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Enteric methane mitigation strategies through manipulation of feeding systems for ruminant production in Southern Australia

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Executive summary

This project has identified and quantified feed supplements, grain treatments and novel forages that enable improved strategies for enhancing milk and meat production while simultaneously reducing methane intensity and possibly greenhouse gas emissions. The knowledge generated from this project will be invaluable to the Australian dairy and livestock industries and dairy and livestock farmers in the temperate zone of Australia will benefit in the long term. Policy experts will have information to enable improved inventory techniques for estimating methane emissions.

Refinements to methodologies (both *in vitro* and *in vivo*) have resulted in greater confidence in the data being generated from more cost effective scientific techniques, which in turn will provide greater opportunity for other research groups (nationally and internationally) to utilise such methods and screen other feed options.

Research on the commonly fed supplement wheat, has raised the possibility of using this supplement at different feeding rates to significantly reduce methane emissions and intensity from dairy cows. However, based on data from this project it is suggested that further research be undertaken to further elucidate the mechanisms behind such reductions and in particular the influence of wheat type (starch and protein content) and forage structure (short and long chop length).

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1. Background

When cattle and sheep (ruminants) digest feed in their rumen, they produce methane which they then burp or eructate. This enteric methane constitutes approximately 7% of the energy consumed by ruminants. If this enteric methane could be reduced and the saved energy diverted to the production of meat or milk, this would enhance the productivity, efficiency and profitability of Australia's livestock industries. For example, during the last 30 years, the average milk yield of Australian dairy cows has increased from approximately 3000 L/cow/year in 1980 to about 6000 L/cow/year in 2010, and over the this same period, the methane emissions intensity (g methane /L of milk) has decreased from approximately 33 g/L to approximately 24 g/L or a 27% decrease in methane intensity.

Currently, enteric methane contributes about 11% of national greenhouse gas emissions. Worldwide, there is growing concern about greenhouse gas emissions and many of our major trading partners are concerned about the carbon "foot-print" of ruminant products (meat and milk). It is likely that in the future, continuing improvements in productivity of the Australian dairy and livestock industries will be associated with reductions in methane intensity. Continuing increases in the productivity of the Australian dairy and livestock industries are also desirable because economists predict they will become necessary to meet the export demands of a world becoming increasingly food insecure. The dairy industry and the temperate livestock industries in Australia are in the relatively high rainfall zones with intensively grazed improved pastures, or in feedlots. In these situations, dietary and management interventions for enhancing animal productivity while simultaneously reducing methane are more feasible than in extensive rangeland zones.

Previous DEDJTR research into the use of dietary supplements to reduce enteric methane has, to date, provided the only practical and profitable mitigation strategies in Australia. In addition, significant capability has been developed in measurement and understanding of rumen gases. The work undertaken in this project has focused on research to enhance productivity without substantially increasing methane emissions from ruminants.

The experimental work has included the use of *in vitro* and *in vivo* techniquesto examine the production responses and effects on methane emissions from a range of forages that are not commonly used at present (e.g. chicory, planatin, brassicas, vetch), various novel feed supplements including almond hulls, citrus pulp, grape marc, algae meals, and oil seeds and also the use of novel grain treatments to alter the site and extent of starch digestion. Research has also examined fundamental digestive processes and elucidated the dynamics of ruminal digestion and enteric methane production in dairy cattle.

2. Methodology

This project builds on previous research in which the project team have established considerable experience in quantifying production responses to supplementary feeding and elucidating the responsible digestive and metabolic mechanisms involved. The approach taken in this project was to utilise multiple methodologies including *in vitro* and *in vivo* approaches to measure methane emissions. Initially *in vitro* studies were used to rank a wide range of forages and supplements. Subsequently *in vivo* studies were undertaken in sheep and lactating dairy cows which used face masks, SF_6 and calorimeters to measure methane emissions form animals.

In vitro

Two automated gas production systems developed by ANKOM^{RF} Technology were used for all *in vitro*fermentations.

Each system runs up to 50 modules containing pressure transducers that record variations in pressure within an attached fermentation vessel. All data is remotely recorded to computer spreadsheets using radio frequency transmission. Each module was set to release gas through a valve when the pressure within the modules reached 1psi, in order to prevent pressure build up in the

modules and the saturation of gas into the fluid. The gas released was automatically calculated and accounted for within the ANKOM^{RF} program.

In vitro fermentations were conducted using a modified technique described by Pell and Schofield (1993) and Schofield et al. (1994). Rumen fluid was collected via rumen fistula from two mid to late lactation dairy cows, fed on a ration containing 4kg of wheat and Hi-Milker mineral mix, offered in two equal portions at both the morning and afternoon milking. Furthermore they received *ad libitum* pasture and silage which totalled 12-14kg DM per day. The rumen fluid was collected per fistula from multiple sites within the rumen. Fluid was obtained using a copper pipe and a 100ml plastic syringe. The fluid was immediately placed in glass bottles that had been pre-warmed to 39˚C, samples from both cows were mixed in equal proportions. A handful of rumen content was placed in the fluid and the bottles were fitted with modified lids containing a one way gas valve. The modified lids prevented the build-up of gases within the headspace as fermentation continued during transportation, as well as enabling any remaining oxygen to be expelled from the bottle. The rumen fluid was transported in an incubator set at 39˚C for 1.5 h. These storage conditions and time prior to the fluids use as inoculum for an *in vitro* incubation have been shown to have no impact on subsequent 48h digestions (Robinson et al., 1999). Rumen fluid was allowed to drain freely through layered cheesecloth while being saturated with CO_2, allowing the separation and elimination of solids from the rumen fluid.

Samples were weighed and allocated to individual 250ml glass bottles. Kansas state buffer was prepared using the method described by Marten and Barnes (1980) and 75ml was added to each fermentation bottle already containing the substrate. The bottles were placed in a water bath set at 39˚C to pre-warm before the addition of rumen fluid. The rumen fluid was then added in 25ml aliquots to each vessel and carbon dioxide was passed through the bottle's headspace before being sealed with an ANKOM^{RF} module and returned to the water bath.

