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Reducing Emissions from Livestock Research Program:

Enteric methane abatement strategies for ruminant production systems in south eastern Australia

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Abstract

Agriculture produces 16% of national greenhouse gas emissions in Australia mainly as methane and nitrous oxide. Prior research has shown that dietary supplements can reduce methane emissions from cattle by 20%. This project evaluated a range of dietary oil supplements, tannin, micro algae oils and grape marc for effects on methane emissions, milk yield and milk composition in dairy cows. The findings of the project has also improved the methods for measuring methane from individual animals (SF₆ tracer) and groups of animals in the field (Open Path Tracer). Oil supplements and grape marc have shown potential to reduce methane without negative impacts on productivity, allowing the development of an offset method that will allow farmers to claim carbon offset credits under the Carbon Farming Initiative. Further research is now required to investigate combinations of forages and supplements for reducing methane and improving productivity in sheep and cattle.

Executive summary

Agriculture produces 16% of national greenhouse gas emissions in Australia mainly as methane (11.6%) and nitrous oxide (2.8%). Based on literature reviews and findings from this project and prior research at Department of Primary Industries (DPI) Ellinbank, dietary supplements have the potential to reduce enteric methane emissions from ruminants by 20%. This report covers research activities between January 2009 and December 2011.

The main objectives of this project were:

- 1) To evaluate forages and dietary supplements for methane mitigation and production impacts
- 2) To evaluate Open Path Tracer methods for measurement of methane from grazing ruminants.

An additional aim, as detailed in the project research agreement, was to share biological samples with other RELRP projects to further elucidate the modes of action by which supplements influence methane abatement.

This report describes findings from four experiments at DPI Ellinbank measuring methane and productivity responses to feeding different types of dietary supplements to dairy cows, as well as from research at DPI Hamilton evaluating an Open Path Tracer technique for measuring methane emissions from grazing sheep.

The four experiments on lactating dairy cows compared the effects on methane emissions, milk yield and milk composition of a basal diet of lucerne hay with the following dietary supplements:

- 1) Crushed wheat, brewers grains, hominy meal or cold-pressed canola;
- 2) Four rates of algae meal containing 20% docosahexanoic acid (DHA);
- 3) Cottonseed oil, crude tannin, or both the cottonseed oil and the tannin;
- 4) Dry grape marc (the skins and seeds after grapes are pressed to make wine) or ensiled grape marc.

Samples of rumen fluid, faeces, blood, hair and feeds from these experiments were provided to scientists working on RELRP projects (B.CCH) 1005, 1007, 1008, 1010, 1013, 1014 and 1025.

Research on the Open Path FTIR Tracer technique focused on development of novel methods to enable it to be used to measure methane emissions from flocks of grazing sheep.

Summary of achievements

Key findings of this project have already been reported in the scientific literature, and further scientific publications are expected within the next 12 months. This research has shown that:

 Fatty feed supplements, especially by-products such as brewers grains, hominy meal and cold-pressed canola can reduce methane emissions and the effect is sustained for over 9 weeks. When a fatty feed supplement is added to a diet, methane emissions are reduced by 3.5% for every 1% increase in the total fat concentration in the diet. These findings are expected to form the scientific basis for a dairy industry offset methodology under the Carbon Farming Initiative.

- Feeding cows on algae meal containing high concentrations of DHA does not reduce methane emissions but results in milk with lowered milk fat concentration. This finding is important because it is contrary to international *in vitro* studies that had reported algae meal high in DHA had potent anti-methanogenic properties with the potential to decrease methane emissions by 75%.
- Dietary supplements of fat and tannin have been shown to inhibit methane emissions from ruminants. This research has shown that when supplements of fat and tannin are fed simultaneously, as extracts, the inhibitory effect was not additive. This novel finding has important implications for dietary methane mitigation strategies that are based on multiple dietary supplements.
- When either dried grape marc or ensiled grape marc were fed to cows, methane emissions were reduced by approximately 20% without decreasing feed intake, or milk yield, or having adverse impacts on milk composition.
- The open path FTIR technique can be used to measure methane emissions from flocks of grazing sheep. The technique could detect differences of less than 10% in the methane emissions from different flocks and from flocks grazing different pasture types. The technique can be used as a tool to demonstrate methane abatement strategies on commercial farms.
- The SF₆ tracer technique can be used to accurately measure methane emissions from dairy cows housed indoors. In this research we showed that background concentrations of both SF₆ and methane are elevated indoors compared to outdoors, and that concentrations indoors can vary spatially. We also showed that the release rate of SF₆ from permeation tubes can be more accurately described by Michaelis-Menten kinetics. Accounting for indoor background concentrations of SF₆ and methane and using Michaelis-Menten kinetics to describe SF₆ release rates has the potential to improve the accuracy of the SF₆ technique by 25%. These findings will help to improve the accuracy of subsequent research projects using this technique.

Benefits to Industry

The findings from this research form the scientific basis on which the dairy industry can now demonstrate real reductions in enteric methane, without affecting productivity. This research will underpin at least two offset methods under the Carbon Farming Initiative, using either oils or grape marc; thus providing farmers with financial incentives to reduce enteric methane. Discussions are well advanced between PICCC, Dairy Australia, Murray Goulburn Co-op, Fonterra and DCCEE, with an agreement to co-invest in a project to develop these offset methods.

Recommendations

It is notable that, apart from herd management efficiencies, dietary supplementation is the only other method under RELRP where significant methane mitigation has been achieved. Given the success of dietary supplementation for mitigating methane emissions without affecting productivity, we recommend that supplementation work be continued and extended to explore combinations of forages and a wider range of supplements for their effect on productivity and methane emissions.

The Open Path FTIR Tracer method has been shown to be useful in estimating methane emissions from herds and flocks in grazing situations, and can be used to quantify the effect of mitigation strategies in a herd or flock context. However, the technique must be used in context, as it is highly dependent on near ideal weather conditions. Therefore the method is more suited to demonstration sites than accurately quantifying dietary mitigation strategies on commercial farms.

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1 Background

1.1 **Project Identification**

Project Title	Enteric methane abatement strategies for ruminant production systems in south eastern Australia
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Commencement date	1/1/2009
Completion date	30/12/2011

1.2 Project Background

Enteric methane (CH₄) contributes about 11% of national greenhouse gas emissions; the majority of which comes from beef, sheep and dairy cattle. Methane is predominantly produced in the rumen or first stomach of ruminants during the fermentation of ingested feed. In ruminants, between 4 and 10% of ingested energy is eructated as CH₄. Thus, CH₄ contributes not only to global climate change, but presents an opportunity for CH₄ mitigation as well as gains in efficiency in the beef, sheep and dairy industries. In south eastern Australia, these industries occupy relatively high rainfall, improved pasture zones or feedlots, where dietary and management interventions for reducing CH₄ are more feasible than in extensive rangeland zones.

In Victoria, the dairy industry contributes approximately 38% of all greenhouse gas emissions from agriculture. Literature reviews and research conducted at Department of Primary Industries (DPI) Ellinbank prior to this project indicate that nutritional abatement strategies have the potential to reduce enteric CH_4 emissions by up to 20% with concomitant productivity gains.

Prior to this project, CH_4 research at DPI Ellinbank focused on the development of the capacity and skills to accurately measure CH_4 emissions from dairy cows. Two respiration chambers were built at Ellinbank and initial experiments focused on quantifying CH_4 emissions in response to supplements of tannin from black wattle and whole cottonseed fed in summer. In addition, the sulphur hexafluoride (SF₆) tracer technique for measuring CH_4 emissions from grazing cattle was established at Ellinbank. This report covers research conducted principally at DPI Ellinbank between January 2009 and December 2011. During this period, research at DPI Ellinbank has focussed on quantifying CH_4 emissions from by-products and fatty feed supplements. This research has been co-funded by the Department of Agriculture, Fisheries and Forestry under its Climate Change Research Program, The Department of Primary Industries Victoria, Meat and Livestock Australia and Dairy Australia.

1.3 Key Stakeholders

The prime beneficiaries of this research will be State and Federal policy makers. At the Federal level, the Department of Climate Change and the Department of Agriculture, Fisheries and Forestry will be beneficiaries in that they will be provided with information that can be used to develop more accurate national inventories of greenhouse gas emissions, determine appropriate mitigation policy targets for livestock industries, develop offset methods for the Carbon Farming Initiative, plus informing future research priorities in this area.

The dairy industry is also a beneficiary of this research through understanding the mitigation potential of dietary supplements, the development of scientifically based methodologies for the Carbon Farming Initiative, and assisting them in negotiating the contribution of the dairy industry to national mitigation targets and time frames.

The next users of this research will be DCCEE, Fonterra, Murray Goulburn Co-op and Dairy Australia in using the published information to develop offset methods for dairy farmers.

2 Project objectives

- 1. Evaluate forages and dietary supplements for CH₄ mitigation and production impacts
- 2. Evaluate Open Path Tracer methods for measurement of CH₄ from grazing ruminants

3 Methodology

This project built on previous research on the impacts of various dietary supplements on CH₄ mitigation potential for ruminants. An early element of this project was a workshop with key members of the Reducing Emissions from Livestock Research Project (RELRP) to establish a national approach to *in vitro* screening of various forage options (drawing on forages evaluated in the 30:30 and EverGraze projects and other sources), plus a range of dietary supplements (oils/fats, tannin extracts, forage tannins, algae, by-products and grape marc) for their CH₄ abatement potential. *In vitro* studies were carried out by Dr. Phillip Vercoe and Dr. Zoe Durmic at the University of Western Australia.

From *in vitro* screening, some promising forages and supplements were then tested *in vivo* using the SF_6 method (see Figure 1). The SF_6 tracer technique is generally regarded as not being as accurate as the Open Circuit Calorimetry Chamber, but the

 SF_6 technique is much less expensive and it can be used with grazing ruminants. In order to utilize the SF_6 technique, we had to carry out some method refinement. This included some fundamental research on the influence of background concentrations of SF_6 and CH_4 on the calculated estimates of CH_4 emissions. This method refinement improved the accuracy of predicting the rate of release of SF_6 from the SF_6 permeation tubes. This improved SF_6 technique was employed to measure CH_4 emissions from cows fed grape marc (Experiment 4).



Figure 1. Dr. Peter Moate with a cow fitted with SF₆ equipment

The two Open Circuit Calorimetry Chambers at DPI Ellinbank (see Figure 2) were used to measure CH_4 emissions from lactating dairy cows. These are the only calorimeters in Australia set up to measure CH_4 emissions from lactating dairy cows. These chambers enable measurement of CH_4 emissions (g/cow/day), CH_4 efficiency (g/kg DMI) and CH_4 intensity (g/L milk).



Figure 2. A cow in one of the calorimeters at DPI Ellinbank

An aim of this project and other projects within the RELRP consortium was to understand the impact of various forages and CH_4 mitigating supplements on rumen microbial populations. Such research requires the collection of samples of rumen fluid. At DPI Ellinbank, we have a herd of 30 rumen fistulated dairy cows, and in a number of experiments rumen fluid was collected from these animals and sent to Dr Mark Morrison at CSIRO Livestock Industries (Project B.CCGH.1005) and to Dr. Valeria Toroc at SARDI (Project B.CCH.1008) to identify differences in rumen microflora in relation to CH_4 emissions.

This project commissioned an Open Path FTIR for quantifying CH_4 emissions from groups of animals in the field (see Figure 3).



Figure 3. The FTIR instruments (left) and sheep fitted with canisters of tracer gas (right).

Further information on the specific methodologies used in each experiment are detailed in the journal papers listed in the references.

4 Results

4.1 *In vitro* screening of forages and diet additives.

4.1.1 Background and Research

During the course of this project 46 samples of feeds were collected and sent to Dr Phillip Vercoe and Dr Zoe Durmic (B.CCH.1010) at the University of Western Australia. These samples were screened by the *in vitro* technique to ascertain their CH_4 mitigation potential.

The testing included six forage species and nine types of feed additives sourced from DPI Ellinbank. The material was tested using an *in vitro* batch fermentation assay where forages were included as sole substrate, while additives were mixed with a concentrate-based diet included at levels that correspond to the recommended levels of intake reported in the literature.

4.1.2 Results

Forages varied in their fermentability as estimated by gas pressure, and CH₄ production (Table 1, Figure 4).

Table 1.	. Overall fe	rmentability	(gas pressu	re, kPa) and	d methane	production ((mL/g
DM sup	plied) of for	ages incuba	ated in vitro.				-

Diant name	Gas pressure (kPa)		Methane (mL/g DM)		Reduction (%)*	
Plant name					Gas	Methane
Clover 1	121	abcd	48	cd	-	-
Clover 2	122	abcd	49	cd	-	-
Turnip bulb 1	119	abcde	44	defg	ns	ns
Turnip leaf 1	118	abcdef	54	bc	ns	ns
Turnip bulb 2	116	bcdefg	38	fgh	ns	22
Turnip leaf 2	114	cdefg	37	fgh	ns	24
Chicory 1	95	jk	37	fgh	22	24
Chicory 2	91	k	46	cde	25	ns
Chicory 3	110	efghi	44	defg	9	ns
Hunter	115	bcdefg	42	defg	ns	ns
Plantain	101	ij	36	fgh	17	25
Winfred leaf 1	108	fghi	39	efgh	11	19
Winfred stem 1	103	hij	42	defg	15	ns
Winfred whole plant 1	122	abcd	57	ab	ns	ns
Winfred whole plant 2	106	ghi	42	defg	13	ns

Significance – within the same column, values not sharing same superscript letter are significantly different (P < 0.05)

* when compared to clover average values; ns- non significant difference compared to clover

Gas pressure in incubations on two chicory samples, Plantain, and three Winfred (excluding whole plant 1), were lower than in a clover control. The highest amounts

of CH₄ were produced by Winfred whole plant 1 (57 mL/g DM) and Turnip leaf 1 (54 mL/g) and the lowest with Turnip leaf and bulb 2, Chicory 1, Plantain, and Winfred leaf 1 (36 – 39 mL/g DM). All plants that had significantly reduced CH₄ values also had reduced gas values. Plantain was the forage with the lowest CH₄ production (36 mL/g DM), with gas reduction of less than 20%. Different varieties of the plant also performed differently in this test. Leaf material from the ATP variety of turnip (Turnip 1) produced more CH₄ than Marco variety (Turnip 2), Chicory 1 was significantly lower than the other two chicories. Variability was also observed between different parts of the same plant - Turnip leaf 1 was more methanogenic than Turnip bulb 1, and leaf material of Winfred 1 plant was less methanogenic than its stem.



