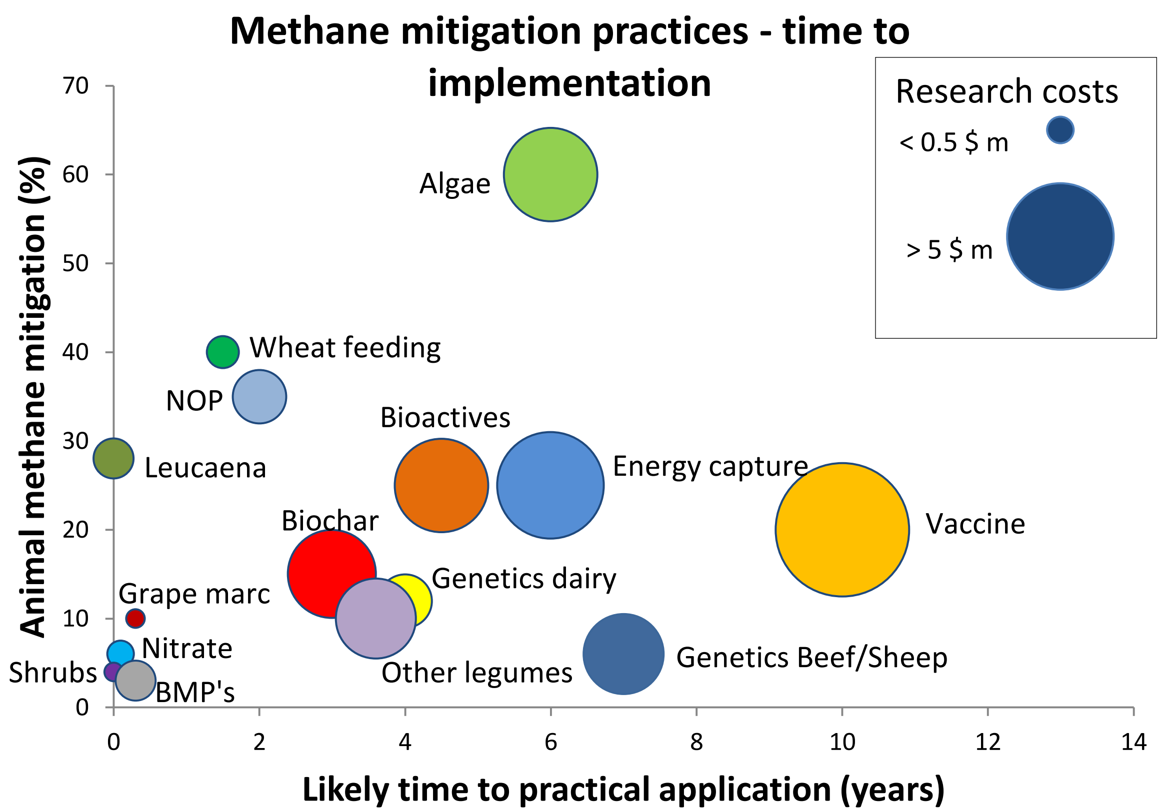


Figure 10. Relationship between the methane mitigation potential in individual animals and likely time to first implementation on farm after considering additional research needed for a range of methane mitigation practices. The size of the bubble-dot represents a relative estimate of the likely cost and risk of further research required.

Red algae from the *Asparagopsis* genus appear from current research projects to have the greatest potential to reduce methane emissions from ruminants (Figures 10 and 11). Small doses of algae are required and it can most likely be used as a supplement across all Australian ruminant production systems. The biggest risk to its widespread adoption is cost and practicality of supplementing cattle and sheep grazed under rangeland conditions. Research is needed in conjunction with potential commercial partners to determine the most effective and economical method for growing, harvesting and preparing the algae for feeding. If the algae can be grown effectively in conjunction with polluting aquaculture industries, the prospects for producing it at sufficiently low price are enhanced. In addition to growing the algae, research is needed to demonstrate its effectiveness in a range of ruminant production systems and demonstrate its impact on productivity. If the research is successfully integrated with research into capturing methane energy for productivity, the effectiveness of algae as a viable production option would be greatly enhanced. An estimate of the time needed for research until algae supplementation could be first implemented on farm is approximately 4 years. This research is regarded as being of high priority.