Each sample was replicated a minimum of 4 times in individual fermentation vessels, and often repeated over two or more separate runs for estimating gas kinetic parameters. Gas samples for gas composition analysis were collected from 4 replicates. Modules containing no feed but solely buffered rumen fluid were also included in 5 replicates in each run, in order to identify any gas and VFA production from unidentified substrate. Incubations were run for 48 or 24 h and cumulative gas pressure was automatically recorded every 5 minutes. The pressure transducers fitted to each module digitally record the pressure in the module's headspace in (psi). The gas pressure was then converted to moles of gas produced using the 'ideal' gas law (n = p (V/ RT)), and then converted to millilitres (ml) of gas produced per 1 gram of DM using Avogadro's Law.

Gas composition analysis

At the end of the fermentation runs, 20ml samples of headspace gas were collected from individual fermentation vessel through a septa port using a syringe, which was then compressed into an evacuated collection tube. These samples were stored at room temperature for later analysis of gas composition. Each 20mL gas sample was analysed for CO_2 and CH_4 concentrations determined by gas chromatography using a 7890A Agilent (USA), with Gilson GX-271 auto sampler, equipped with 4 columns (HayeSep® N 80/100 mesh, 0.5 m x 1/8 in. SST (precolumn for both channels); Porapak® QS 80/100 mesh, 2 m x 1/8 in. SST (analytical on TCD – FID channel); HayeSep® D, 80/100 mesh, 2 m x 1/8 in. SST (analytical to uECD) 6 feet 80/100 1/8" OD mesh Molsieve 13 X) and three detectors (TCD, FID and ECD). With the injector and column oven maintained at 70˚C. Gas standards used for the calibration curve were obtained from Air Liquide (US) with a purity of ±0.05%.

As each of the fermentation modules is designed to vent gas when the pressure within the jar reaches 1psi a method was required for estimating the amount of CH_4 produced by the substrate in each module to include vented gas. The following calculations were used based on the data produced from the ANKOM system and from the headspace gas sample taken at the end of the fermentation. The pressure data was used to estimate the volumes of gas produced by the substrate between ventings, and to infer a concentration of CH_4 produced by substrate that would be required to arrive at the concentration of CH_4 measured in the headspace after these ventings at the conclusion of the run. In order to do this an assumption is made that the ratio of CH_4 to other gases (mainly CO_2) produced by the substrate remains constant, and that the time profile of adsorption or desorption of CO_2 between

the headspace and rumen fluid in blank control modules represents that in other modules in the same run.

Suppose that during the interval leading up to the i^{th} venting, pressure in the headspace rises from y_i to Y_i, then falls to y_{i+1} after the i^{th} venting. Thus, for time interval i between venting events, i=1…n, let:

y_i = pressure of gas in headspace at the start of the interval

Y_i = pressure of gas in headspace at the end of the interval.

n = number of ventings, including a final notional venting at the end of the run.

C_i = concentration of CH_4 in headspace gas on the i^{th} venting.

c = concentration of CH_4 in gas produced by substrate, c assumed constant.

The concentration of CH_4 in headspace at start of experiment is C_0 = 0, and C_n = the measured concentration of CH_4 in headspace at the conclusion of the run. Y_n is the pressure in the headspace at the conclusion of run (typically 24h).

For blank, untreated, control modules, let X_i represent the (cumulative) pressure at a time corresponding to the i^{th} vent of a treatment module. (Usually, pressure does not build and there are no ventings in control modules, so pressure and cumulative pressure are usually the same. But if there are control ventings, X is the re-constructed cumulative pressure.) Differences between the control-module X_i are then used to estimate net adsorption or desorption of CO_2 to or from solution between ventings in the treatment module.

In treatment modules, the amount of gas produced by substrate between ventings is the change in the amount of gas in the headspace, plus the amount of gas (CO_2) absorbed by solution. This amount of gas (mol) produced by substrate between ventings is given by,

$$a_i = \frac{[(Y_i - y_i) - (X_i - X_{i-1})]V}{RT} \qquad (1)$$

where V (m^3) and T (°K) are headspace volume and temperature, and R the ideal gas law constant, and pressures in Pa. In these experiments, V and T are constant across modules.

The CH_4 concentration in the headspace on the i^{th} vent would be the amount of CH_4 in the headspace at the start of the i^{th} period, plus the amount of CH_4 produced during the period, divided by the final total amount of gas in the headspace:

$$C_i = \frac{C_{i-1}y_i + c[(Y_i - y_i) - (X_i - X_{i-1})]}{Y_i}$$.

That is,

$$C_i = C_{i-1}r_i + cs_i$$, where $r_i = \frac{y_i}{Y_i}$ and $s_i = \frac{Y_i - y_i - X_i + X_{i-1}}{Y_i}$. (2)

It turns out that we can calculate c using the pressure ratios r_i and s_i, and the final CH_4 concentration, C_n. But to do this we need the following theorem.

Theorem:

$C_i = cK_i$, where $K_0 = 0$ and $K_i = r_iK_{i-1} + s_i$, i=1…n. (3)

Proof:

The initial concentration of CH4 is 0, so $C_0 = 0$, and $cK_0 = 0$ by definition of K_0. So (3) holds for i=0.

If $C_i = cK_i$ then by equation (2),

$$\begin{aligned} C_{i+1} &= r_{i+1}C_i + cs_{i+1} \\ &= c(r_{i+1}C_i/c + s_{i+1}) \\ &= c(r_{i+1}K_i + s_{i+1}) \\ &= cK_{i+1}. \end{aligned}$$

That is, (3) holds for i+1. But (2) does hold for i =0. Thus (3) holds for all i=0…n. (End of proof)

Application:

The pressure data, y and Y, are available from each treated module, and the pressure data, X, are available from the control module measurement. Hence r_i and s_i can be calculated using definitions in (2), and K_i can be calculated recursively using our theorem, $K_i = r_iK_{i-1} + s_i$, starting with $K_0 = 0$, up to and including K_n.

The concentration (ppp) of CH_4, C_n ,is observed in the module headspace of each treatment module at the conclusion of the experiment. Thus, using (3), the concentration (ppp) of gas produced by substrate can be calculated as

$$c = C_n / K_n \tag{4}$$

The total produced CH_4 (mol) is then,

$$n_{CH4} = c\sum_{i=1}^{n} a_i \tag{5}$$

where a_i is defined at (1).