Figure 4. Methanogenic potential (mL/g DM supplied) of forages fermented in vitro.

Inclusion of some feed additives with control diet (pellet) resulted in significant reductions (P < 0.05) in CH₄ production when compared to the control (Table 2, Figure 5).

Table 2. Fermentability (total gas pressure, kPa) and methane production (mL/g DM
supplied) of control diet (pellet) supplemented with different additives and incubated
in vitro.

Treatment	Gas		Methane	
	(kPa)		(mL/g DM)	
Control (pellet)	121	abcd	60	abc
Almond hulls*	107	ghi	44	efg
DHA	117	bcdef	43	efgh
DHA oxidised	128	а	65	а
Grape marc crumble**	115	bcdefg	37	ghi
Grape marc pellets**	103	hij	39	fghi
Monensin	122	abcd	58	abc
N. oculata	113	defgh	36	hi
Tannin	101	ij	34	i

Significance – within the same column, values not sharing same superscript letter are significantly different (P < 0.05)

* included as 50:50 with pellet, ** included as sole substrate



Figure 5. Methane production (mL/g DM) of the control diet (pellet) supplemented with different additives and incubated *in vitro*.

Almond hulls, DHA, Nannochloropsis oculata and Tannin caused 30 to 40% reductions in CH_4 when compared to control diet without the additive, with the lowest CH_4 values recorded with Tannin (34 mL/g DM), followed by N. oculata (36 mL/g DM). However, Almond hulls, and Tannin also caused reductions in total gas production (Table 2). When included as sole substrate, Grape mark crumble and Grape mark pellet also had low methanogenic potential (37 mL/g DM and 39 mL/g DM respectively) with Grape marc crumble also supporting normal rumen fermentation (as indicated by total gas pressure). Monensin was ineffective at reducing CH_4 at the levels applied here. Furthermore, oxidized DHA was also ineffective and produced 34% more CH_4 than non-oxidized DHA.

4.1.3 Implications and recommendations

Some novel forages, such as turnip, Winfred, Chicory and Plantain, demonstrated the potential to significantly reduce CH₄ production by rumen microbes. However, this effect was accompanied by a small but significant reduction in overall microbial activity, as measured by reduced gas production. While it can be concluded that the inhibitory effect was non-selective, the fact that CH₄ was reduced to a greater extent than total gas may be used as an argument in support of more specific action against methanogens. Plant bioactivity is a complex issue, relying on plant secondary compounds as well as nutritive composition of the plant. We still do not understand why some varieties of turnips are more methanogenic than other varieties or why some chicory varieties are more methanogenic than others. However, in vitro fermentation techniques are the most cost effective for investigating the reasons for these effects. As different plant secondary compounds may act with different mechanisms in the microbial ecosystem, further in vitro investigations including continuous flow studies (Rusitec) into their combined effect is warranted to enable selections of plants that may provide useful tools to manipulate rumen microbial fermentation. However, sufficient evidence now exists to warrant the in vivo evaluation of some of these forages for their CH₄ mitigation potential. This should be a priority of the next phase of this research.

The supplementation of a concentrate-based diet with selected feed additives resulted in significant reductions in CH_4 *in vitro*. Additives such as Tannin and marine extracts were effective, causing approximately 40% reduction in CH_4 , without

severely disrupting normal gas production. Industry by-products such as Almond hulls and Grape marc also demonstrated the potential to alter CH₄ output when mixed with the rumen microbes. Further investigation is required to pursue these candidates in a continuous flow system (Rusitec) and *in vivo* to confirm these findings. Dr Durmic and Dr Vercoe are currently drafting a manuscript on this research.

4.2 Influence of feeding fatty by-products on methane emissions from dairy cows

4.2.1 Background and Research

Previous research at DPI Ellinbank and elsewhere had shown that feeding dairy cows supplements containing a high concentration of fat can result in reduced CH₄ emissions. However, no research had quantified CH₄ emissions in response to feeding cows either brewers grains, cold-pressed canola or hominy meal. Between February and May 2009, an *in vivo* feeding experiment was conducted in which CH₄ emissions were measured from individual cows in calorimeters. The experiment compared three dietary supplements for their CH₄ mitigation potential. Feeding cows either brewers grains, cold-pressed canola or hominy meal instead of crushed wheat resulted in decreased emissions of CH₄. A meta-analysis including results from 15 additional experiments published in the scientific literature showed that CH₄ emissions are decreased by 3.5% for every 1.0% increase in the dietary fat concentration. The results of this experiment were presented at an International Conference: Greenhouse Gases (GHG) in Animal Agriculture in Banff, Canada in October 2010 and subsequently published in a scientific journal (see Appendix 1).

4.2.2 Implications

The findings from this research will be incorporated into a number of decision support programs (DairyMod, DGAS). The findings from this research will also constitute the scientific basis for a 'Carbon Farming Initiative offset Methodology'.

4.3 Influence of feeding algal meal on methane emissions

4.3.1 Background and Research

Companies are currently establishing facilities at coal-fired power stations which pump the flue gases through growth vessels containing algae, and thereby capture carbon dioxide. Dried algae can be used as a feed supplement for cattle. Some of the algae species intended to be grown for this purpose contain high concentrations of the long chain fatty acid called docosahexanoic acid (DHA). When European researchers incubated dried algae high in DHA, *in vitro* in rumen fluid, CH₄ emissions were reduced by approximately 75%.

In this project we carried out the first *in vivo* experiment to measure the effects on CH_4 emissions of feeding dried algae high in DHA to dairy cows. In early 2010, cows were fed diets containing either 0, 25, 50 or 75 g/day of DHA. Feed intake, milk production and milk composition were measured. Methane production from individual cows was measured in chambers. Samples of rumen fluid and faeces were sent to collaborating RELRP scientists (Dr. Valeria Toroc (SARDI), Dr Athol Klieve (UQL)) for bacterial/DNA studies.

Dry matter intake, CH_4 emissions and milk yield were not influenced by DHA, but milk fat % and yield of milk fat were decreased by 28%. An interesting finding from this research was that there were substantial increases in the concentrations of some 'healthy' fatty acids in milk. At the highest dose of DHA, the concentration of DHA in

milk was increased 30 fold, cis-9, trans11-conjugated lineoleic acid (CLA) increased 5 fold and the concentration of poly-unsaturated fatty acids increased 75%. A manuscript on this research is in preparation.

4.3.2 Implications

The feeding of dried algae high in DHA is not a practical means for reducing CH_4 emissions from dairy cows; nor is it likely to be an appropriate CH_4 mitigation strategy for other ruminant production systems. Additional research is still required to quantify the CH_4 mitigation potential of feeding dairy cows on dried algae containing lower concentrations of DHA or perhaps with fatty acids profiles similar to those found in the supplements tested in previous successful experiments.

4.4 Effect of feeding two methane mitigants on methane emissions

4.4.1 Background and Research

There is general consensus amongst ruminant nutritionists that there will not be a single nutritional 'silver bullet" that will result in complete or even substantial (> 50%) reduction of enteric CH₄ emissions from ruminants. Instead, the hope is that a number of dietary interventions may individually bring about modest (10 - 20%) reductions in CH₄ emissions and that when combined, the effects of these dietary interventions will be additive so that substantial reductions in enteric CH₄ emissions may be achieved.

During late 2010, a feeding experiment was carried out to examine whether the simultaneous feeding of two dietary feed supplements known to be CH₄ mitigants would result in an additive inhibitory effect against enteric CH₄ production. The experiment involved 8 rumen fistulated lactating dairy cows in a 4 x 4 Latin square experiment (4 periods and 4 treatments) in which every cow received each dietary treatment. The cows were offered a basal diet of 6.0 kg DM/day of crushed wheat, and *ad libitum* lucerne hay. The treatments were administered to individual cows via their rumen fistulae: CON, 800 ml/cow/day of water; TAN, 400 g/cow/day of tannin (from *Acacia mearnsii*); FAT, 800 g/cow/day of cottonseed oil; F&T, 400 g/cow/day of tannin and 800 g/cow/day of cottonseed oil.

The FAT supplement alone resulted in the expected 12% reduction in CH_4 emissions, while the TAN treatment resulted in a 3% reduction in CH_4 emissions. The most unexpected finding from this last experiment was that the F&T treatment resulted in a 4% reduction in CH_4 emissions. We interpret these findings as indicating the presence of tannin in the diet negated the CH_4 inhibitory effect of fat. This experiment is one of the first *in vivo* experiments to investigate the combined effects of the simultaneous dietary administration of two putative CH_4 mitigants.

4.4.2 Implications

The unexpected small effect on CH_4 production by the F&T treatment in this experiment brings into doubt the fundamental assumption that multiple dietary treatments and management strategies will have additive effects against CH_4 emissions. It is recommended that future research should elucidate the mechanisms involved when mitigants successfully reduce CH_4 emissions and when and if multiple mitigation dietary treatments and management strategies result in additive effects against CH_4 emissions.

4.5 Influence of feeding grape marc to dairy cows on their methane emissions.

4.5.1 Background and research

Each year, the Australian wine industry produces approximately 200,000 tonnes of grape marc (the skins and seeds remaining after grapes are pressed to make wine). Thus grape marc is a substantial resource which could be utilised by the dairy and beef industries. There are three types of grape marc that are currently commercially available. Fresh grape marc (FCM) is the fresh grape skins and seeds after the grapes have been pressed. Usually, fresh grape marc is ensiled to preserve it. Ensiled grape marc (EGM) has good long term stability and is suitable for feeding to animals. An alternative commercial product is dried grape marc 'crumble' (DGM). There is no information in the scientific literature concerning the effects on CH₄ emissions when grape marc is fed to ruminants, nor effects on dairy production when grape marc is fed to dairy cows.

During autumn 2011, an indoor feeding experiment was carried out involving 32 lactating dairy cows fed either a control diet, or a diet supplemented with either dry grape marc or ensiled grape marc (see Figure 6). Methane emissions were measured using the SF_6 technique.



Figure 6. Ensiled grape marc

Both dry and ensiled grape marc reduced CH_4 emissions by approximately 20% (see Table 3). This represents perhaps one of the largest ever reductions in CH_4 emissions from dairy cows *in vivo* resulting from a nutritional intervention. The milk from cows fed grape marc was significantly (*P*<0.05) enhanced in concentrations of healthy fatty acids including conjugated linoleic acid (CLA), mono-unsaturated and poly-unsaturated fatty acids.

Item	Control	Dry Grape Marc	Ensiled Grape Marc
Number of cows	12	10	10
Crushed wheat (kg DM/day)	5	5	5
Lucerne (kg DM/day)	13.2	8.1	8.6
Wet grape (kg DM/day)	0	5	0
Dry grape (kg DM/day)	0	0	5
Total DMI (kg DM/cow/day)	18.2	18.1	18.6
Milk (L/cow/day)	13.4ab	15.0b	11.5a
Methane (g/cow/day) (% decrease)	470b	375a (20%)*	389a (17%)*
Methane (g/kg DMI/day)	26.1c	20.2a (23%)*	21.5b (18%)*
Methane (g/L milk)	35.3b	26.1a (26%)*	35.2b (0%)*

Table 3. Influence of dry or ensiled grape marc on dairy production and methane emissions from late lactation dairy cows.

Means in the same row followed by different letters differ significantly P < 0.05

4.5.2 Implications and recommendations

These findings indicate that grape marc is a useful dietary supplement to mitigate CH_4 emissions from ruminants. Future research to determine the effects on CH_4 emissions, milk yield and milk production when grape marc is fed to dairy cows in early lactation is warranted. Research is also required to understand the mode of action of grape marc, as the supplement contains both fat and tannin but clearly these were not interacting as was evident in the earlier fat and tannin experiment.

4.6 Improvements to the SF₆ tracer technique for measuring methane emissions from ruminants

4.6.1 Background matters

Quantification of CH_4 abatement potential of nutritional feeding strategies requires an accurate and inexpensive method for measuring CH_4 emissions. The SF_6 tracer technique is much less expensive than the calorimetry chamber technique, but its accuracy has been questioned by a number of researchers. We have carried out research to increase the accuracy of the SF_6 technique. In this regard, we investigated the influence of background concentrations of CH_4 and SF_6 on the estimation of CH_4 emissions by the SF_6 technique. We showed that correcting for background concentrations of CH_4 and SF_6 our findings in a scientific journal (see section 6.1).

4.6.2 Michaelis Menten kinetics describe SF₆ release from permeation devices

Perhaps the greatest factor that can impact on the accuracy of the SF_6 tracer technique is the estimation of the release rate of SF_6 from the permeation tube. Since its development in 1993, it has been the practice of most researchers to assume that the release of SF_6 from permeation devices is linear (zero order kinetics). Working

with colleagues from Teagasc in Ireland, we determined that the release of SF_6 from permeation devices is in fact non-linear. An abstract of our findings was published¹.

At DPI Ellinbank, we have made a further major advance with the SF₆ technique in that we have recognised that the release of SF₆ from permeation devices is described by Michaelis-Menten (MM) kinetics. We have employed MM kinetics in the SF₆ technique and estimate that in some circumstances this modification can remove errors of up to 25% in the estimated CH₄ emissions. We have written four draft chapters (see bibliography) of a manual intended for researchers using the SF₆ technique. It is hoped this manual will help researchers improve the accuracy of their estimates of CH₄ emissions. The manual will be an on-line peer reviewed manual hosted by the New Zealand Agricultural Greenhouse Gas Research Centre.

4.6.3 Implications

It is expected that our findings and modifications to the implementation of the SF_6 method will help researchers obtain more accurate estimates of CH_4 emissions.

4.7 Use of the FTIR technique to measure methane emissions from ruminants.

4.7.1 Research

An Open Path FTIR was purchased and commissioned at DPI Hamilton. Three experiments were conducted at DPI Hamilton by Dr Frances Phillips from the University of Wollongong (March 2009, November 2009 and November 2010).

The original contract indicated that this method would be developed for sheep and cattle. Due to problems with the tracer gas canister placements, the method had to be refined to be used for sheep for the March 2009 and November 2009 experiments. As the method had already been developed for use with cattle in a previous contract, a decision was made to continue to develop the method for sheep in the final measurement period in November 2010. This was confirmed with the RELRP program coordinator in an email on 1st February 2011.