Biochar theoretically could be an effective way for reducing methane emissions, capturing energy from methane through methanotrophs, increasing rumen microbial growth and enhancing feed digestion and animal productivity. Evidence to date is based on an extremely limited number of *in vitro* and one animal experiment. However, with the assumptions made from these experiments and the predicted methane mitigation and animal productivity gains, an argument can be made for additional research to better evaluate the effectiveness of biochar. Initial research evaluating existing hypotheses could be completed within a few years, but if validated, ongoing research would be required. Research into biochar may best be integrated into research on energy capture within the rumen, because it primarily involves manipulation of rumen function. Further investment into biochar has been given a medium-high ranking at least for testing the initial hypotheses. The initial hypothesis testing research is considered to be of high priority because of potential application across all ruminant sectors.

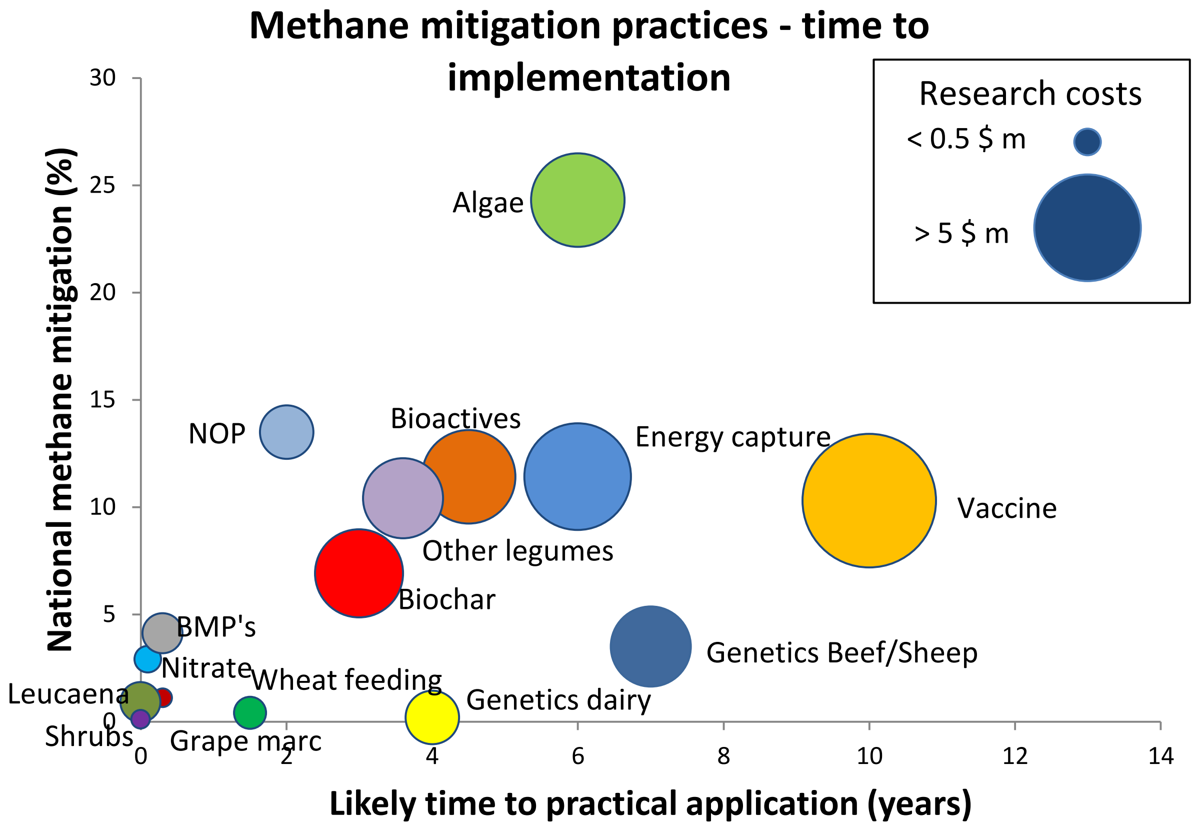


Figure 11. Relationship between the methane mitigation potential nationally across all ruminant sectors, expressed as a percentage of the potential total mitigation from all practices considered (12.97 m tonnes annually), and likely time to first implementation on farm after considering additional research needed for a range of methane mitigation practices. The size of the bubble-dot represents a relative estimate of the likely cost and risk of further research required.

Plant bioactive compounds are similar to biochar in priority for evaluation of further investment because results to date have only been obtained from longer-term *in vitro* experiments. These experiments are highly promising based on evaluation of previous compounds assayed *in vitro* and also tested in animals. The methane mitigation potential assumed is based on these comparisons. The bioactive compounds examined would have a substantial impact of methane mitigation in individual animals and nationally if verified in animal experiments. Initial research testing the existing hypotheses would be relatively short term. However, if these bioactive compounds are proved to be effective in substantially reducing methane emissions from ruminants, research across each ruminant sector would be required once a possible route to commercialisation has been identified.