In vivo

The project team has continued to refine and develop capability and expertise in measuring enteric methane emissions from dairy cows using both the SF_6 technique (Deighton et al. 2014 Animal Feed Science and Technology 197: 47-63) and Open Circuit Respiration Chambers (Williams et al. 2013, Journal of Dairy Science 96:484-494).These techniques have been used to assess the methane mitigation potential of supplements and forages when fed to dairy cattle.

An integral part of this research undertaken in this project involves quantification of the effects of different potential methane mitigants on dry matter intake, milk production, milk composition and functionality.

Fundamental studies to elucidate the mechanisms responsible for enhanced productivity involve measuring not just the daily feed intake and production of methane, but also the dynamic diurnal patterns in feed intake, (every 15 seconds), the diurnal pattern of rumen pH (every 30 seconds) and the diurnal pattern in methane production (every 15 seconds). These fundamental studies on the pattern of methane emissions may provide a theoretical basis for the development of a simple and inexpensive method to measure and predict methane emissions from grazing ruminants.

Throughout this project, samples of blood and hair have been collected from all cows that are measured for methane emissions in the respiration chambers. These blood and hair samples will provide DNA which will be added to the DEPI Victoria database, and this is expected to be crucial for the identification of high producing dairy cows that are genetically predisposed to emitting low amounts of methane.

The third component of this project used sheep to test the efficacy of novel treatments of cereal grains, novel forages and grape marc as methane mitigants. Gas exchange (CH_4, O_2 and CO_2concentrations) and spirometry parameters (respiratory rate, respiratory exchange ratio (RER), gas temperature and tidal volume) were measured in real time via an indirect respiration method using face masks in an open circuit system (PowerLab exercise physiology kit; ADInstruments, Bella Vista, NSW, Australia). Measurements were conducted over 3 d to allow the measurement of all sheep. While not specifically used for methane analysis, the PowerLab system is regularly used for the measurement of respiratory and spirometry parameters in sheep. The system records inspired or expired air using a pneumotach, gas concentrations via a mixing chamber and gas analyser, gas temperature using a thermistor pod and the measurement of respiration rate using a spirometer and flow head. The simultaneous measurement of respiratory gas concentrations and air flow allows for the calculation of metabolic values. The sheep were acclimated to the use of the face masks for two weeks prior to the experimental measurement days. Gas exchange measurements were collected twice daily for each

sheep on consecutive days (to allow all animals to be measured) and each period of measurement were at least 20 min in duration (no longer than 30 min per animal per measurement period). The first measurement period occurred prior to the morning feed (AM measure) beginning at approximately 07.30. Two animals were measured at one time and were returned to their pens following their measurement period. Animals were fed (feeds are described in individual experimental descriptions) in a staggered manner to allow time for the measurement periods. The second measurement period (PM measure) began 4 h post feeding and was designed to capture any differences in gas production occurring due to fermentation of feedstuffs.

The gas analyser (ADInstruments MLT205) utilises an infrared detector to measure CO_2 and a paramagnetic cell to measure O_2 with detection ranges of 0-10% and 0-100% respectively. A custom made QUBIT no dispersive infrared (NDIR) analyser was used for the detection of CH_4 with a range of 0-3000ppm and a resolution of +/- 2ppm (Qubit systems, Inc. Ontario, Canada). An additional system, the ZRE infrared gas analyzer (Fuji Electric Systems, Australian Dynamic Technologies Co, Sydney, Australia) with a range of 0-3000ppm +/-1% was also paired with a PowerLab system and used in conjunction with the Qubit system to concurrently measure 2 sheep.

3. Results

In vitro experiments

Grape marc

Experiment 1

This experiment investigated the mitigation potential of grape marc selected from several grape varieties and prepared in a number of ways. It was hypothesised that factors such as colour, part (skin, seed, stalk, whole) and preparation method (steamed, ensiled, crimped, distilled, fresh) would impact the level of methane produced *in vitro*, thus allowing for more accurate selection of grape marc in future *in vivo* trials. A wide variety of samples were selected by Australian Wine Research Institute (AWRI) that covered both fresh and processed marc, red, white and mixed grape varieties as well as individual components of marc; seed, skin and stalk (Table 1.1). Each grape marc variety was incubated in 1 g dry matter (DM) replicates as sole substrate in the vessels over a 48 h fermentation.

Table 1.1. Description of the type of grape marc varieties and their preparation

Grape Marc	Description
1	Steam distilled, dried, marc
2	White ensiled marc
3	White crimped marc
4	White steam distilled marc
5	Mix of white and red ensiled marc
6	Mix of white and red steam distilled marc
7	Red ensiled marc
8	Red crimped marc
9	Red steam distilled marc
10	Fresh white marc, Riesling
11	Fresh white marc, Chardonnay
12	Fresh white marc, Sauvignon Blanc
13	Fresh white marc, skin only
14	Fresh white marc, seed only
15	Fresh red marc, Pinot Noir
16	Fresh red marc, Shiraz
17	Fresh red marc, Cabernet Sauvignon
18	Fresh red marc, skin only
19	Fresh red marc, seeds only
20	Fresh red marc, stalks only

Both the percentage of gas and the total volume of methane per g DM incubated are reported. The volume of gas produced is dependent on the amount of substrate fermented and could therefore vary if fermentation is incomplete; hence the percentage of methane in the fermentation gas is reported alongside the cumulative amount of all gases.

The colour (red or white) of the grape marc variety had no significant effect on methane production or concentration. However, the part of the plant (Table 1.2) that comprised that grape marc proved significant ($P<0.001$) with skins being highly fermentable and yet resulting in the lowest concentration of methane (16.3%). Marc derived from seeds alone had the lowest rate of fermentation and the highest concentration of methane (47.6%).

The greater total gas production from grape skins compared to grape seeds is not surprising because grape seeds contain greater concentrations of lignin and fat than do grape skins, and it is well known that lignin and fat are not fermented by rumen microorganisms. The smaller methane concentration in the fermentation gas from grape skins compared to the methane concentration in the fermentation gas from grape seeds suggests there is a component of grape skins that has a specific anti-methanogenic effect.