Improvements were made to this technique so that it could be used on sheep. Sheep were fitted with canisters on their backs which released nitrous oxide at a steady known rate. The nitrous oxide acts as a tracer to enable accurate estimation of CH_4 emissions. The method provides a herd or flock-average animal emission rate and includes information on the distribution of emissions over the day (3 minute time resolution typical). For sheep, the method has an error of < 10 %, dependent on experimental design or conditions such as the number of sheep in the flock, grazing area and geometry.

In March 2009 and November 2009, experiments measured the CH₄ emissions from flocks of sheep grazing ryegrass pasture, chicory and lucerne. Merino sheep grazing on pasture emitted CH₄ at 19.7 ± 7.4 and 23.0 ± 5.7 g/head/day, while Coopworth sheep emitted 28.0 ± 5.9 g/head/day, in 3 experiments. A flock of Coop-worth sheep grazing chicory emitted CH₄ at an estimated 37.0 ± 9.2 g /head/day, compared with

¹ Deighton, M.H., Boland, T.M., O'Loughlin, B.M., Moate, P.J. and Buckley, F. (2011). Assessing enteric methane emissions from ruminants using a calibrated tracer: effects of nonlinear sulphur hexafluoride release and permeation tube rumen residence time. Proceedings of the 62nd Annual Meeting of the European Federation of Animal Science, Stavanger, Norway, 29th August – 2nd September, p228.

 37.8 ± 8.1 g/head/day while initially grazing lucerne following grazing on dry ryegrass, reducing to 27.3 ± 5.2 g/head/day after 3 days grazing on lucerne. These are plausible estimates for sheep eating approximately 1 to 1.6 kg DM /day. A comprehensive report on this work is attached in Appendix 2.

4.7.2 Conclusions

The Open Path FTIR system has now been evaluated on cattle and sheep, with appropriate modifications applied to adapt between animal sizes and grazing areas. The method requires appropriate weather conditions (wind speeds between 2 and 8 ms⁻¹ and absence of fog or heavy rain) and experimental duration must allow for non-measurement days. While the precision of the method is less than that of chambers, it does provide a valuable addition to the suite of measurement tools available for quantifying CH_4 emissions and changes in those emissions over the day in the field. The method can and should be used in the appropriate context.

5 Discussion/ Conclusion

5.1 General Discussion

This research project has evaluated a number of dietary supplements for their CH_4 mitigation potential and productivity effects. Dietary oils have now been evaluated in a range of experiments, including a number of oil sources and duration of effects. Together with the meta-analysis of all available studies, this now provides a sufficient body of evidence in the peer reviewed literature to progress this research through to a CFI offset methodology. To this end, a consortium has been formed between PICCC, Dairy Australia, Fonterra, Murray Goulburn Co-op and DCCEE, together with the project team, to develop this method. This will provide dairy farmers with an incentivised option to reduce CH_4 emissions.

The issue of additivity of mitigation options still requires further research. Mixing tannin extract with fat had no effect on CH_4 production, perhaps due to the binding of the tannin and fat, thereby neutralising both. However, adding grape marc, which contains both tannin and fat, produced the largest reduction in CH_4 from all experiments conducted. Clearly this is an area that requires further investigation.

The project has also addressed some key issues and sources of error in the SF_6 technique. Pervious international workshops have concluded that the permeation tubes and associated release of SF_6 is likely to be the biggest source of error in the method. The improvements proposed by this project's research, using MM kinetics was shown to remove errors of up to 25% in the estimated CH_4 emissions. This has significant implications for improving confidence in the future use of the technique.

It is notable that, apart from herd management efficiencies, dietary supplementation is the only other method under RELRP where significant CH_4 mitigation has been achieved. Given the success of dietary supplementation as a means of reducing CH_4 emissions without affecting productivity, we recommend that this supplementation work be continued and extended to explore combinations of forages and a wider range of supplements for their effect on productivity and CH_4 emissions.

The Open Path FTIR Tracer method has been shown to be useful in estimating CH_4 emissions from herds and flocks in realistic grazing situations. This technique can therefore be used to quantify the effect of mitigation strategies in a herd or flock context. However, the technique must be used in context, is highly dependent on near ideal weather conditions, and cannot be easily replicated. Therefore the method

is more suited to demonstration sites than accurately quantifying dietary mitigation strategies.

5.2 **Project Impact on Policy:**

The research findings and scientific publications (Eckard et al. 2010; Moate et al. 2011) from this project were cited on page 42 of the submission by Australian Wool Innovation to The Australian Government Department of Climate Change and Energy Efficiency, Design of the Carbon Farming Initiative, Consultation Paper: Carbon Farming Initiative, January 2011.

It is notable that, apart from obvious animal management, the research conducted here has provided the only option sufficiently viable to be used as a basis for a CFI offset method. The findings from this research form the scientific basis on which the dairy industry can now demonstrate real reductions in enteric CH₄ emissions, without affecting productivity. This research will underpin at least two offset methods under the Carbon Farming Initiative, using either oils or grape marc, providing farmers with financial incentives to reduce enteric CH₄. Discussions are well advanced between PICCC, Dairy Australia, Murray Goulburn Co-op, Fonterra and DCCEE, together with the project team, with an agreement to co-invest in a project to develop these offset methods.

This research also provides a growing database of emissions research that will be used to improve biophysical modelling of CH_4 emissions and inform the development of a Tier 3 inventory methodology for dairy cattle.

6 Appendices

6.1 Bibliography of publications during this research project.

Project personnel are shown in bold type.

Peer reviewed papers in scientific journals

- Browne, N.A., **Eckard, R.J.**, Behrendt, R., Kingwell, R.S., 2011. A comparative analysis of on-farm greenhouse gas emissions from agricultural enterprises in south eastern Australia. Animal Feed Science and Technology 166-167, 641-652.
- Ching-Seng Ang, Steve Binos, Matthew I Knight, **Peter J Moate**, Benjamin G Cocks and Matthew B McDonagh. (2011). Global Survey of the Bovine Salivary Proteome: Integrating Multidimensional Prefractionation, Targeted and Glycocapture Strategies. Published in September 2011 in: Journal of Proteome Research. DOI: 10.1021/pr200516d.
- Christie, K.M., Rawnsley, R.P., **Eckard, R.J.** (2011). A whole farm systems analysis of the greenhouse gas emissions of 60 Tasmanian dairy farms. Animal Feed Science and Technology 166-167, 653 -662.
- Cullen, B. R., **Eckard, R. J**. (2011) Impacts of future climate scenarios on the balance between productivity and total greenhouse gas emissions from pasture based dairy systems in south-eastern Australia. *Animal Feed Science and Technology*, 166-167, 721-735.
- **Eckard, R.J.**, Grainger, C., de Klein, C.A.M. (2010) Options for the abatement of methane and nitrous oxide from ruminant production: A review, Livestock Science (in press), doi: 10.1016/j.livsci.2010.02.010.
- Grainger, C., Clarke, T., Auldist, M. J., Beauchemin, K. A., McGinn, S. M., Waghorn, G. C., and Eckard, R. J. (2009). Potential use of Acacia mearnsii condensed tannins to reduce methane emissions and nitrogen excretion from grazing dairy cows. Canadian Journal of Animal Science 89 (2), 241-251. doi:10.4141/CJAS08110
- Grainger, C., **Williams, S. R. O.**, **Eckard, R. J.**, and Hannah, M. C. (2010) A high dose of monensin does not reduce methane emissions of dairy cows offered pasture supplemented with grain. Journal of Dairy Science 93, 5300-5308. doi:10.3168/jds.2010-3154
- Grainger, C., Williams, S. R. O., Clarke, T., Wright, A. D. G., and Eckard, R. J. (2010) Supplementation with whole cottonseed causes a long-term reduction of methane emissions from dairy cows offered a forage and cereal grain diet. Journal of dairy Science 93, 2612-2619. doi:10.3168/jds.2009-2888
- Henry, B., **Eckard, R.J.** (2009) Greenhouse gas emissions in livestock production systems. Tropical Grasslands, 43, 232-238
- Moate, P.J., Williams, S. R. O., Grainger, C., Hannah, M. C., Ponnampalam, E. N., and Eckard, R. J. (2011). Influence of cold-pressed canola, brewers grains and hominy meal as dietary supplements suitable for reducing enteric methane emissions from lactating dairy cows. Animal Feed Science and Technology 166-167:254-264.
- Williams, S.R.O., Moate, P.J., Hannah, M. C., Ribaux BE., Wales, W. J., and Eckard, R.J., (2011). Background matters with the SF₆ tracer method for estimating enteric methane emissions from dairy cows. Published on 17/9/2011: Animal Feed Science and Technology, 170, 265-276. DOI: 10.1016/j.anifeedsci.2011.08.013.

Abstracts of published papers

Browne, N.A., **Eckard, R.J.**, Behrendt, R., Kingwell, R.S., 2011. A comparative analysis of on-farm greenhouse gas emissions from agricultural enterprises in south eastern Australia. Animal Feed Science and Technology 166-167, 641-652.

Abstract: Agriculture in Australia contributes 16% of national greenhouse gas (GHG) emissions, with enteric CH₄ and N₂O contributing 10.4% and 2.8% of national emissions, respectively. If agriculture is to face an emissions constrained future then it is important to understand the emission profiles of different agricultural land uses and emissions associated with different production systems. Using the Australian National Inventory methodology, whole farm GHG emissions were calculated for different farm types in south eastern Australia. Fourteen representative farms were examined that included production of Merino fine wool, prime lamb, beef cattle, milk, wheat and canola. These farm systems were defined by the production parameters of an average farm and a top producing farm, ranked on gross margin/ha/100 mm rainfall in benchmarking studies. Emissions from the systems were allocated to the primary product from each farm such as wool, meat, milk fat plus protein (MFP) or grains. The biophysical models GrassGro and DairyMod were used to simulate the livestock systems and model outputs were then used in an emissions calculator. This calculator used a yearly time frame and employed the International Panel on Climate Change methodology, as currently used in the Australian National Inventory. The calculator included CH_4 and N_2O on-farm emissions but excluded emissions from pre- and post-farm processes, such as meat processing and fertiliser production. Energy and transport emissions were also excluded because they are not defined as agricultural emissions in the Australian National Inventory. Dairy farms produced the highest emissions/ha (8.4–10.5 t CO₂-eqv/ha), followed by beef (3.9–5.1 t CO₂eqv/ha), sheep (2.8–4.3 t CO₂-eqv/ha) and grains (0.1–0.2 t CO₂-eqv/ha). When compared on an emissions intensity basis (i.e., t CO₂- eqv/t product), cow/calf farms emitted the most (22.4–22.8 t CO₂-eqv/t carcass weight) followed by wool (18.1–18.7 t CO₂-eqv/t clean fleece), prime lamb (11.4–12.0 t CO₂-eqv/t carcass weight), dairy (8.5–9.4 t CO₂-eqv/t milk fat + protein), steers (6.3–6.7 t CO₂-eqv/t carcass weight) and finally grains (0.04–0.15 t CO₂-eqv/t grain). Emissions intensities of top farms were not always less than average farms. If a C price were imposed on agriculture, emissions intensity provides insight about relative cost impacts of the C price on production of different agricultural products under different production systems. The incidence of the C price on different products and production systems could trigger land use change.

Ching-Seng Ang, Steve Binos, Matthew I Knight, **Peter J Moate**, Benjamin G Cocks and Matthew B McDonagh. (2011). Global Survey of the Bovine Salivary Proteome: Integrating Multidimensional Prefractionation, Targeted and Glycocapture Strategies. Published in September 2011 in: Journal of Proteome Research. DOI: 10.1021/pr200516d.

Abstract: Saliva is easily obtainable from a large number of animals in a noninvasive manner and contains a wide diversity of compounds including hormones, metabolites, and proteins that may be a good source of biomarkers of health and disease. Here we have used a combination of multidimensional prefractionation, targeted, and glycocapture methodologies to profile the bovine salivary proteome. The nontargeted approach used four different separation methodologies consisting of SDSPAGE, Off-gel fractionation, RP-HPLC, and SCX-HPLC. In the targeted approach, we've employed a hypothesis-based methodology by only selecting extracellular proteins from in silico data. Finally, the hydrazide capture methodology not only enabled us to identify formerly N-linked glycoproteins but it also provided a selective enrichment process for the identification of low abundance proteins. Together, the three different approaches identified 402 salivary proteins and 45 Nlinked glycoproteins. A large number of these proteins have previously been uncharacterized in bovine saliva. To date, this is the largest global survey of the bovine salivary proteome and expands the potential of the diagnostic utility of this fluid to guide development of experiments seeking biomarkers for health traits (i.e., disease resistance) as well as feed conversion efficiency and productivity traits in dairy and beef cattle.



Christie KM, Rawnsley RP, **Eckard RJ** (2011). A whole farm systems analysis of the greenhouse gas emissions of 60 Tasmanian dairy farms. Animal Feed Science and Technology 166-167, 653 -662.

Abstract: The Australian dairy industry contributes 1.6% of the nation's greenhouse gas (GHG) emissions, emitting an estimated 8.9 million tonnes of CO₂ equivalents (t CO₂e)/annum (DCC, 2008). This study examined GHG emissions of 60 Tasmanian dairy farms using the Dairy Greenhouse gas Abatement Strategies (DGAS) calculator, which incorporates International Panel on Climate Change (IPCC) and Australian inventory methodologies, algorithms and emission factors. Sources of GHG emissions including pre-farm embedded emissions associated with key farm inputs (i.e., grains/concentrates, forages and fertilizers) and on-farm emissions from CO₂, CH₄ and N₂O are estimated by DGAS software. A detailed description of GHG calculations and functionality of DGAS software are provided. Total farm GHG emissions of 60 Tasmanian dairy farms, as estimated with DGAS, ranged between 704 and 5839 t CO₂e/annum, with a mean of 2811 t CO₂e/annum. Linear regression analyses showed that 0.93 of the difference in total farm GHG emission was explained by milk production. The estimated mean GHG emission intensity of milk of production was 1.04 kg CO₂e/kg fat and protein corrected milk (FPCM; ranged between 0.83 and 1.39 t CO₂e/t FPCM) with a standard deviation of 0.13. Stepwise multiple linear regression analysis showed that feed conversion efficiency (kg FPCM/kg dry matter (DM) intake) and N based fertilizer application rate explained 0.60 of the difference in the GHG emissions due to milk production from these pastoral based dairy systems. Estimated per cow and per hectare emission intensity was 6.9 ± 1.46 t CO₂e/cow and 12.6 ± 4.37 t CO₂e/ha, respectively. Stepwise multiple linear regression analysis showed that DM intake per cow (t DM intake/cow/lactation) explained 0.86 of the variability in per cow GHG emissions intensity, while milk production/hectare (t FPCM/ha) explained 0.92 of the variability in per hectare GHG emission intensity. Given the influence that feed conversion efficiency and/or N based fertilizer application rates had on all GHG emissions intensities, it is clear that these factors should be key target areas to lower the intensity of emissions associated with dairying in Tasmania.