Other legumes with effective tannin or other methane mitigating compounds exist in Australia and other countries. Many of these plants have been shown to have favourable growth and quality characteristics within one NLMP project. The major advantage of these plants is through increased feed yieldat specific times of the year and feed quality improving animal productivity. The impact on methane mitigation has not been studied widely. Further research may be warranted investigating the effectiveness of agronomically superior strains with identified compounds for reducing methane emissions in ruminants, for example low methane high Lucerne accessions. Further research is also required to evaluate the use of red-clover as an option for reducing methane emissions. An agronomic assessment is then needed to determine the regions where introducing red clover may be an appropriate farm practice.A breeding program is needed for biserrula to improve its growth performance, improve its early growth palatability and remove its propensity to cause photosensitivity in sheep, while maintaining its ability to reduce methane emissions. Furthermore, chicory, although not a legume, fills a similar role as lucerne and has a real potential as a valuable feed supply, particularly over winter and summer feed-gap periods and could assist farmers in southern Australia adapt to anticipated changes in climate. Investment is required for a substantial plant breeding program to exploit the variability that has been identified and allow the development of persistent cultivars with growth characteristics suitable for filling identified annual feed gaps and maintaining high nutritional quality.

Little further research is needed to prove the benefits from growing rows of Australian native shrubs with inter-row pastures for grazing during the autumn feed-gap period. However, investment is required to assist adoption of the process and this would be assisted if the shrubs could be accurately represented within plant based whole farm models. For all the potential forage systems used to reduce methane emissions, research will be required to provide dose responses or quantitative relationships to allow development of ERF methodologies for producers to have a means for claiming carbon credits.

Research into fundamental mechanisms of rumen function has lead to an enhanced understanding and to the hypothesis that the amount of energy captured from digestion of feed in the rumen could be greatly increased. With an appropriate change in biochemical pathways within the rumen, energy capture could be enhanced and methane emissions substantially reduced. The MACC analyses conducted with different proportions of saved methane energy from mitigation practices being captured for animal productive functions, quantified the importance of capturing this energy in terms of productivity and enterprise profitability. Several hypotheses have been developed on possible methods for manipulating the rumen to achieve these desired outcomes. The hypotheses will not have been fully evaluated during current projects. Further investment in this area is considered to be of high priority because of the potential benefits in both national methane mitigation and animal productivity.