The marc variety had a significant effect (P<0.001) on the amount of methane produced, the percentage of methane produced, the rate of gas production and the total amount of gas produced. The concentration of CH_4 ranged from 12% to 51%, and the total amount of methane (ml/g DM) ranged from 6.2 to 27.8 ml/g DM. There was a strong linear relationship (R^2 = 0.93) between the final pH and the total VFA concentration. Moreover, there was a strong linear relationship between the total VFA concentration and the total gas production (R^2 = 0.96) but a less strong relationship between total VFA production and total methane production (R^2 = 0.63). There were particularly low concentrations of methane in the fermentation gas from grape marc varieties numbers 15 and 20.

Grape marc varieties numbers: 1, 2, 3, 5, 7, 8, 14, 17 and 19 all produced less than 50 ml of total gas /g of incubated substrate compared to variety number 13 which produced 124.3 ml of gas/g of incubated substrate. This observation suggests the former varieties were not very fermentable. Varieties number 15 and 20 produced fermentation gas containing less than 20% methane, suggesting that these two varieties may have contained specific anti-methanogenic substances.

Table 1.2. Effect of grape part on gas production kinetics and gas composition. Means followed by the same letter are not significantly different at P=0.05

	Rate constant (h^{-1})	Total gas production (ml/g incubated DM)	CH_4 (ml/g incubated DM)	loge CH_4 (% of total gas production)	CH_4 (% of total gas production)
Seed	0.941[a]	22.5[a]	8.9[a]	-0.877[a]	43
Skin	0.941[a]	101.1[b]	19.5[ab]	-1.643[b]	20.0
Whole	0.918[a]	60.6[c]	15.7[c]	-1.227[b]	30.0
sed	0.0106	13.76	2.86	0.1518	
P-value	0.0090	<0.001	<0.001	0.008	

Table 1.3. Effect of grape marc variety on gas production kinetics and gas composition

Grape marc variety	Rate constant (h^{-1})	Total gas production (ml/g incubated DM)	CH_4 (ml/g incubated DM)	log_e CH_4 (% of total gas production)	CH_4 (% of total gas production)
1	0.876	33.6	12.2	-1.026	36
2	0.929	48.0	15.3	-1.143	32
3	0.916	41.3	14.3	-1.063	35
4	0.929	59.4	17.5	-1.228	29
5	0.924	45.9	16.0	-1.057	35
6	0.925	68.0	19.3	-1.267	28
7	0.878	49.4	18.5	-0.975	38
8	0.866	44.9	16.3	-0.993	37
9	0.915	65.3	19.4	-1.222	30
10	0.944	84.5	18.3	-1.544	21
11	0.932	114.7	26.2	-1.522	22
12	0.947	89.2	20.2	-1.484	23
13	0.923	127.9	30.8	-1.468	23
14	0.970	20.7	6.8	-1.13	32
15	0.932	57.3	11.3	-1.632	20
16	0.923	60.4	18.7	-1.166	31
17	0.936	47.3	16.1	-1.082	34
18	0.944	103.9	24.0	-1.467	23
19	0.914	24.2	12.9	-0.623	54
20	0.956	71.6	9.8	-1.995	14
sed	0.0108	0.27	0.99	0.05118	
P-value	<0.001	<0.001	<0.001	<0.001	

Table 1.4. Effect of grape marc variety on concentration and proportions of volatile fatty acids in the rumen fluid at the end of the fermentation

Grape marc variety	Acetic (mmol/L)	Propionic (mmol/L)	Butyric (mmol/L)	Total VFA (mmol/L)	Ac:Pr	Final pH
1	8.3	3.6	0.64	12.8	2.2	6.30
2	15.7	4.6	0.51	21.0	3.3	6.13
3	12.7	3.9	0.53	17.3	3.1	6.18
4	16.1	5.1	0.87	22.3	3.1	6.10
5	15.4	4.0	0.61	20.4	3.7	6.18
6	18.6	5.3	1.27	25.4	3.4	6.09
7	13.6	3.3	0.80	18.4	4.1	6.23
8	14.4	3.3	0.96	19.3	4.3	6.23
9	15.8	4.2	1.17	21.5	3.7	6.19
10	17.5	12.3	0.70	30.7	1.3	5.81
11	20.1	14.2	3.11	38.3	1.3	5.65
12	19.2	12.6	1.55	33.5	1.4	5.78
13	25.0	15.7	3.36	45.0	1.5	5.57
14	8.2	3.3	0.00	11.4	2.5	6.24
15	16.7	9.8	0.02	26.4	1.6	5.92
16	16.4	3.4	0.74	21.5	4.9	6.20
17	14.8	3.5	0.37	19.2	4.2	6.17
18	20.0	12.2	2.24	34.8	1.6	5.75
19	10.9	3.1	0.48	14.9	3.4	6.26
20	15.6	9.9	1.18	27.6	1.8	5.91
sed	1.05	0.68	0.276	1.70	0.20	0.020
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Experiment 2

Based on the results from experiment 1 as well as information surrounding the potential of varieties to be used as animal feed sources (availability, storage potential etc.) eight grape marc varieties were selected for further screening. Grape marc products selected were: steam distilled dried marc, red ensiled marc, red crimped marc, fresh white marc – Riesling and Sauvignon blanc, and fresh red marc – skin only, seeds only and stalks only (Table 2.1). This involved the fermentation of the grape marc with three types of common forages (perennial ryegrass (PRG), chicory and a low N concentration pasture hay (HAY)) to investigate any interaction effects. All combinations of grape marc products and forages were replicated within the *in vitro* system. Each fermentation module contained a total of 1 g DM of substrate, comprising varying levels of grape marc (0, 25, 50, 75, 100%).

Table 2.1. Description of the grape marc varieties used in experiment 2

Grape Marc	Description
1	Steam distilled, dried, marc
7	Red ensiled marc
8	Red crimped marc
10	Fresh white marc, Riesling
12	Fresh white marc, Sauvignon Blanc
18	Fresh red marc, skin only
19	Fresh red marc, seeds only
20	Fresh red marc, stalks only

Table 2.2. Chemical composition of the three forages used in the *in vitro*fermentations.