Cullen, B. R., **Eckard, R. J**. (2011) Impacts of future climate scenarios on the balance between productivity and total greenhouse gas emissions from pasture based dairy systems in south-eastern Australia. *Animal Feed Science and Technology*, 166-167, 721-735.

Abstract: The challenge for agriculture is to increase production in warmer climates in order to meet the demands of an increasing global population, while also meeting targets for reduced greenhouse gas (GHG) emissions. Our aim was to quantify the net effect of future climate scenarios on the productivity and total GHG emissions from pasture based dairy systems in 4 regions of south-eastern Australia using the biophysical model DairyMod. In each region, a single paddock in the grazing rotation of a dairy farm was simulated. This paddock was grazed at a stocking density of 50 lactating dairy cows/ha for a period of one day each time the pasture biomass reached 2.2 t dry matter (DM)/ha. In this way, the annual stocking rate (i.e., cows/ha) reflected the number of times that the paddock was grazed annually. No supplementary feed was offered to the animals. Model estimates of annual pasture intake, stocking rate, milk production, CH_4 and N_2O emissions were compared at each site in a baseline climate (1971-2000) and 3 future climate scenarios representing increasingly warm and dry conditions, termed the '2030', '2070 mid' and '2070 high' scenarios. At Kyabram (northern Victoria) summer irrigated perennial pastures were modelled in the baseline scenario, with supplementary irrigated annual pasture systems simulated in the baseline scenario for comparison, and in the future scenarios. At Terang (south-western Victoria), Ellinbank (south-eastern Victoria) and Elliott (north-western Tasmania) dryland perennial pastures were modelled. In dryland systems, increased pasture intake, stocking rate and milk production was modelled in all future scenarios for the cool temperate climate at Elliott, with reduced production in the '2070 mid' and '2070 high' scenarios at Ellinbank and Terang. At Kyabram, productivity of the annual system was lower than the perennial system in the baseline scenario, but increased in future climates, assuming adequate irrigation water availability. Among sites and climate scenarios, annual per cow GHG emissions were 3.4–5.5 t CO₂ equivalents (CO₂e), with CH₄ making 0.63–0.89 of total emissions. Annual emissions per unit area ranged from 2.6 to 13.1 t CO2e/ha among sites and climate scenarios, and generally reflected stocking rates. However, in the future scenarios, there were changes in N_2O emissions at dryland sites due to increased direct N₂O losses and lower indirect N₂O through volatilisation and leaching. Annual emission intensities ranged from 7.5 to 10.9 t CO₂e/t milk protein plus fat among sites and climate scenarios. The lowest emissions intensity was at Elliott, which also had little change in future climates. At Terang and Ellinbank, the emission intensity was 8.8 t CO₂e/t MS in the baseline climate, but this increased by more than 20% in the 2070 high scenario. Results suggest that pasture based production systems can continue as the basis of the dairy industry in north-western Tasmania, but lower production and higher emission intensity at Terang and Ellinbank suggest that systems adaptations are required to meet future GHG emissions reduction goals.

Eckard R.J., Grainger C., de Klein C.A.M. (2010) Options for the abatement of methane and nitrous oxide from ruminant production: A review, Livestock Science (in press), doi: 10.1016/j.livsci.2010.02.010.

Abstract: Agriculture produces ~10%–12% of total global anthropogenic greenhouse gas emissions, contributing ~50% and ~60% of all anthropogenic methane (CH₄) and nitrous oxide (N_2O), respectively. Apart from their significant contribution to anthropogenic greenhouse gas emissions, the energy lost as CH₄ and total N losses are two of the most significant inefficiencies remaining in ruminant production systems. A number of options are reviewed to reduce production of enteric CH₄ and N₂O from ruminant production systems, mainly focusing on breeding, feeding, animal management, soil and fertilizer management, and rumen manipulation. To fully assess the net abatement potential, each strategy must be subjected to whole-farm systems modelling and a full life-cycle assessment, to ensure that a reduction in emissions at one point does not stimulate higher emissions elsewhere in the production system. Most of the options reviewed require many years of research before practical strategies and commercially viable products are available for use on farms. This paper reviews the options available for livestock production to reduce CH_4 and N₂O emissions while improving production, and highlights research issues and the need for a systems approach to the evaluation of the relative merits of abatement options.

Grainger C., Clarke T., Auldist M.J., Beauchemin K.A., McGinn S.M., Waghorn G.C. and **Eckard R.J.** (2009). Potential use of Acacia mearnsii condensed tannins to reduce methane emissions and nitrogen excretion from grazing dairy cows. Canadian Journal of Animal Science 89 (2), 241-251. doi:10.4141/CJAS08110

Abstract: We measured the effect of condensed tannins (CT) extracted from the bark of the Black Wattle tree (Acacia mearnsii) on the milk production, methane emissions, nitrogen (N) balance and energy partitioning of lactating dairy cattle. Sixty lactating cows, approximately 32 d in milk grazing ryegrass pasture supplemented with 5 kg d 1 cracked triticale grain, were allocated to three treatments: Control, Tannin 1 (163 g CT d 1) or Tannin 2 (326 g CT d 1 initially, reduced to 244 g d 1 CT by day 17). Cows were dosed twice daily after milking for 5 wk with the powdered CT extract (mixed 1:1 with water). Low and high CT supplementation reduced (PB0.05) methane emissions by 14 and 29%, respectively (about 10and 22% on an estimated dry matter intake basis). However, milk production was also reduced by the CT (PB0.05), especially at the high dose rate. Milk yields were 33.0, 31.8 and 29.8 kg cow 1 d 1. Tannin 2 also caused a 19% decline in fat yield and a 7% decline in protein yield, but protein and lactose contents of milk were not affected by CT supplementation. After the initial 5-wk period, five cows representative of each treatment group were moved to metabolism facilities to determine effects of CT on energy digestion and N balance over 6 d. The energy digestibility was reduced (PB0.05) from 76.9 (Control) to 70.9 (Tannin 1) and 66.0% (Tannin 2) and the percentage of feed N lost to urine was reduced (PB0.05) from 39 to 26% and 22% for the respective treatments. The CT also caused a reduction (PB0.05) in intake during the metabolism study, effectively increasing CT as a percentage of intake. Although CT can be used to reduce methane and urinary N losses from cows fed pastures with a high crude protein (CP) concentration, reduced milk yield in this study suggested the dietary concentration was too high. If CT are to be considered as a means for lowering methane emissions further research is needed to define impacts of lower doses of A. mearnsii CT on methane production and cow productivity. Dairy producers will be reluctant to adopt feeding practices that compromise profitability.

Grainger C., **Williams SRO.**, **Eckard RJ** and Hannah MC. (2010) A high dose of monensin does not reduce methane emissions of dairy cows offered pasture supplemented with grain. Journal of Dairy Science 93, 5300-5308. doi:10.3168/jds.2010-3154

Abstract: The primary objective of our research was to determine the effect of a high dose of monensin supplementation on enteric CH₄ emissions of dairy cows offered a ryegrass pasture diet supplemented with grain. An additional objective was to evaluate effects on milk production and rumen function, because a commensurate improvement in milk production could lead to adoption of monensin as a profitable strategy for methane abatement. Two experiments were conducted (grazing and respiratory chambers) and in both experiments monensin (471 mg/d) was topdressed on 4 kg (dry matter)/d of rolled barley grain offered in a feed trough twice daily at milking times. In the grazing experiment, 50 Holstein-Friesian cows were assigned randomly to 1 of 2 groups (control or monensin). Cows grazed together as a single herd on a predominantly ryegrass sward and received monensin over a 12-wk period, during which time measurements of milk production and body weight change were made. The SF₆ tracer technique was used to estimate methane production of 30 of the 50 cows (15 control cows and 15 monensin cows) for 3 consecutive days in wk 3, 5, 8, and 12 of treatment. Samples of rumen fluid were collected per fistula from 8 of the 50 cows (4 per diet) on 2 consecutive days in wk 3, 5, 8, and 12 of treatment and analyzed for volatile fatty acids and ammonia-N. In the metabolic chamber experiment, 10 pairs of lactating dairy cows (control and monensin) were used to determine the effects of monensin on methane emissions, dry matter intake, milk production, and body weight change over a 10-wk period. Methane emissions were measured by placing cows in respiration chambers for 2 d at wk 5 and 10 of treatment. Cows received fresh ryegrass pasture harvested daily. Monensin did not affect methane production in either the grazing experiment (g/d, g/kg of milk) or the chamber experiment (g/d, g/kg of dry matter intake, g/kg of milk). In both experiments, milk production did not increase with addition of monensin to the diet. Monensin had no effect on body weight changes in either experiment. Monensin did not affect volatile fatty acids or ammonia-N in rumen fluid, but the acetate to propionate ratio tended to decrease. Monensin did not improve milk production of grazing dairy cows and no effect on enteric methane emissions was observed, indicating that monensin cannot be promoted as a viable mitigation strategy for dairy cows grazing ryegrass pasture supplemented with grain.

Grainger C., **Williams SRO**, Clarke T, Wright ADG, and **Eckard RJ.** (2010) Supplementation with whole cottonseed causes a long-term reduction of methane emissions from dairy cows offered a forage and cereal grain diet. Journal of dairy Science 93, 2612-2619. doi:10.3168/jds.2009-2888

Abstract: The objective of our work was to supplement a forage and cereal diet of lactating dairy cows with whole cottonseed (WCS) for 12 wk and to determine whether the expected reduction in CH₄ would persist. A secondary objective was to determine the effect of supplementing the diet with WCS on milk yield and rumen function over the 12-wk feeding period. Fifty lactating cows were randomly allocated to 1 of 2 diets (control or WCS). The 2 separate groups were each offered, on average, 4.2 kg of DM/cow per day of alfalfa hay (a.m.) and 6.6 kg of DM/cow per day of ryegrass silage (p.m.) on the ground in bare paddocks each day for 12 wk. Cows in each group were also individually offered dietary supplements for 12 wk in a feed trough at milking times of 5.4 kg of DM/cow per day of cracked wheat grain and 0.5 kg of DM/cow per day of cottonseed meal (control) or 2.8 kg of DM/cow per day of cracked wheat grain and 2.61 kg of DM/cow per day of WCS. The 2 diets were formulated to be similar in their concentrations of CP and ME, but the WCS diet was designed to have a higher fat concentration. Samples of rumen fluid were collected per fistula from the rumen approximately 4 h after grain feeding in the morning. Samples were taken from 8 cows (4 cows/diet) on 2 consecutive days in wk 2 of the covariate and wk 3, 6, 10, and 12 of treatment and analyzed for volatile fatty acids, ammonia-N, methanogens, and protozoa. The reduction in CH4 emissions (g/d) because of WCS supplementation increased from 13% in wk 3 to 23% in wk 12 of treatment. Similarly, the reduction in CH₄ emissions (g/kg of DMI) increased from 5.1% in wk 3 to 14.5% in wk 12 of treatment. It was calculated that the average reduction in CH4 emissions over the 12-wk period was 2.9% less CH₄ per 1% added fat, increasing from 1.5% in wk 3 to 4.4% less CH₄ in wk 12. There was no effect of WCS supplementation on rumen ammonia- N, rumen volatile fatty acids, rumen methanogens, and rumen protozoa. On average over the 12-wk period, supplementation with WCS decreased the yield of milk (10%), fat (11%), protein (14%), lactose (11%), and fat plus protein (12%) and BW gain (31%). The WCS supplementation had no effect on milk fat concentration but resulted in a decrease in concentration of protein (5%) and lactose (11%). The major finding from this study is that addition of WCS to the diet of lactating dairy cows resulted in a persistent reduction in CH_4 emissions (g of CH_4/kg of DMI) over a 12-wk period and that these reductions in CH₄ are consistent with previous work that has studied the addition of oilseeds to ruminant diets.

Henry, B. **Eckard, R.** (2009) Greenhouse gas emissions in livestock production systems. Tropical Grasslands, 43, 232-238

Abstract: Agriculture is responsible for a significant proportion of total anthropogenic greenhouse gas emissions (perhaps 18% globally), and therefore has the potential to contribute to efforts to reduce emissions as a means of minimising the risk of dangerous climate change. The largest contributions to emissions are attributed to ruminant methane production and nitrous oxide from animal waste and fertilised soils. Further, livestock, including ruminants, are an important component of global and Australian food production and there is a growing demand for animal protein sources. At the same time as governments and the community strengthen objectives to reduce greenhouse gas emissions, there are growing concerns about global food security. This paper provides an overview of a number of options for reducing methane and nitrous oxide emissions from ruminant production systems in Australia, while maintaining productivity to contribute to both objectives. Options include strategies for feed modification, animal breeding and herd management, rumen manipulation and animal waste and fertiliser management. Using currently available strategies, some reductions in emissions can be achieved, but practical commercially available techniques for significant reductions in methane emissions, particularly from extensive livestock production systems, will require greater time and resource investment. Decreases in the levels of emissions from these ruminant systems (i.e., the amount of emissions per unit of product such as meat) have already been achieved. However, the technology has not yet been developed for eliminating production of methane from the rumen of cattle and sheep digesting the cellulose and lignin-rich grasses that make up a large part of the diet of animals grazing natural pastures, particularly in arid and semi-arid grazing lands. Nevertheless, the abatement that can be achieved will contribute significantly towards reaching greenhouse gas emissions reduction targets and research will achieve further advances.

Moate P.J., Williams S.R.O., Grainger C., Hannah M.C., Ponnampalam E.N., and Eckard R.J. (2011). Influence of cold-pressed canola, brewers grains and hominy meal as dietary supplements suitable for reducing enteric methane emissions from lactating dairy cows. Animal Feed Science and Technology 166-167:254-264.