The concept of vaccinating ruminants against methanogenic organisms has been proven. The major limitation to developing an effective vaccine has been identifying appropriate antigens and effective routes for the vaccine to ensure high levels of antibodies are delivered within the rumen. Research within NLMP and related projects has identified potential antigens to target *Archaea*. Research within New Zealand is evaluating different vaccination protocols, but the success of this research is currently unknown. Figures 10 and 11 indicate that an effective vaccine reducing methane emissions from all ruminant sectors by around 15% would be of considerable national value. Ease of implementation and relatively low costs of vaccines per animal treated makes this approach attractive, although development and commercialisation costs are initially high. It is argued here that future investment, preferably in collaboration with New Zealand, would be worthwhile. Such investment has been given medium priority, partly because of the risks in not achieving the desired outcomes and partly because of the high cost of the proposed research.

7 Investment priority summary

The priorities for investment into methane mitigation projects based on the capacity to reduce methane, the potential productivity gain, barriers to implementation and time and cost of research are as follows:

High priority

- Develop a dose response curve for leucaena to allow development of an ERF methodology

- Red marine macro-algae evaluation in different classes of ruminants and development of a commercial growing, harvesting and drying process, plus a method of supplementing to grazing livestock
- Manipulation of rumen function and biochemical pathways to allow markedly enhanced capture of energy from digestion and reduced methane emissions
- Evaluate two selected plant bioactive compounds in sheep for reducing methane emissions and quantify effects on productivity. If positive results, pursue commercialisation plan

Medium-High

- Evaluate characteristics of biochar and effects in vitro; undertake a dose-response experiment with ruminants to identify changes in rumen function, methane emissions and animal productivity
- Evaluate Archaea surface peptides for development of vaccines; evaluate effective routes for vaccination against rumen Archaea; collaborate with New Zealand team

Medium

- Evaluate NOP under Australian forage conditions
- Genetic selection for low methane emissions in dairy cows
- Determine specifications of wheat for reducing methane when fed at high rates to dairy cows on pasture; determine dose response or other information needed for development of an ERF methodology and explore for feedlot application
- Evaluate legumes, specifically lucerne and red clover, with superior agronomic characteristics for methane mitigation properties in vitro and subsequently in animals if positive
- Plant breeding programs for i) biserrula to improve productivity and palatability and reduce photosensitivity in animals and ii) chicory for improving persistence and ability to fill identified feed gaps

Low

- Grape marc - most information already obtained and national mitigation potential is low
- Nitrates - much information already obtained, but could examine effective ways for reducing methaemoglobin concentrations, but national mitigation potential is low
- Best Management Practices - Not research but adoption and extension needed

Several areas were identified for investment in the short term irrespective of the rank given for longer-term research because the research is likely to lead either to improved application of several methane mitigation practices or provide evidence needed to show more detailed research is warranted.

- Wheat feeding to dairy cows at pasture: specifying the quality of wheat needed for the methane mitigation effects; developing best management practices for introducing wheat feeding at high rates; drafting an ERF methodology,
- Leucaena: dose response curve for methane mitigation and verification of faecal NIR for predicting proportion of leucaena in the diet; final draft of an ERF methodology,
- Nitrate: evaluation of the effectiveness of providing with nitrates antioxidants and N-acetylcysteine to reduce the rise in methaemoglobin,
- Bioactives: evaluate compounds C, L and G, extracted from Australian native plants for anitmethanogenic effects in sheep,
- Grape marc-tannins: apply the new tannin analytical techniques to other animal feed ingredients known to impact on animal productivity across domestic animal species;

- Shrubs: incorporate shrubs effectively into plant physiological simulation models for effective use in whole farm economic analyses.

Other NLMP projects not directly related to methane mitigation

High

- NIR for predicting digestibility and nutrient content of forages using handheld and real-time response technology

Medium

- Incorporation of information from the ELLE database on forage growth and nutritional value into plant physiologicy-based whole farm simulation models

Low

- Intra-ruminal device for measuring methane has low priority because of technical and animal welfare issues, but high priority for adaptation for use in human medicine.

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