Plant Type	% DM	% CP	%ADF	% NDF	%Ash	% IVDMD	%IVDOMD	Est ME (MJ/kg DM)	% WSC
Chic	94.4	20.3	12.9	20.8	15.0	83.4	74.2	12.7	16.9
Hay	95.8	6.5	27.5	53.6	5.3	64.2	61.4	9.4	18.8
PRG	93.5	21.8	24.5	44.6	12.8	82.9	75.1	12.6	13.5

Tables 2.3 to 2.5 report the rate constant, total gas production, methane production and proportion of methane in total gas production across the eight grape marc products and the three forages. There were significant effects of grape marc, forage and rate of marc inclusion, with all marc products generally showing a decline in total gas production and methane production as the rate of inclusion increased. In general, total gas and methane production tended to be higher when grape marc was incubated with chicory than for PRG or HAY. There was however greater variation in the impact of grape marc on the proportion of methane in total gas production with some marc products showing an increase as the amount of marc increased and others a decline. For example products 1, 7, 8 and 19 showed an increase ($P<0.001$) in methane as a proportion of total gas production across all forage

types as the amount of grape marc incubated increased, while products 12 and 20 consistently showed a decline (P<0.001).

Table 2.3. Effect of grape marc variety and percentage of substrate on gas production kinetics and gas composition when combined with PRG

GM Variety	GM (%)	Rate Constant (h^{-1})	Total gas production (ml/g incubated DM)	log_e CH4 (ml/g incubated DM)	CH_4 (ml/g incubated DM)	CH_4 (% of total gas production)
	0	0.903	82.2	3.25	25.8	32.6
1	25	0.898	83.4	3.48	32.3	38.2
	50	0.891	68.2	3.30	27.1	41.3
	75	0.880	49.7	3.03	20.7	43.9
7	25	0.908	86.9	3.34	28.2	34.6
	50	0.896	76.2	3.46	31.8	45.0
	75	0.888	69.0	3.30	27.1	41.3
8	25	0.907	83.8	3.37	29.0	35.9
	50	0.895	79.6	3.41	30.2	38.5
	75	0.881	62.0	3.25	25.8	44.1
10	25	0.920	95.9	3.49	32.8	36.3
	50	0.906	94.1	3.39	29.7	33.0
	75	0.907	87.8	3.36	28.8	34.3
12	25	0.907	92.3	3.51	33.3	38.3
	50	0.913	96.4	3.39	29.6	33.0
	75	0.899	92.0	3.37	29.0	32.2
18	25	0.906	92.3	3.40	30.0	31.7
	50	0.920	95.3	3.28	26.7	28.1
	75	0.913	93.9	3.32	27.5	28.3
19	25	0.906	81.3	3.36	28.8	36.4
	50	0.900	69.0	3.31	27.3	39.8
	75	0.887	46.7	3.13	22.9	51.8
20	25	0.900	92.8	3.46	31.7	35.5
	50	0.913	94.1	3.27	26.4	29.3
	75	0.887	80.9	3.15	23.3	31.0

Table 2.4. Effect of grape marc variety and percentage of substrate on gas production kinetics and gas composition when combined with chicory

GM Variety	GM (%)	Rate Constant (h^{-1})	Total gas production (ml/g incubated DM)	log_e CH4 (ml/g incubated DM)	CH_4 (ml/g incubated DM)	CH_4 (% of total gas production)
	0	0.883	92.5	3.21	24.8	28.5
1	25	0.876	96.6	3.51	33.3	34.9
	50	0.871	76.5	3.37	29.0	40.4
	75	0.850	52.0	3.16	23.6	50.1
7	25	0.889	99.9	3.52	33.8	33.9
	50	0.889	83.7	3.44	31.2	38.6
	75	0.874	66.7	3.35	28.6	46.0
8	25	0.891	98.9	3.51	33.3	35.4
	50	0.877	87.7	3.48	32.4	38.7
	75	0.868	66.3	3.34	28.1	46.3
10	25	0.893	106.1	3.48	32.5	31.8
	50	0.899	100.5	3.52	33.7	35.0
	75	0.906	82.4	3.15	23.3	29.6
12	25	0.900	102.6	3.42	30.7	29.1
	50	0.883	104.3	3.53	34.1	34.9
	75	0.889	88.8	3.34	28.2	33.9
18	25	0.891	92.3	3.26	25.9	27.1
	50	0.899	100.7	3.41	30.2	30.1
	75	0.905	96.7	3.28	26.5	28.0
19	25	0.892	89.6	3.34	28.3	31.7
	50	0.885	71.5	3.36	28.6	40.2
	75	0.859	47.4	3.18	24.0	49.2
20	25	0.884	105.0	3.40	29.8	30.2
	50	0.894	94.9	3.34	28.3	31.0
	75	0.881	72.7	3.03	20.6	37.0

Table 2.5. Effect of grape marc variety and percentage of substrate on gas production kinetics and gas composition when combined with a low N hay

GM Variety	GM (%)	Rate Constant (h^{-1})	Total gas production (ml/g incubated DM)	log_e CH4 (ml/g incubated DM)	CH_4 (ml/g incubated DM)	CH_4 (% of total gas production)
	0	0.924	90.8	3.25	25.8	29.5
1	25	0.923	76.1	3.11	22.3	30.3
	50	0.911	59.6	3.02	20.5	33.7
	75	0.902	43.7	2.77	16.0	35.2
7	25	0.930	84.3	3.14	23.1	26.8
	50	0.919	69.4	3.20	24.4	34.2
	75	0.920	57.2	2.97	19.6	33.6
8	25	0.919	75.3	3.10	22.2	28.5
	50	0.923	73.7	3.12	22.6	31.4
	75	0.919	56.9	2.95	19.2	33.1
10	25	0.940	97.1	3.17	23.8	24.5
	50	0.938	84.5	3.09	22.0	23.6
	75	0.944	95.6	2.89	18.1	19.8
12	25	0.932	91.6	3.15	23.4	25.0
	50	0.921	84.0	3.12	22.6	26.2
	75	0.933	87.9	2.98	19.7	21.3
18	25	0.911	72.3	3.15	23.3	31.8
	50	0.900	78.4	3.23	25.2	34.2
	75	0.895	70.9	3.03	20.7	30.3
19	25	0.897	65.5	3.24	25.6	38.0
	50	0.895	46.8	3.00	20.1	43.5
	75	0.859	33.5	2.98	19.8	59.6
20	25	0.935	92.0	3.19	24.3	26.1
	50	0.953	102.8	2.92	18.6	17.7
	75	0.942	78.1	2.38	10.8	14.0