Abstract: There are limited data in the literature concerning in vivo effects of dietary fat supplementation on enteric CH₄ emissions from lactating dairy cows. The purpose of this experiment was to evaluate four dietary treatments designated as control (CON), brewers grains (BG), hominy meal and cold-pressed canola (HCC) and hominy meal only (HM) for their effects on CH₄ emissions and milk production. Sixteen late lactation Holstein cows were used in pairs, in a double 4 × 4 Latin square experiment with the four dietary treatments fed as total mixed rations over 24 d treatment periods. All diets contained ~600 g forage/kg dry matter (DM; 5 kg DM of alfalfa hay and 7 kg DM of perennial ryegrass silage/day). The CON diet contained 303 g/kg DM of cracked wheat grain and 70 g/kg DM of solvent extracted canola meal and the CON diet was formulated to contain ~26 g total fat/kg DM. For the BG, HCC and HM diets, part of the cracked wheat and solvent extracted canola was substituted with the designated fat supplement so that the resulting diets contained 51, 52 and 65 g total fat/kg DM respectively. Fat supplementation did not influence DM intake and there were only small (P<0.05) positive effects on milk yield and negative effects on concentrations of milk fat and milk protein. The HM diet reduced (P<0.05) CH₄ emissions when expressed either as g CH₄/cow/d, g CH₄/kg DM intake, or g CH₄/L milk. The BG diet also (P<0.05) reduced CH₄ emissions when expressed as g CH₄/cow/d or g CH₄/L milk, while the HCC diet decreased CH₄ emissions in terms of g CH_4/L milk. Combining data from the fat supplemented diets enabled comparison of CH_4 emissions from the CON diet with CH_4 emissions from the fat supplemented diets. Fat supplementation reduced (P<0.05) CH₄ emissions: 500, 462 g CH4/cow/d; 25.0, 23.2 g CH₄/kg DM intake and 23.3, 20.5 g CH₄/L milk for the CON and fat supplemented groups respectively. Similarly, by combining data from all fat supplemented groups, regression analysis revealed that fat supplementation reduced CH₄ emissions for at least 7 wk. Combining results of this investigation with data from the literature, we conclude that for each increase of 10 g/kg DM in dietary lipid concentration, enteric emissions are reduced by 0.79 g CH4/kg DM intake or ~3.5% thereby allowing estimation of the magnitude of enteric CH₄ abatement based on dietary fat supplementation.

Williams SRO., Moate PJ., Hannah MC., Ribaux BE., Wales WJ., and Eckard RJ., (2011). Background matters with the SF₆ tracer method for estimating enteric methane emissions from dairy cows. Published on 17/9/2011: Animal Feed Science and Technology, 170, 265-276. DOI: 10.1016/j.anifeedsci.2011.08.013.

Abstract: Since its inception, the sulfur hexafluoride (SF₆) tracer technique for estimating ruminal methane (CH_4) emissions has undergone several refinements. One key divergence from the original description of the method has been its use with animals housed indoors. Given the different molecular masses of CH₄ (16 g/mol) and SF₆ (146 g/mol) it is possible that these gases could disperse and accumulate differentially within animal houses. The purpose of this study was to examine and compare the ambient outdoor concentrations of CH₄ and SF₆ with background concentrations measured during indoor experiments. A literature search found 52 scientific papers which reported use of the SF₆ tracer technique with 17 reporting use indoors, 31 outdoors and 4 were desktop reviews or an uncommon implementation of SF₆ as a tracer. Complete details of where background concentrations were measured, and how they were used, were not provided in any of the papers. Concentrations of CH₄ in open air at Department of Primary Industries, Ellinbank, Victoria, Australia (38°14 S, 145°56 E) were variable at about 2.6 mol/mol which was about 50% higher than those of 1.73 measured at the Cape Grim Baseline Air Pollution Station (40°41_S, 144°41_E). This difference was thought to be due to the CH_4 emissions from cows in the Ellinbank area. During the same period, the SF₆ concentration in open air at DPI Ellinbank increased from 4.9 pmol/mol in November 2003 to 6.8 pmol/mol in March 2010. This trend was similar to those measured at Cape Grim. Inside the DPI Ellinbank animal house, which is open to atmosphere on 2 sides, the accumulation of gases during experiments varied in a guadratic manner along the line of feeding stalls with the CH₄ concentration ranging from 4 to 10 mol/mol and SF6 ranging from 4 to 26 pmol/mol. Vertically, background concentration of CH4 trended from 4.6 _mol/mol at 225 mm above the floor to 12.3 mol/mol at 1775 mm while SF6 trended from 8.2 to 14.9 pmol/mol at the same heights. Calculations showed that use of inappropriate background values to calculate CH₄ emissions could lead to discrepancies ranging from -6.2% to +0.8% on an emission of 500 g CH₄/cow/d. Thus, we recommend use of distributed sentinel canisters for monitoring accumulation of gases within animal houses, and using local

background values to correct CH4 and SF₆ measurements from individual animals.

Manuscripts submitted to peer reviewed scientific journals

- Elizabeth M. Ross, **Peter J Moate**, Carolyn R Bath, Sophie E Davidson, Tim I Sawbridge, Kathryn M Guthridge, Ben G Cocks & Ben J Hayes (2011). Untargeted massively parallel sequencing reveals bovine rumen metagenome variation. Submitted in October 2011 to PLoS ONE.
- Williams, S. R. O., Clarke, T., Hannah, M. C., Marett, L. C., Wales, W. J., Moate, P. J., and Auldist, M. J. (2011). Energy partitioning in herbage-fed dairy cows offered supplementary grain during an extended lactation. Submitted in September 2011 to Journal of Dairy Science.

Manuscripts in preparation

- Durmic, Z., **Moate, P. J.**, Arash, A., Revell, D. K., and Vercoe, P. E. (2012). *In vitro* screening of plant essential oils, dietary additives, plant extracts and industry by-products for methane mitigation from ruminants in Australia. To be submitted to: Animal Feed Science and Technology.
- Moate, P. J., Williams, S. R. O., Hannah, M. C., Ponnampalam, E., Eckard, R. J., Auldist, M., Ribaux, B. E., Wales, W. and Jacobs J. (2012) Influence of dietary DHA supplementation on feed intake, milk production and methane emissions of dairy cows. To be submitted to Journal of Dairy Science.
- Moate, P. J., Williams, S. R. O., Hannah, M. C., Eckard, R. J., Auldist, M., Ribaux, B. E., Wales, W. (2012). Grape marc reduces methane emissions of dairy cows when fed as a feed supplement. To be submitted to Journal of Dairy Science.
- Williams, S.R.O., Berrisford, T., Fisher, P.D., Moate, P. J., Reynard, K. Reducing methane on-farm can reduce net global emissions. To be submitted to The International Journal of Life Cycle Assessment.

Book chapters

The following four book chapters have been submitted for publication in 'Guidelines for using the SF_6 technique to measure methane emissions from ruminants' to be published by The Global research Alliance on Agricultural Greenhouse Gases. Palmerston North, New Zealand.

Chapter 5 Calibration and Performance of Permeation Tubes. By **Peter J. Moate**, **S. Richard O. Williams**, Matthew H. Deighton and Keith Lassey.

- Chapter 8 Sampling background air. **S. Richard O. Williams**, **Peter J. Moate** and Matthew H. Deighton
- Chapter 11. Measurement of methane emission rates and methane yield. **Peter J. Moate**, **S. Richard O. Williams**, Matthew H. Deighton, Cesar Pinares-Patino, Ben Vlaming and Keith Lassey.
- Chapter 13 What SF₆ detail should be reported in publications? **S. Richard O. Williams** and **Peter J. Moate**

Conference papers and abstracts

Aprianita A, Donkor N, Moate P, Auldist M, Greenwood J, Wales B, Vasiljevic T (2011). Effect of methane emission reducing diet on coagulation properties of bovine milk OF. American Dairy Science Association annual meeting. New Orleans, Louisiana, 10 - 14 July, 2011 (Abstract).

- Cowie A., **Eckard R**, Eady S (2011). Greenhouse Gas Accounting for inventory, emissions trading and lifecycle assessment in the land-based sector. Proceedings of the CCRSPI Conference, Melbourne, 15th to 17th February 2011, p108.
- Deighton, M.H., Boland, T.M., O'Loughlin, B.M., Moate, P.J. and Buckley, F. (2011). Assessing enteric methane emissions from ruminants using a calibrated tracer: effects of non-linear sulphur hexafluoride release and permeation tube rumen residence time. Proceedings of the 62nd Annual Meeting of the European Federation of Animal Science, Stavanger, Norway, 29th August – 2nd September, p228
- Durmic Z., Revell D.K., Charmley E., Tomkins N., **Eckard R., Moate P.**, Vercoe P.E (2011) Antimethanogenic plants for grazing systems. Proceedings of the CCRSPI conference, Melbourne, 15th to 17th February 2011, p40.
- **Eckard RJ** and Barlow EWR (2011) Can we achieve net reductions in greenhouse gas emissions and meet global food demand? Proceedings of the CCRSPI conference, Melbourne, 15th to 17th February 2011, p49.
- Hannah, M., Moate, P. J., (2011) Fitting Michaelis-Menten directly to substrate concentration data. Australasian Applied Statistics Conference (GenStat & ASReml). 12 - 15 July, 2011, Palm Cove, Queensland.
- Henry B., Charmley E., **Eckard R.**, Gaughann J., Hegarty R. (2011). Livestock production in a changing climate. Proceedings of the CCRSPI conference, Melbourne, 15th to 17th February 2011, p38.
- Moate PJ, Williams SRO, Grainger C, Hannah MC, Eckard RJ. (2011) By-products fed to cows reduce methane emissions. Proceedings of the CCRSPI conference, Melbourne, 15th to 17th February 2011, p43.
- Moate PJ, Williams SRO, Hannah MC, Eckard RJ, Jacobs J. (2011) Methane emissions unaffected when DHA fed to cows. Proceedings of the CCRSPI conference, Melbourne, 15th to 17th February 2011, p41.

News paper and magazine articles, radio interviews

- 30th September 2009: ABC radio interview ABC Ballarat & S/West Vic. Richard Eckard.
- 28th February 2010: ABC Landline interview and TV segment. Peter Moate and Richard Eckard. http://www.abc.net.au/landline/content/2010/s2832333.htm
- 2nd March 2010: ABC Country Hour interview, Richard Eckard.
- Peter Moate was interviewed by Darren Gray of The Age and an article about the research from this project was published in the Age on 23/5/2011. This article or variations of this article appeared in at least 11 other newspapers across Australia including: Cohuna Farmers Weekly (1/6/2011 p6); Wimmera Mail Times (1/6/2011 P91); Bairnsdale Advertiser (30/5/2011, P28); Colac Herald (30/5/2011 P12); Hamilton Spectator (28/5/2011 P20); Portland Observer (27/5/2011 P27); Warnambool Standard (26/5/2011 P4); Stock & Land (26/5/2011 P62); The Warragul Gazette (31/5/2011 P38); Bendigo Advertiser (25/5/2011 P34); Sheparton News (25/5/2011 P8).
- Peter Moate was interviewed by Angus Thompson of The weekly Times and an article was published in The Weekly Times on 1/6/2011. Following the above articles in The Age and The Weekly Times, Peter Moate was also interviewed by 7 radio stations regarding Methane Abatement from dairy cows. These

included: 3AW Melbourne Breakfast (5/23/2011 7:19AM); ABC Ballarat 7.30 News (5/24/2011 733AM); ABC Ballarat Country Hour 5/23/2011 12:50PM; ABC Statewide Drive (5/23/2011 3:06 PM); ABC Riverina (Wagga Wagga NSW Statewide Drive 5/23/2011, This interview was syndicated to the following 8 stations ABC Central NSW, ABC Coffs harbour, ABC Illawarra, ABC Taree, ABC Tamworth, ABC Lismore, ABC Bega, ABC Dubbo); 2UE Sydney 12:00 News (5/23/2011 12:02 PM); ABC Gippsland (Sale 7:30 News 5/26/2011 734); ABC South Western Victoria Warnambool (6:30 news 5/24/2011 6:32 AM); 3WM Horsham (Country Today 5/26/2011 12:33 PM); 2NM (Muswellbrook) Southern Cross rural News 5/23/2011 12:17PM).

- Peter Moate was interviewed by Angus Thompson of The weekly Times and an article was published in The Weekly Times on 1/6/2011.
- Peter Moate was interviewed in July 2011 by a reporter and an article: 'Curbing dairy emissions a question of diet' was published on page 41 of the Nov/Dec edition of The Australian Dairy Farmer.
- Richard Eckard was interviewed on SBS radio on the 24th May 2011.
- Richard Eckard was interviewed for ABC Landline on the Carbon Farming Initiative. The shows aired as follows: Carbon's Bill Broadcast: 5/06/2011 1:59:21 PM; Carbon's Bill Broadcast: 12/06/2011 2:01:54 PM; Carbon's Bill 3 Broadcast: 19/06/2011 2:50:01 PM.
- Richard Eckard profiled the methane research on Channel 10 SCOPE Science program, screened on Saturday 11th June, 2011.

Extension activities

Project personnel have attended a large number of conferences, workshops, seminars, industry forums, farmer groups, students and field days and given many presentations and talks to promote awareness of this project and other methane abatement research being conducted nationally. These events included

- 20th April 2009. How dairy impacts climate The real carbon cycle in dairy. Australia and New Zealand Co-Operative Leaders Forum, Aitken Hill Conference Centre Melbourne.
- 5th May 2009. BMP's What can be achieved by Australian farmers? National Climate Change Technical Working Group, Canberra.
- 12th May 2009. Agricultural Greenhouse Emissions what's current & implications of the CPRS. Field Day, Ventnor, Phillip Island.
- 21st July 2009. The methane story. Training session for 25 extension staff in DPI, Hamilton.
- 31st July 2009. Greenhouse Gas Emissions Abatement Options and Likely Policy Constraints. New Farmer Inductions, Western Port Agricultural Greenhouse Emissions and Resource Efficiency Project. Koo Wee Rup.
- 3rd August 2009. Overview of methane and nitrous oxide research for Dairy. Murray-Goulburn MG FarmC@re - Environment Advisory Meeting, Melbourne.
- 17th August 2009. Impact of CPRS on Cattle and Sheep Farmers implications for Ruminant Nutrition. Keynote speaker, Australian Association of Ruminant Nutrition.