Table 2.6. The main and interaction effects of the combined results in tables 2.3-2.5

	Rate Constant (h^{-1})	Total gas production (ml/g incubated DM)	log_e CH_4 (ml/g incubated DM)	CH_4 (% of total gas production)
sed	0.0132	5.74	0.10	3.90
Forage	<0.001	<0.001	<0.001	<0.001
GM variety	<0.001	<0.001	<0.001	<0.001
GM %	<0.001	<0.001	<0.001	<0.001
Forage*GM Variety	<0.001	<0.001	<0.001	<0.001
Forage*GM %	0.958	<0.001	<0.001	0.011
GM variety*GM %	<0.001	<0.001	0.310	<0.001
Forage*GM variety*GM %	0.977	0.63	0.153	0.82

Tables 2.7 to 2.9 present the volatile fatty acids concentrations and final pH within the fermentation vessels. Acetate concentration and total VFA concentration were higher (P<0.001) for chicory than other forages. Grape marc products 7 (Red ensiled marc), 8 (Red crimped marc) and 19 (Fresh red marc, seeds only) showed a decrease (P<0.001) in acetate, propionate and total VFA concentration across all forage types as the proportion of grape marc increased. In contrast marc 10 and 12 (fresh white marc) showed no effect on acetate, propionate and total VFA concentrations. The acetate:propionate ration increased (P<0.001) for products 7, 8 and 19 as the amount of grape marc increased across all forage types, while this ratio remained unaffected by other marc products.

Table 2.7. The effect of grape marc variety and percentage of substrate on concentration of volatile fatty acids (mmol/L) and pH in the final rumen fluid inoculum when combined with PRG

GM Variety	GM (%)	Acetic	Propionic	Butyric	Total VFA	Ac:Pr	pH
	0	20.2	8.8	2.5	32.0	2.3	6.0
1	25	18.1	7.2	2.5	28.4	2.5	6.0
	50	16.1	6.0	2.0	24.7	2.7	5.9
	75	12.1	4.2	1.5	18.3	2.8	6.0
7	25	20.1	7.7	2.3	30.6	2.6	5.8
	50	17.7	5.8	2.2	26.4	3.1	5.9
	75	15.7	4.2	1.7	22.2	3.8	6.0
8	25	19.9	7.7	2.2	30.4	2.6	6.1
	50	17.0	5.5	2.0	25.1	3.1	5.9
	75	14.4	3.8	1.6	20.3	3.8	6.1
10	25	19.2	7.6	2.8	30.1	2.6	6.1
	50	19.6	8.2	2.8	30.8	2.4	6.1
	75	16.8	7.7	2.4	27.0	2.2	5.8
12	25	19.4	7.9	3.0	30.7	2.5	5.8
	50	19.6	8.6	2.9	31.3	2.3	5.9
	75	17.5	8.1	2.9	28.6	2.2	6.1
18	25	21.6	9.3	3.3	34.6	2.3	5.8
	50	21.5	9.8	2.7	34.2	2.2	6.2
	75	18.2	9.4	2.8	30.6	2.0	6.2
19	25	20.1	8.2	2.7	31.6	2.5	6.2
	50	17.0	6.5	2.3	26.4	2.6	5.9
	75	12.2	4.0	1.0	17.6	3.0	5.8
20	25	20.4	7.8	3.2	31.8	2.6	5.9
	50	19.6	8.1	2.5	30.3	2.4	6.3
	75	17.0	7.5	2.0	26.6	2.3	5.9

Table 2.8. The effect of grape marc variety and percentage of substrate on concentration of volatile fatty acids (mmol/L) and pH in the final rumen fluid inoculum when combined with chicory

GM Variety	GM (%)	Acetic	Propionic	Butyric	Total VFA	Ac:Pr	pH
	0	24.1	11.3	2.4	38.5	2.1	5.4
1	25	19.3	7.8	2.5	30.4	2.5	6.0
	50	17.6	6.5	1.9	26.5	2.7	5.9
	75	13.5	4.3	1.5	19.8	3.1	5.9
7	25	22.9	8.7	2.7	35.2	2.7	5.8
	50	19.5	6.5	1.9	28.5	3.0	5.8
	75	16.3	4.2	1.7	22.8	3.9	5.9
8	25	23.0	8.7	2.6	35.0	2.6	6.0
	50	19.3	6.0	2.1	28.2	3.2	5.8
	75	15.6	4.0	1.7	21.9	3.9	6.1
10	25	19.5	8.3	2.5	30.6	2.4	6.0
	50	20.1	8.0	3.0	31.5	2.7	6.0
	75	16.9	8.8	1.5	27.4	1.9	5.8
12	25	23.9	10.8	2.6	37.7	2.2	5.8
	50	21.1	9.1	3.1	33.6	2.3	5.9
	75	17.9	9.0	2.3	29.4	2.0	6.1
18	25	23.8	11.9	2.5	38.5	2.0	5.8
	50	22.1	10.9	2.8	36.1	2.1	6.2
	75	19.9	10.4	2.9	33.4	2.0	6.2
19	25	23.0	9.7	2.4	35.8	2.4	6.2
	50	18.8	6.9	1.7	27.9	2.7	5.8
	75	12.2	3.9	0.9	17.3	3.1	5.8
20	25	23.5	9.4	3.4	36.7	2.5	5.9
	50	20.6	8.7	2.6	32.2	2.4	6.2
	75	16.9	7.3	2.0	26.4	2.4	6.0

Table 2.9. The effect of grape marc variety and percentage of substrate on concentration of volatile fatty acids (mmol/L) and pH in the final rumen fluid inoculum when combined with low N hay

GM Variety	GM (%)	Acetic	Propionic	Butyric	Total VFA	Ac:Pr	pH
	0	18.8	10.5	2.6	32.3	1.8	6.0
1	25	16.0	7.6	2.4	26.4	2.1	6.2
	50	15.1	6.0	2.1	23.6	2.5	6.1
	75	12.1	4.8	1.5	18.6	2.5	6.2
7	25	18.4	8.2	2.7	29.8	2.2	6.1
	50	16.3	5.7	2.4	25.0	2.9	6.1
	75	13.5	3.8	1.5	19.3	3.5	5.9
8	25	18.4	8.0	2.6	29.6	2.3	6.0
	50	16.5	5.6	2.2	24.8	2.9	6.1
	75	13.9	3.9	1.7	20.1	3.6	6.3
10	25	18.8	9.7	2.9	31.7	2.0	6.3
	50	16.9	8.2	2.5	27.9	2.1	6.3
	75	17.0	9.5	2.1	28.8	1.8	6.2
12	25	19.6	9.8	3.3	33.1	2.0	6.2
	50	17.1	9.6	2.4	29.3	1.8	6.0
	75	16.6	9.8	2.2	29.0	1.7	6.1
18	25	17.0	10.2	1.8	29.3	1.7	6.2
	50	16.1	9.4	2.2	27.8	1.8	6.4
	75	14.8	9.6	1.4	25.9	1.5	6.4
19	25	15.3	8.0	2.1	25.7	1.9	5.3
	50	13.1	5.6	1.2	20.2	2.3	6.1
	75	10.5	3.7	1.0	15.4	2.8	6.1
20	25	18.7	9.6	3.1	31.8	2.0	6.0
	50	17.4	9.6	2.2	29.5	1.8	6.3
	75	13.6	10.3	1.1	25.3	1.3	6.2

Table 2.10. The main and interaction effects of the combined results in tables 2.7-2.9

	Acetic (mmol/L)	Propionic (mmol/L)	Butyric (mmol/L)	Total VFA (mmol/L)	Ac:Pr	pH
sed	1.17	0.66	0.47	1.98	0.14	0.24
Forage	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
GM variety	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
GM %	<0.001	<0.001	0.039	<0.001	<0.001	<0.001
Forage*GM Variety	<0.001	<0.001	0.297	<0.001	<0.001	0.893
Forage*GM %	<0.001	<0.001	0.696	<0.001	0.211	0.03
GM variety*GM %	<0.001	<0.001	0.058	<0.001	0.004	0.378
Forage*GM variety*GM %	0.859	0.929	0.897	0.976	0.452	0.927

Experiment 3

Further work was conducted using the same eight grape marc varieties and three forages. This experiment investigated the effects of varying the level of grape marc while maintaining a constant amount of forage. All grape marc varieties where incubated with each of the forage types. The forage within each fermentation module remained at 0.5g DM while the level of grape marc was either 0, 0.125 or 0.25g DM.

Table 3.1. Effect of grape marc variety and percentage of substrate on gas production kinetics and gas composition when combined with PRG

GM Variety	GM (g)	Rate Constant (h^{-1})	Max gas (ml/g DM)	CH_4 (ml/g)	CH_4 (% of total gas production)
None	0	0.906	93.6	44.8	39.5
1	0.125	0.920	83.8	45.1	50.0
	0.25	0.916	76.5	32.4	38.8
7	0.125	0.927	122.0	44.1	44.9
	0.25	0.935	116.9	44.9	56.3
8	0.125	0.910	101.4	51.4	49.8
	0.25	0.905	100.0	41.1	45.2
10	0.125	0.910	98.2	48.1	37.9
	0.25	0.911	98.6	41.4	31.8
12	0.125	0.907	99.0	44.3	44.2
	0.25	0.898	85.4	40.7	44.4
18	0.125	0.922	112.0	41.1	45.8
	0.25	0.913	116.7	41.7	42.3
19	0.125	0.915	91.3	45.5	40.4
	0.25	0.905	80.0	35.1	38.5
20	0.125	0.914	92.0	39.6	32.4
	0.25	0.915	87.7	38.0	31.2

While there were significant effects of forage type, grape marc and rate of marc inclusion for the majority of marc products tested, there was no effect on total gas production, methane production or the proportion of methane in total gas produced. For products 7 (Red ensiled marc) and 18 (Fresh red marc, skin only) there was an increase ($P<0.001$) in total gas production across all forage types.

Table 3.2. Effect of grape marc variety and percentage of substrate on gas production kinetics and gas composition when combined with chicory

GM Variety	GM (g)	Rate Constant (h^{-1})	Max gas (ml/g DM)	CH_4 (ml/g)	CH_4 (% of total gas production)
None	0	0.892	107.4	53.2	40.0
1	0.125	0.901	94.6	46.0	45.0
	0.25	0.901	89.5	39.5	44.1
7	0.125	0.906	121.5	54.0	49.7
	0.25	0.915	117.3	47.7	45.9
8	0.125	0.895	113.3	58.0	53.6
	0.25	0.900	108.1	51.4	49.8
10	0.125	0.892	108.6	50.7	40.1
	0.25	0.899	102.7	42.5	34.1
12	0.125	0.895	111.1	57.8	48.6
	0.25	0.881	100.8	43.4	45.7
18	0.125	0.887	116.2	54.6	48.7
	0.25	0.893	110.5	42.0	44.6
19	0.125	0.897	103.2	52.6	40.7
	0.25	0.898	99.5	45.1	41.7
20	0.125	0.891	106.6	47.5	39.1
	0.25	0.893	100.6	30.9	30.7

Table 3.3. Effect of grape marc variety and percentage of substrate on gas production kinetics and gas composition when combined with low N hay

GM Variety	GM (g)	Rate Constant (h^{-1})	Max gas (ml/g DM)	CH_4 (ml/g)	CH_4 (% of total gas production)
None	0	0.908	74.6	47.0	52.0
1	0.125	0.914	69.5	44.7	60.0
	0.25	0.907	65.1	36.0	50.7
7	0.125	0.915	105.4	55.7	66.0
	0.25	0.923	93.2	44.1	55.8
8	0.125	0.907	94.7	55.2	60.0
	0.25	0.895	86.3	49.1	66.6
10	0.125	0.897	84.8	50.5	47.5
	0.25	0.909	95.7	40.3	39.4
12	0.125	0.915	97.2	51.9	49.4
	0.25	0.900	74.8	43.5	42.9
18	0.125	0.923	105.8	48.6	51.0
	0.25	0.911	111.5	44.1	48.8
19	0.125	0.889	76.4	53.2	48.6
	0.25	0.886	73.2	38.0	46.8
20	0.125	0.897	82.4	42.9	38.9
	0.25	0.894	70.6	41.2	37.3