- In June and July 2009 Richard Eckard met with researchers in Brazil and Canada to further develop international collaboration with the RELRP team. These visits included:
- On 23rd to 25th June 2009, Richard Eckard met with Prof Adibe Abdulla, University of Saõ Paulo, Brazil and discussed research on methane emissions from sheep fed dietary supplements and *in vitro* techniques.
- On 29th June to 1st July 2009, Richard Eckard met with Drs Sean McGinn and Karen Beauchemin, Agriculture and Agri-Foods, Lethbridge, Canada, to discuss their Open Path techniques and dietary supplement research
- 7th October 2009: Marcus Oldham college Industry forum. "Australian Agriculture in an Emissions Constrained Future", Richard Eckard.
- 15th October 2009: ADIC forum, Farm and Carbon emissions. "Abatement options: what is possible? Richard Eckard.
- 20th October 2009: Dairy Farmers Forum, Opal Cove Resort Coffs Harbour, "Climate Change, Emissions and the Dairy Industry", Richard Eckard.
- 2nd December 2009: DPI Climate Change Showcase, Canberra, "Overview of FFSRD Climate Change Research in Victoria", Richard Eckard.
- 2nd December 2009: DPI Climate Change Showcase, Canberra, "Mitigation in the Dairy industry", Joe Jacobs.
- 8th February 2010: Local farmer group at Ellinbank, Richard Eckard.
- 12th February 2010: Greenhouse in Agriculture extension messages day, Melbourne, "Options for agricultural greenhouse gas mitigation", Richard Eckard.
- 2nd March 2010: ABARE conference Canberra. "Options for agricultural greenhouse gas mitigation", Richard Eckard.
- 7th to 10th March 2011. Peter Moate and Richard Williams attended the Global Alliance SF6 workshop, at Palmerston North, New Zealand, and presented results of improvements to the SF6 method developed at DPI Ellinbank. The objective of this workshop is to produce a technical manual of the use of the SF6 technique to measure methane from ruminants. Peter Moate and Richard Williams have co-authored four chapters in a manual describing techniques for measuring methane from ruminants using the SF6 method.
- 20th April 2011. Richard Eckard demonstrated the Ellinbank chambers and the methane research to the Victorian Minister of Agriculture and Food Security, Peter Walsh.
- 26th May 2011. Richard Eckard was guest speaker at the Future Ready Dairy Systems Project day at Ellinbank.
- 12th May 2011. Reducing Emissions from Livestock Program, Terang field day,. 30 producers attended. Richard Eckard was keynote speaker "More than just hot air, Get the goods on greenhouse gases and working with climate variability".
- 16th June 2011. Richard Eckard and Peter Moate attended a DA sponsored meeting (Dairying for Tomorrow/FRDS Workshop) in Melbourne. The meeting was for high level executives from Dairy Companies and Company Extension personnel. Richard and Peter gave presentations describing the major findings from this project.
- 16th June 2011. Richard Eckard and Peter Moate presented the research at a Dairying for Tomorrow Coordinators meeting in Melbourne.

- 21st and 22nd June 2011. Richard Eckard presented results from this project to the Intelact Conference in Melbourne.
- Data from this research has been included in the DAFF National CFI Training program, developed and currently being presented by Richard Eckard to all National Landcare Facilitators in the major capital cities in July, August and September 2011.
- 2nd of August 2011. Peter Moate and Richard Eckard attended a "RELRP Whole Program Science Review" at MLA headquarters in Brisbane.
- 12th October 2011. Information from this project was used in a presentation by Richard Eckard at the Central Victorian Highlands Agribusiness forum.
- 24th October 2011. Summary results presented to Industry and Policy (DCCEE and DAFF) in Canberra at the PICCC Climate Change Showcase.

6.2 Methane emission from sheep grazing two pasture systems, ryegrass and plantain measured using Open-Path FTIR spectroscopy

Report provided by Dr Frances Phillips, Centre for Atmospheric Chemistry, University of Wollongong in August 2011, in completion of a sub-contract agreement between University of Wollongong and DPI Victoria, dated 26 October 2010 and being part of MLA contract BCCH 1009. Contact details: <u>francesp@uow.edu.au</u>, telephone 0242 214104

Aim:

The project aimed to demonstrate the capability of open path FTIR (OP-FTIR) spectroscopy to measure methane (CH_4) emissions from sheep in a grazing environment, develop the procedures and protocols for measuring those emissions and to define the uncertainty in the emission estimates. Part III of the project aimed to determine if differences in emissions from sheep grazing two pasture systems, plantain and ryegrass can be detected using the OP-FTIR system and to define the uncertainty in those emission estimates.

Introduction

This report details the third and final measurement trial designed to develop the techniques and protocols to measure CH_4 emissions from sheep and to define the uncertainty in those emission estimates. Emissions of CH_4 from sheep in a grazing environment are particularly difficult to measure due to the low emissions per animal and the distribution of the sheep over an extended grazing area. In this third trial, based on the techniques and experience developed in the previous two trials, the CH_4 emissions from a mob of sheep grazing two pastures, plantain and ryegrass were measured. Plantain was thought to result in lower CH_4 emissions due to the high levels of tannins present in the leaf, however experimental data is limited to quantify any reduction in emissions (Pers. Comm. Dr Matt Robinson, DPIVic). Ryegrass is a commonly used pasture for sheep production in the southwest Victorian region.

Methods

The trial took place in October and November 2010 at the DPI Victoria's Hamilton Research Centre, on the north-east corner of the EverGraze site, in a similar location to Part 1 of this project conducted in March 2009. The site is part of the Adaptation and Mitigation project (Dr Matt Robinson, DPIVic), a replicated pasture trial including plantain, ryegrass and Lucerne and designed to identify pasture species and mixture of species to provide resilience to climate variability and reduce greenhouse gas (GHG) emissions from the sheep industry in the south-west region of Victoria.

The project used OP-FTIR spectroscopy to measure the CH_4 mixing ratio in the air downwind from the grazing animals. Measurements are made in conjunction with a tracer gas released at a known rate close to the animal's mouth and measured simultaneously with the CH_4 at the instrument path. The tracer gas models the dispersion of the CH_4 plume between the animals and the instrument and allows the emission rate to be derived from the mixing ratio. A full description of the OP-FTIR instrument, the tracer-gas release system and the backpacks used to mount the tracer-gas canisters onto the sheep, plus factors that influence the paddock layout are given in the previous reports, and only a brief description is included below.

Pasture system and paddock design

The flock used comprised 30 Merino sheep, with measurements made from the same sheep for the two pastures. Animals were acclimatised to the plantain for 1 week before start of measurements and to the ryegrass for 48 hours between measurements. This may not be sufficient time for the rumen microbes to fully adjust to the new feed.

Each of the plots within the Adaption and Mitigation trial are approximately nine meters wide and several hundred meters long. This experiment utilised the northern ends of plot 4 replicate 4 for the plantain and plot 2 Replicate 4 for ryegrass. Two enclosures, approximately 9 x 100 m, for each pasture (Figure 1) were constructed using electric mesh fencing, with each 900 m2 area expected to provide 4 to 5 days grazing. Dr Matthew Robertson (DPI Victoria) noted that the plantain was more mature than anticipated and would be less palatable for the animals with much of the beneficial chemicals in the leaves of the plantain possibly degraded. With heavy rain in the area previous to the start of measurements the plantain quickly became trampled and after 3 days grazing the pasture guality had degraded considerably (Figure 2A and B). On 1st November 2010, day 4, the length of the enclosure was increased by 5 m towards the instruments to provide additional grazing area, but this was again badly degraded within 12 hours. Sheep were moved to the second enclosure on the morning of the 2nd November 2010, where the grazing was restricted to only 48 hours, until 9:00 4th November, when sheep were moved onto ryegrass pasture to acclimatise to the new pasture. Measurements recommenced 48 hours later around 9:00 6th November with the ryegrass providing greater pasture mass with less degradation of the pasture (Figure 2C). Grazing was restricted to 3 days per enclosure with the animals moved to the second enclosure on the morning of the 9th November, and measurements continuing until approximately 9:00 12th November.



Figure 1 Paddock layout showing the northern end of Replicate 4, with the position of the 2 animal enclosures and OP-FTIR + reflectors positioned in plot 4 (plantain + Lucerne). The sheep were moved into the portable yard located at the end of the plot each morning to have the canisters exchanged



Figure 2 Plantain (A) pre-grazing, (B) overgrazed and trampled following heavy rain, and (C) ryegrass after 3 days grazing.

OP-FTIR Instrumentation

The OP-FTIR instrument consists of an FTIR spectrometer, (Matrix IR-Cube, Bruker Optik GmbH, Ettlingen, Germany) equipped with a mechanically cooled (-196°C, RicorK508) MCT detector (Infrared Associates Inc., Florida, USA, or Judson Industries, Montgomeryville, PA, USA) coupled to a 250 mm Schmidt-Cassegrain telescope (LX 200ACF, Meade Instruments Corporation, Irvine California, USA). The telescope has been modified to function as a parallel beam expander, expanding the beam from 25 to 250 mm diameter and reducing beam divergence by a factor of ten to 2 mradians. The system is mounted onto a heavy duty tripod (Gibralter model 4-60450-OA, Quickset International Inc., Illinois, USA) with, currently, a manually adjustable head (model 4-62926-7) to allow alignment of the beam between spectrometer and retro-reflector. The spectrometer scans continuously and, in typical operation, records a time-averaged (nominally every 3-minutes) infrared absorption spectrum of the open atmospheric path between spectrometer and retro-reflector located 100 to 130m from the instrument. Each spectrum is analysed immediately after collection using the MALT analysis program to provide path-averaged concentrations of NH₃, N₂O, CO₂, CH₄, CO and water vapour (Griffith, 1996). Operation of the system is fully automated under the control of a laptop computer running a program written at the University of Wollongong (OSCAR, G. Kettlewell).

Two instruments were initially deployed for the trial (serial numbers OP2 and OP3), with a third instrument, owned by DPI Victoria (serial number OP4) included from 7th November until 12th November and operated adjacent to OP2. The instruments were located in the laneway (15 m wide) between the two enclosures for the pasture (Figure 1) on the eastern and western sides of the pasture plots locating the two measurement-paths perpendicular to the predominant wind direction. The geographic bearing of the measurement-path, between the instrument and retro-reflector, was 165, with the orientation of the instruments rotated 180° when sheep were moved between the enclosures. The concentration of any CH₄ originating from an upwind source was measured by both instruments with the CH₄ from experimental animals assumed to be the difference in the CH_4 mixing ratio at the two paths. However as the two instruments are sampling different regions of the CH₄ plume originating from the remote source, the CH₄ mixing ratio at the two paths will differ slightly, potentially introducing an error in the estimated emissions. This error increases with increasing distance between the measurement paths, increasing strength of the upwind source, decreasing distance between the experimental animals and the upwind source and decreasing wind speed. The experimental site at Hamilton Research Centre is embedded within the EverGraze project and surrounded by small groups of sheep and cattle, which due to operational requirements of EverGraze, are difficult to

relocate. The very narrow plots employed here are an advantage as the difference in the CH_4 mixing ratio from an external source measured at the two instrument paths is minimised, and dispersion of the plume from the experimental animals at the instrument is limited.

Tracer-gas

The tracer-gas canisters are 240 x 60 mm diameter aluminium canisters commonly used as "paint ball" canisters fitted with a head encompassing a capillary tube (PEEKsil HPLC capillary tubing, 0.025mm inner diameter, SGE Analytical Science Pty Ltd, Ringwood, Vic. Australia) to limit the flow-rate of tracer gas to around 10 gh⁻¹. Laboratory tests showed an increase in flow rate with the canister in a horizontal position, as on the sheep's back, compared to vertical as attached to cattle, and the length of the capillary was increased from 25 to 35 mm to ensure a flow rate of ~10 gh⁻¹. The canisters were mounted on the sheep's back using a purpose built canvas backpack designed by staff at DPI Victoria, and attached via Velcro strips glued onto the back (Figure 3). As the wool of the sheep was only a few millimetres long, the wool did not require clipping and the Velcro was glued directly to the wool. A full gas canister weighs between 900 and 1100 g, and once the sheep are trained to the canister, appeared to have minimal detrimental effect on the sheep's behaviour. A neoprene sleeve (~3 mm thick) provided some protection from temperature extremes,

Tracer gas canisters were attached to the back of, initially, 20 sheep. This was reduced to 19 on the 31st November when a problem was identified with one of the backpacks. Each canister was filled with approximately 300 g of N_2O (liquid nitrous oxide, engine boost grade, product code 624, BOC Australia, Sydney, NSW, Australia) as the tracer gas, with canisters replaced each day when the sheep were moved into a portable yard located adjacent to the plots. The animals were absent from the enclosure for ~30 minutes while the canisters were exchanged.

The average tracer-gas flow-rate for each canister is determined from the weight loss of gas and the release time. However, as the instantaneous flow-rate of the gas varies with temperature the canister temperature is monitored using temperature logging buttons (logging interval 3 minutes; Thermocron eTemperature model TCS, OnSolutions, Baulkham Hills, NSW, Australia) attached to each canister.



Figure 3. The sheep with the backpack and canister mounted to their backs.

Calculation of CH₄ flux

The time (t, hours) and temperature (T, $^{\circ}$ C) dependent flow rate of the N₂O from a canister, F(t), (gh⁻¹) can be calculated from the relationship:

$$F(t) = F_0 + \frac{dF}{dT} (T(t) - T_0) \quad \text{Eq. 1}$$

Where $\frac{dF}{dT}$ is the temperature dependence of the flow rate, determined in the

laboratory by monitoring the flow rate at a range of temperatures as 0.184 \pm 0.036 g h⁻¹°C⁻¹, F₀ is the canister flow rate (gh⁻¹) at T₀=0°C.

As the integrated flow rate over the release time, $t_{r},$ is equal to the mass of gas lost Δm (g),

$$\int_{0}^{t_{r}} F(t) dt = \Delta m \qquad \text{Eq. 2}$$

 F_0 can be calculated from Eq. 1 and 2 such that:

$$F_0 = \frac{\Delta m}{t_{t_r}} - \frac{dF}{dT} \int_0^{t_r} \left(T(t) - T_0 \right) dt \qquad \text{Eq. 3}$$

 F_0 is calculated for each canister from Eq. 3, allowing F(t) to be calculated at temperature T and time t from Equation 1. The time-temperature dependent N₂O emission rate is the sum of the flow the total number of canisters, n

$$Q_{N_2O(t)} = \sum_{i=1}^{n} F_i(t)$$
 Eq. 4

 $Q_{N2O}(t)$ is interpolated from the time resolution of the temperature buttons to that of the CH₄ and N₂O volume mixing ratio data.

The CH₄ emission, Q_{CH4} , at time t is calculated from the relationship:

$$Q_{CH_4} = \frac{\Delta[CH_4]}{\Delta[N_2O]} Q_{N_2O} * \frac{MWt_{CH_4}}{MWt_{N_2O}} * \frac{24}{n_{animals}}$$
 Eq. 5

where: $Q_{CH4} = \text{flux CH}_4$ at time t (ghead⁻¹day⁻¹), $Q_{N2O} = \text{time-temperature emission of}$ the tracer gas, N₂O at time t as calculated above (gh⁻¹), $\Delta [CH_4] = \text{enhancement in}$

CH₄ mixing ratio over local background mixing ratio (ppbv) and $\Delta [N_2 O] =$

enhancement in N₂O mixing ratio over local background mixing ratio (ppbv) both at time t, MWt_{CH4} and MWt_{N2O} are the molecular weights of CH₄ (16.0 gmol⁻¹) and N₂O (44.0 gmol⁻¹), n_{animals} is the number of animals, and 24 (hday⁻¹) converts the flux from per hour to per day.

Weather Station and Meteorological Criteria

A weather station, installed close to and north of the enclosures, provided 3dimensional wind speed and wind direction data at 10 Hz resolution and averaged to 15 minutes (sonic anemometer, CSAT3, Campbell Scientific Inc, Logan Utah, USA), plus wind direction and speed from a wind sentry and cup anemometer (03001 RM Young Wind Sentry set, Campbell Scientific Inc, Logan Utah, USA) air temperature (T107, Campbell Scientific Inc, Logan Utah, USA) and humidity (HMP55C, Campbell Scientific Inc, Logan Utah, USA) measured each minute and averaged to 5 minutes. All data were recorded to a data logger (CR5000, Campbell Scientific Inc, Logan Utah, USA) and downloaded daily.

Emission data quality from the technique is influenced by the weather conditions. To ensure efficient mixing of the CH_4 and tracer gas, and to limit influence of upwind CH4 sources, data are rejected when wind speed < 1-2.0 ms-1. Rain and fog decrease the signal strength at the detector, decreasing the precision of the data. The loss in precision accelerates when signal strength decreases to less then half

the maximum signal strength, when data are rejected. Data collected when the wind direction is within 20° of the measurement path is rejected as the area of the paddock sampled may not be representative of the flock of sheep.

Results

Field Day

The project featured as part of a field day on the 11th November, when several photographers, reporters and others were close to the instruments. Although the instruments were not interfered with, the infra-red beam was interrupted during much of the day with guests inspecting the site.

Meteorological conditions

The weather was generally cool and wet. Measurements were halted and the animals moved to alternate comparable pasture on the 30th November due to predicted heavy rain and strong winds. Maximum temperatures ranged from 12 to 26°C, with minimum temperatures from 4 to 10°C.

The wind speed during the day was generally above 2 ms^{-1} but decreased to $< 2 \text{ ms}^{-1}$ on several evenings with wind speeds $< 1 \text{ ms}^{-1}$ on around 4 nights (Figure 4). Several mornings were marked by fog. With the very narrow plots the wind speed selection criteria was reduced to 1 ms^{-1} . During the first measurement period (plantain) 85% of the data were retained following wind speed and direction selection criteria, and 70% retained for measurement period 2 (Ryegrass). The majority of the rejected data (>90%) were collected during the evening (Figure 4).



Figure 4. Meteorological data, wind speed and direction, while sheep grazed plantain (28/10/2010 to 31/10/2010) and ryegrass (6/11/2010 to 12/11/2010). The dashed lines indicate the limits for the wind direction selection criteria for the two instruments.

Tracer-Gas Flow Rate

The backpacks held the canisters in a horizontal position with the nozzle vertical. Initially 20 canisters were deployed however on the 31st one backpack was found to be faulty when a canister was found on the ground close to where the sheep were camping, after which 19 canisters were deployed. Three sets of canisters were used in rotation, with 231 canisters deployed over 12 days.

With the layer of insulation incorporated into the canister backpacks the variation in temperature measured by the e-temperature buttons was < 1°C (1 σ). The recorded temperature of the canisters was consistently higher then the air temperature, presumably due to the influence of the animal's body warmth and solar radiation. Calibration in the laboratory confirmed the difference in temperature recorded by the e-temp button and the T107 temperature sensor from the weather station was <1°C. Flow rates varied between canisters with differences in capillary length but were consistent for each canister with lower flow rates associated with lower temperatures. Rotation 3 canisters showed consistently lower flow rates (Figure 5). Previously reported problems with blockages of the flow restrictor have largely been overcome

with the replacement of the laser drilled stainless-steel disk with the capillary tubing as the flow restrictor.



Figure 5. The averaged temperature measured by the e-temperature buttons on the canisters compared with the air temperature as measured by the T107 temperature sensor (at height ~1.8 m) and the corresponding time and temperature dependent tracer-gas flow rate averaged over the 20 (19) canisters. The Top Panel is while sheep are grazing plantain and the Lower Panel grazing Ryegrass.

Data: CH₄ and N₂O Mixing Ratio

Data collection started on the morning of the 27th October and continued until approximately 10:00 on the 4th November, with data collected by the two instruments on the 27th, when animals were absent from the site, compared to ensure the two data sets were co-calibrated. The linear regression of CH_4 mixing ratio from the two instruments indicates good agreement with a difference between the two data sets of 0.2% (Figure 6). With the limited range in the N₂O mixing ratio, a regression of the two data sets is not appropriate; however the average difference in the measured N₂O mixing ratio for the two instruments over this time was 0.2±1.0 ppb.



Figure 6. From the linear regression between CH_4 mixing ration measured at OP2 and OP3 instrument paths the difference in retrieved CH_4 is <1% at the two instrument paths.

Plantain

Animals first entered the enclosures at 9:00 28th October and left for the last time on 4th 9:00 November. There is evidence in the data of other sources of CH₄ in the surrounding area, in particular on the 27th October with a source to the south-east to north-west. There isn't any evidence of other significant N₂O sources (Figure 7). Enhancement in CH₄ above background concentration was generally between 25 and 75 ppb, increasing to 150 ppb during the final two days of measurement, and 25 to 150 ppb in N2O. To limit the error in measuring Δ CH4 a minimum increase of 50 ppb is preferred.

Following quality control criteria ~75% of the available CH_4 and N_2O mixing ratio data were retained. Data were not collected on the 30th October due to predicted heavy rain and strong winds, and while data collection resumed on the 31st October continuing rain had a negative impact on data quality and emission estimates are limited to the afternoon and early evening.

Ryegrass

On the 4th November the instruments were relocated to the ryegrass plot. Measurements recommenced on the morning of the 6th November with sheep grazing the southern end of the plot from the 4th to the 6th November to acclimatise to the different pasture. Enhancement above background concentration was generally strong for N₂O at around 50 to 150 ppb, but was lower for CH₄ compared to that from plantain with enhancement around 25 to 50 ppb. There is some evidence of the influence of CH₄ from other animals in the area, particularly when the wind was from the east (Figure 8). Weather conditions were variable, with data quality adversely affected by low wind speeds, fog and rain. In particular available data between midnight and 6 am is limited and of lower quality with increased variability.



Figure 7. Plantain Pasture : CH_4 and N_2O mixing ratio data: Top panels N_2O mixing ratio (ppb), lower panels CH_4 mixing ratio (ppb). Data from 11 are red, 12 in blue, with data filtered from analysis in pale tones, the data from downwind from the animals – Enhanced, in mid tones, and from upwind of the animals, Background, in dark tones.



Figure 8. Ryegrass : CH_4 and N_2O mixing ratio data: Top panels N_2O mixing ratio (ppb), lower panels CH_4 mixing ratio (ppb). Data from 11 are red, 12 in blue, with data filtered from analysis in pale tones, the data from downwind from the animals – Enhanced, in mid tones, and from upwind of the animals, Background, in dark tones.

Measurement Errors and Uncertainty in Emission Estimates

The measurement error in ΔCH_4 and ΔN_2O depends on instrumental precision, 0.6 and 5 ppb for N₂O and CH₄ respectively and the precision in the calibration of the data from two instruments. From the comparison of data collected on the 27th October the error in ΔCH_4 and ΔN_2O is estimated as 6 and 1.0 ppb respectively. The error in the daily average value of Q_{N2O} is low with both the weight loss of tracergas, Δm , and release time, tr being well defined. Sources of uncertainty in the time and temperature dependent flow-rate, Q_{N20 t}, include the temperature of the tracergas, the flow rate temperature dependence dF/dT, and any disruption to the gas flow due to obstructions. An error in dF/dT will introduce a bias or offset in Q_{N2O(t)} which is maximum when $\Delta Tt = |T_t - T_{ava}|$ is maximum, where T_t is the temperature at time t and T_{avg} is the daily average temperature, and decreases as T_t approaches T_{avg} . Sensitivity analysis for these data, where the flow rates were compared when calculated with dF/dT =dF/dT \pm error (dF/dT= 0.148, 0.184 and 0.220 g(°C)-1, Fig. 9) demonstrated the maximum error in $Q_{N2O(t)}$ was ±5% (Δ Tmax=15°C at t= 13:42 28/10/2010) with a corresponding error in Ft. The introduced error in the dailyaveraged emission is reduced from the maximum of 5% with the increasing available data over the release time.

The standard deviation in the average temperature of the canisters, monitored using logging buttons, is < 1° or 6%. Most of this variation will be driven by the exposure of the canister to solar radiation. The error introduced by assuming the CH_4 mixing ratio, originating from a distant source and measured at the two instrument paths are equal is difficult to quantify. However the limited distance between the two instrument paths in this experiment limits the error that may be introduced.

The errors in Figure 10 and 11 are the measurement uncertainty in the emission estimate (purple) and the uncertainty in the emissions derived from the 3-point variation (1 σ) in the individual emission estimates. The variability is typically 1.5 to 2 times greater then the measurement uncertainty. All CH₄emissions are quoted as g CH₄ animal⁻¹day⁻¹, for all averaging periods with uncertainties quoted as ±1 σ of the variation (Tables 1 and 2).



Figure 9. Q_{N2O} was calculated with dF/dT = 0.148 (blue, dF/dT+uncertainty), 0.184 (black, dF/dT) and 0.220 (red, dF/dT-uncertainty)g(°C)⁻¹. The maximum error in $Q_{N2O(t)}$ due to uncertainty in dF/dT was maximum when ΔT (T_{t} - T_{avg}) was maximum. In this work this occurred when t= 28/10/2010 13:42, with a resulting maximum error in $Q_{N2O(t)} = \pm 5\%$. When T=T_{avg}, $Q_{N2O(t)}=Q_{N2O(avg)}$ (black dashed line).

CH₄ Emissions

Plantain

A daily average emission was estimated for 5 days while the animals grazed plantain (Figure 10, Table 1). The increase in emissions on days 1 compared to days 2, 6 and 7 is significant, but differences in emissions on days 2, 6 and 7 are not significant. Although an average daily emission could not be calculated for day 4 (31st October) due to insufficient data, comparison of the average emission between 12:00 and 20:00 demonstrates emissions on days 4 and 5 are similar but higher compared with other days. This may relate to the degradation of plantain quality following grazing and trampling by the animals in the waterlogged conditions. On the 2nd, day 6, the animals were moved onto fresh pasture and emissions returned to similar to that on days 1 and 2. The daily average emission for all available data is 26.9 ± 0.33 g CH₄ animal⁻¹day⁻¹ (column 8 Table 1). If data from day 4 and 5 is excluded this is reduced to 24.7 ± 0.37 g CH₄ animal⁻¹day⁻¹ (last column Table 1). Note data were not collected on day 3 due to heavy rain.

Ryegrass

A total of just under 1300 emission estimates were successfully calculated over the available 7 days (Table 2, Figure 11). However loss of data was concentrated in the early hours of the morning, with only 90 of the possible 720 emission estimates available between 3 to 8 am, largely due to fog and low wind speeds. Due to insufficient data to define the variation in emissions over the day a daily-averaged emission estimate was calculated for only day 1. However comparing emission estimates between 7:00 and 23:00 showed considerable day-to-day variation in emissions. The emission estimate from all available data was 29.9 ± 0.75 gCH₄ animal⁻¹day⁻¹(final column Table 2).

The difference in the daily averaged CH_4 emission from the sheep grazing plantain and ryegrass is significant. The diurnal distribution of the emissions is similar for both pastures (Figure 12, 13), with maximum emissions in the early morning around 6:00 to 7:00am, a minimum near midday, increasing after 14:00 and decreasing again after 20:00. Higher emissions from ryegrass are evident in the early hours of the morning, between midnight and 6:00. This corresponds to the period when limited data while grazing ryegrass are available and the variation in the emission estimates is high. However comparing emissions between 7:00 and 23:00 the difference in emission rates remains significant with 25.4±0.37and 27.4±0.45 gCH4 animal⁻¹day⁻¹ with sheep grazing plantain and ryegrass respectively. **Table 1.** The hourly-averaged CH₄ emission estimates $(gCH_4animal^{-1}day^{-1}\pm 1\sigma)$ from sheep while grazing plantain are calculated by averaging available $Q_{CH4(t)}$ for each hour. An hourly-averaged flux is not calculated when < 4 values for $Q_{CH4(t)}$ are available in that hour. A daily-averaged emission is calculated when sufficient data are available to define the diurnal variation. An averaged-hourly CH₄ emission (included in Table as "All Data") is also calculated by averaging all data over all days for each hour, and the average-emission (gCH₄animal⁻¹day⁻¹) calculated from the average of these data. The emissions measured on day 4 and 5 were noted to be high compared with remaining days and the hourly and daily averaged-emission are also calculated with all data but days 4 and 5 excluded (Excl. 4.5).

Date	28/10	29/10	31/10	1/11	2/11	3/11	All Data	Excl. 4,5
Day	1	2	4	5	6	7		
Hour of	gCH₄animal⁻	gCH₄animal⁻	gCH₄animal⁻	gCH₄animal⁻	gCH₄animal⁻	gCH₄animal⁻	gCH₄animal⁻	gCH₄animal⁻
day	'±1σ	¹ ±1σ	'±1σ	'±1σ	'±1σ	'±1σ	'±1σ	'±1σ
Daily-								
averaged								
flux	26.8±0.6	23.9±1.48	35.6±1.11	30.7±0.68	24.0±0.57	23.6±0.68	26.9±0.33	24.8±0.37
8:00		19±0.81		34.4±1.6			18.3±0.21	17.2±0.24
9:00	23.7±0.52	16.8±1.3		57.1±0.96		17.7±0.86	23.2±0.22	23.8±0.26
10:00	26.2±0.52	25.0±0.92		41.1±0.84		28.4±0.92	25.1±0.33	26.1±0.40
11:00	25.0±0.46	14.2±2.6		55.0±1.1	17.3±0.56	26.1±0.57	21.4±0.27	21.4±.037
12:00	26.9±0.50	20.1±1.2	37.0±1.4	40.1±0.65	17.0±0.51	24.6±0.76	24.4±0.34	25.5±0.45
13:00	23.1±0.38	21.6±0.72	46.8±0.86	35.6±0.67	14.3±0.37	30.6±0.94	21.4±0.25	21.5±0.30
14:00	26.8±0.41	20.6±0.81	34.6±0.62	35.9±0.75	17.7±0.48	22±0.44	25.8±0.45	26.6±0.50
14:00	27.3±0.58	29.8±1.3	33.8±0.70	29.7±0.65	17.1±0.40	35.7±0.82	35.7±0.41	36.2±0.41
16:00	29.7±0.42	49.8±0.79	48.5±1.2	26.2±1.3	20.0±0.54	28.5±0.55	30.6±0.66	32.0±0.70
17:00	30.0±1.1	35.5±0.46	47.2±0.65	31.2±0.61	27.5±0.77	39.3±0.61	25.8±0.77	20.2±0.75
18:00	30.1±0.65	39.3±1.3	44.1±0.80	38.4±0.46	25.9±0.35	22.4±0.46	29.0±0.44	19.0±0.48
19:00	27.0±0.43		32.0±0.62	34.1±0.64	30.1±0.52	30.4±0.57	30.3±0.40	26.5±0.46
20:00	20.2±0.37	29.3±7.4		23.4±0.35	21.7±0.39	19.6±0.30	29.8±0.34	22.1±0.30
21:00	19.9±0.42			21.3±0.48	21.9±0.43	19.6±0.33	27.4±0.30	22.1±0.34
22:00	16.0±0.65	14.7±0.36		23.6±0.40	22.2±0.43	18.6±0.67	28.8±0.29	22.6±0.33
23:00	20.1±0.66	14.2±0.64		22.4±0.42	19.9±0.33	16.1±0.33	26.4±0.24	21.7±0.26
00:00	28.0±0.62	20.8±0.59		20.9±0.37	32.3±0.59	16.2±0.31	28.9±0.29	27.4±0.36
1:00	28.6±0.60	27.0±2.5	24.2±.1.1	20.7±0.31	29.9±1.1	20.4±0.53	32.8±0.31	31.7±0.29
2:00	23.7±0.75			20.9±0.33	23.2±0.49	17.6±0.57	35.9±0.29	33.6±0.35
3:00	34.7±0.53	15.2±1.8		21.7±0.48	25.6±0.96	16.1±0.91	32.7±0.25	27.3±0.27
4:00	28.2±0.59	18.0±1.1	21.5±1.2	21.3±0.49	23.4±0.50	14.7±0.52	31.0±0.26	29.7±0.31
5:00	33.6±0.83			21.1±1.0	24.3±0.42	18.9±1.3	21.9±0.20	21.3±0.22
6:00	37.5±0.63				36.4±0.73	32.8±0.52	20.7±0.21	20.5±0.23
7:00	29.8±1.3		22.3±1.3		37.0±1.1	26.3±1.2	19.3±0.22	18.1±0.26

Table 2. The hourly-averaged CH₄ emission estimates (gCH₄animal⁻¹day⁻¹±1_{σ}) from sheep while grazing ryegrass are calculated by averaging available Q_{CH4(t)} for each hour. An hourly-averaged flux is not calculated when < 4 values for Q_{CH4(t)} are available in that hour. A daily-averaged emission is calculated when sufficient data are available to define the diurnal variation. An averaged-hourly CH₄ emission (included in Table as "All Data") is also calculated by averaging all data over all days for each hour, and the average-emission (gCH₄animal⁻¹day⁻¹) calculated from the average of these data.

Date	6/11	7/11	8/11	9/11	10/11	11/11	All data
Day	1	2	3	4	5	6	1-6
Hour of day	gCH₄animal⁻ ¹±1σ	gCH₄animal⁻ ¹±1σ	gCH₄animal⁻ ¹±1σ	gCH₄animal⁻ ¹±1σ	gCH₄animal⁻ ¹±1σ	gCH₄animal⁻ ¹±1σ	gCH₄animal⁻¹±1σ
Daily- averaged							
	34.3±0.20				00.014.40		29.4±0.75
8:00			40.4.0.00		36.8±1.42		35.1±1.3
9:00	34.6±1.21	26.0±0.71	18.1±0.86		16.8±0.58		24.6±0.46
10:00	29.5±0.59	27.3±1.53	12.0±1.6		12.3±0.91	28.1±1.43	21.8±0.46
11:00	32.2±0.79	32.7±0.71	19.9±1.1		19.4±1.8	34.4±1.29	27.3±0.55
12:00	26.5±2.05		16.9±0.55		22.9±1.0	18.0±0.87	20.7±0.46
13:00	34.5±0.79	29.8±2.05	21.7±0.85	33.5±1.25	20.1±0.94		27.1±0.54
14:00	35.3±0.64	24.4±1.11	18.3±0.67	33.9±0.77	18.7±3.8	15.9±0.40	25.1±0.31
14:00	32.6±0.45	22.4±0.46	24.4±0.52	36.3±0.57	19.6±1.0	19.6±0.44	25.8±0.22
16:00	36.2±0.46	25.0±0.76	28.0±0.54	39.4±0.70		19.3±0.40	29.2±0.26
17:00	33.5±0.47	24.1±0.58	29.3±0.45	32.5±0.74	38.0±1.0	24.1±0.68	29.3±0.26
18:00	40.0±0.42	28.6±0.54	30.9±0.43	32.2±0.48			33.5±0.24
19:00	35.8±0.45	24.2±0.38	27.1±0.51	32.1±0.50			29.6±0.24
20:00	30.6±0.30	26.9±0.37	20.1±0.55		22.4±2.57		25.7±0.24
21:00	33.4±0.74	23.0±0.44	15.4±0.79		18.0±0.41		23.2±0.34
22:00	32.7±0.49		19.8±0.51	23.6±1.2	22.3±1.1		26.5±0.35
23:00	31.7±0.43		21.3±1.0		21.6±0.55		25.8±0.29
00:00	34.4±0.54				24.9±0.80		30.1±0.49
1:00	36.8±0.66				28.7±0.67		32.2±0.48
2:00	37.0±0.83	23.7±2.07			31.7±0.99		32.6±0.63
3:00	36.3±0.76						36.3±0.79
4:00	33.9±0.82			35.2±5.6			34.5±0.82
5:00	36.3±5.26						36.3±5.3
6:00	37.7±1.64						37.7±1.8
7:00	37.1±1.38						35.7±1.2



Figure 10. CH₄ emission from sheep (30) grazing plantain (g CH₄ head⁻¹day⁻¹) estimated from the individual, 3-minute, CH₄ and N₂O mixing ratio data (x) and the hourly-averaged by emission rate calculated from these data(\bullet). Two error bars are includes, red is the calculated measurement, and purple is the variance in the data calculated as 1 standard deviation of the 3 point (9 minute) averaged data The variance is typical 1.5 to 2 x that of the measurement error.



Figure 11. CH4 emission from sheep (30) grazing ryegrass (g CH_4 head⁻¹day⁻¹) estimated from the individual, 3-minute, CH_4 and N_2O mixing ratio data (x) and the hourly-averaged by emission rate calculated from these data(•). Two error bars are includes, red is the calculated measurement, and purple is the variance in the data calculated as 1 standard deviation of the 3 point (9 minute) averaged data The variance is typical 1.5 to 2 x that of the measurement error.



Figure 12 Daily Distribution of CH_4 emissions from sheep grazing Top: Plantain, Lower: Ryegrass. Data are individual emission estimates (3 minute). The high emission from sheep grazing plantain on days 4 and 5, and the greater variability in estimated emissions plus the limited available data when sheep grazed the ryegrass is evident.



Figure 13 A comparison of the distribution in emissions over the day when the sheep grazed plantain (red) and ryegrass (blue) shows a similar pattern with higher emission in the morning, decreasing in mid afternoon and increasing again in later afternoon evening and decreasing again through the night.

Conclusion

It is particularly challenging to measure CH_4 emissions from a mob of sheep in a grazing environment due to the very low emissions per animal and the extensive grazing area required by the animals. Precision of the OP-FTIR system for measuring the CH₄ mixing ratio in air is approximately 5 ppb, with an atmospheric background concentration of ~1720ppb. To measure the CH₄ concentration with acceptable uncertainty requires an increase in CH₄ above background concentrations of ~10 x precision or 50 ppbv. However within a rural environment the variability in atmospheric CH₄ can easily equal this and the anticipated change in emissions from an animal due to a mitigation strategy may not be any greater than 10%. Logistic realities necessitated this research being conducted in the EverGraze site and hence, due to the very close proximity and movement of other animals, distinguishing CH_4 originating from the experimental animals and the surrounding livestock was challenging. The narrow plots used here were an advantage as they limited both the dispersion of the CH_4 plume between the animals and the measurement path and the difference in CH₄ mixing ratio, measured at the two instrument paths, due to surrounding animals.

The use of a backward Lagrangian stochastic (bLs) model, such as implemented in WindTrax, to relate the CH4 mixing ratio to emissions is not suitable in a typical grazing environment due to the non-homogeneous distribution of the sheep in the "source area", the possible presence of obstacles including trees and non-flat terrain. Instead in this work a tracer-gas has been used to model the atmospheric dispersion of the CH₄ plume. This method has been shown to significantly reduce the uncertainty in emission estimates compared to a bLs model (Bai 2011).

A backpack system to attach tracer-gas canisters to the animals while minimising the impact on animal behaviour was developed by staff at DPI Victoria (Andy Phelan and Kirsten Foggerty). Testing in the laboratory at University of Wollongong has adapted the canister head to give well characterised and reliable tracer-gas flow rates in a horizontal, instead of the usual vertical, position. Generally both the backpack and the canister gas release mechanism worked well, with only minor issues that were

quickly remedied. Researchers should, however, be aware of the possible impact on the wool quality, and need to clip the wool with gluing Velcro strips to the sheep's back.

Weather conditions were generally suitable for the technique, however with sheep grazing ryegrass, enhancement in CH_4 was decreased due to the wind direction often aligning more closely to the instrument path and with available data in the early hours of the morning limited by periods of low wind speeds and fog just prior to sunrise. This limited the number of days when a daily averaged emission could be estimated to day 1 only. Daily average emissions while grazing plantain were estimated for 5 days, with differences in emissions on days 2, 6 and 7 not being significant, and a small but significant increase in emissions noted on day 1. A similar distribution in emissions over the day from the two pasture systems was noted with higher emissions in the early morning and evening and lower emissions around midafternoon and during the night.

The project demonstrated a decrease in emissions of ~16% with sheep grazing plantain compared with ryegrass in the spring with emissions measured as 24.8 ± 0.37 g CH₄ head⁻¹day⁻¹ with grazing plantain and 29.4 ± 0.75 g CH₄ head⁻¹day⁻¹ with ryegrass. The emission estimates from plantain were confounded on 2 days when the quality of the plantain was severely degraded, but with the higher emissions measured on these days included (26.9 ± 0.33 g CH₄ head⁻¹day⁻¹) the difference in emissions with grazing the two pastures was still significant. Limited pasture samples were collected during the trial but results are not yet available and it is currently not possible to associate these changes in emissions with changes in dry matter intake or differences in the chemical composition of these pastures.

In comparison, in our previous campaigns with the FTIR technique, the emissions from Coop-worth sheep when grazing chicory and a combination of Lucerne pasture (initial 5 days) and Lucerne hay (final 4 days) were measured. A combination of Lucerne pasture and hay was used due to limited pasture availability, which also necessitated the sheep grazing dry ryegrass in the farm lanes immediately prior to the start of measurements with grazing Lucerne. Emissions were estimated as $37.0\pm9.0 \text{ g CH}_4$ head⁻¹day⁻¹ while grazing chicory. Grazing Lucerne pasture and immediately following grazing dry ryegrass in the lanes, emissions were $37.7\pm8.1 \text{ g}$ CH₄ head⁻¹day⁻¹ which reduced to $27.3\pm5.2 \text{ g CH}_4$ head⁻¹day⁻¹ for the final 4 days when the sheep grazed Lucerne hay. The emissions per animal were anticipated to be higher from Coop-worth sheep due to the higher daily intake. Daily average intake is not available for these data, however the Chicory and Lucerne pasture quality was heavily degraded by the end of measurements whereas the animals intake was not limited when fed Lucerne hay.

This work aims to mimic the normal grazing environment for the animals. In this measurement trial the animals were restricted to an area 100 x 9 m. This raises questions to how closely the system models the standard grazing practices, the impact of preferential grazing on the measured emissions and the possibility of the feed-on-offer being limited. In particular in this work the narrow enclosures and wet conditions resulted in the animals severely trampling the pasture. This was especially marked with grazing plantain, with ryegrass being more resilient and the grazing period decreased.

The work in Parts I and II of this Project has successfully developed the techniques, procedures and protocols required for the OP-FTIR technique to measure CH_4 emissions from sheep in an environment that closely mimics the true grazing environment. The results from Parts II and III have demonstrated the OP-FTIR

technology in association with a tracer gas can detect differences in emissions from sheep in a grazing environment of <10%. This is dependent on the caveats that sufficient measurement time is available to well characterise the emissions allowing for periods of poor or unsuitable weather conditions, the animals are acclimatised to the pasture and the experimental design is sufficiently flexible to respond to changing metrological and pasture conditions.

To minimise the uncertainty in the emissions estimate requires careful design of the experimental site. But the impact on emissions by limitations in the animals grazing environment must also be considered. It is essential that the experimental design and duration be sufficiently flexible to respond quickly to changing pasture quality and weather conditions, which can be difficult with staff limitations. The time commitment of the host organisation staff to exchange the gas canisters on the sheep is less then an hour per day. However maintaining and moving fences for the enclosures plus monitoring and sampling pastures requires substantial staff commitment. Similarly the UoW now operates three OP-FTIR instruments and has increased the staffing levels for this work to give greater flexibility in time-tabling and design of experiments. A scanning instrument is currently in production, and possible future staff increases are under consideration to further increase the flexibility of the experimental design and duration.

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