Table 3.4. The main and interaction effects of the combined results in tables 3.1-3.3

	Rate Constant (h^{-1})	Max gas (ml/g DM)	CH_4 (ml/g)	CH_4 (% of total gas production)
sed	0.0091	9.11	5.23	7.27
Forage	0.261	0.003	<0.001	0.039
GM variety	<0.001	<0.001	<0.001	<0.001
GM (g)	<0.001	<0.001	<0.001	<0.001
Forage*GM Variety	0.113	0.314	0.84	0.812
Forage*GM (g)	0.385	0.556	0.504	0.955
GM variety*GM (g)	0.017	0.555	0.879	0.884
Forage*GM variety*GM (g)	0.992	0.982	0.224	0.752

Experiment 4

Experiment 4 focused on a single grape marc product (grape marc 10 - Fresh white marc, Riesling). The first six treatments included different combinations of Lucerne hay (*Medicago sativa* L.) and grape marc. These were; 1) 0.5g Lucerne hay, 2) 1g Lucerne hay, 3) 0.5g Lucerne hay and 0.5g grape marc, 4) 0.75g Lucerne hay and 0.25g grape marc, 5) 0.5g grape marc and 6) 1g grape marc. The other eight treatments were designed to measure the isolated effect of a specific component of grape marc. These were; 7) 0.5g Lucerne hay, 0.5g grape marc and 0.5g PEG 6000 (Sigma-Aldrich), 8) 0.5g grape marc and 0.5g PEG 6000, 9) 0.5g Lucerne hay and 0.472g defatted grape marc, 10) 0.472g defatted grape marc, 11) 0.5g Lucerne hay, 0.472g defatted grape marc and 0.472g PEG 6000, 12) 0.472g defatted grape marc and 0.472g PEG 6000, 13) 0.5g Lucerne hay and 0.0165g L-Tartaric acid (Sigma-Aldrich, >99.5%), 14) 0.5g Lucerne hay and 42µL grape seed oil (Stoney Creek cold pressed oil).

Nutrient composition of the grape marc is provided in Table 4.1.

Table 4.1. Nutrient concentrations in incubated grape marc (Hixson 2014)

Component	As Fed	DM	Component	As Fed	DM
% Moisture	9.2		% Calcium	.28	.31
% Dry Matter	90.8		% Phosphorus	.17	.19
% Crude Protein	7.0	7.7	% Magnesium	.08	.09
% Available Protein	4.1	4.5	% Potassium	1.57	1.73
% ADICP	2.9	3.2	% Sodium	.012	.013
% Adjusted Crude Protein	5.0	5.5	PPM Zinc	10	11
Soluble Protein % CP		15	PPM Copper	24	26
% Acid Detergent Fibre	21.7	23.9	PPM Molybdenum	<.01	<0.1
% Neutral Detergent Fibre|	26.5	29.2	% Sulfur	.09	.10
% NFC	49.1	54.0	% Chloride Ion	.08	.09
% Starch	.7	.7	DCAD, mEq/100g		36
% ESC (Simple Sugars)	26.8	29.5	Tartrate+Tartaric acid g/kgDM		33.13
% Crude Fat	7.1	7.8	Tannins g/kg DM		92.02

After the 24 h fermentation, total gas production (Table 4.2) was significantly different between treatments ($P<0.001$). Treatments 13 and 14, which included L-Tartaric acid and grape seed oil respectively, produced the lowest amounts of total gas.

Treatment 3 (0.5 g Lucerne hay + 0.5 g grape marc) produced significantly ($P<0.05$) more methane than treatment 2 (1 g Lucerne hay). In addition, there was no difference between treatments 1 and 3 and treatments 2 and 4. Furthermore, the addition of PEG and the process of defatting grape marc

did not result in higher levels of methane than the treatments containing whole grape marc. In contrast, the addition of L-Tartaric acid and grape seedoil did reduce methane production.

Table 4.2. Gas and methane production resulting from substrate incubated when incubated *in vitro* with buffered rumen fluid in the presence of grape marc components

Treatment	Ingredients	Rate[1]	Total gas at 24h(ml)	Total CH_4 at 24h(ml)	CH4% at 24h	pH
1	0.5g Luc	0.97^{b}	39.7^{bc}	28.7^{bc}	73.2^{e}	6.65^{b}
2	1 g Luc	0.96^{b}	62.7^{e}	29.4^{bc}	47.3^{b}	6.44^{b}
3	0.5 g Luc+0.5 g GM	0.95^{b}	79.8^{h}	38.2^{f}	48.2^{bc}	6.34^{b}
4	0.75g Luc+0.25g GM	0.97^{b}	66.4^{ef}	32.1^{cde}	48.4^{bc}	6.41^{b}
5	0.5 g GM	0.91^{b}	43.9^{cd}	30.9^{cd}	71.6^{de}	6.44^{b}
6	1g GM	0.95^{b}	70.4^{fg}	30.8^{cd}	44.1^{b}	6.20^{b}
7	0.5 g Luc+0.5 g GM+PEG	0.95^{b}	76.9^{gh}	35.3^{ef}	45.5^{b}	6.21^{b}
8	0.5 g GM+PEG	0.96^{b}	47.6^{d}	30.4^{c}	64.8^{d}	6.40^{b}
9	0.472g DGM+0.5 g Luc	0.95^{b}	64.7^{ef}	35.1^{def}	54.6^{c}	6.33^{b}
10	0.472 g DGM	0.91^{b}	35.7^{b}	31.4^{cde}	86.2^{f}	6.56^{b}
11	0.472 g DGM+ 0.5 g Luc+PEG	0.96^{b}	70.6^{fg}	35.6^{ef}	50.5^{bc}	6.27^{b}
12	0.472 g DGM+PEG	0.94^{b}	47.0^{d}	33.1^{cde}	70.7^{de}	6.44^{b}
13	0.5 g Luc+L-Tartaric Acid	0.65^{a}	27.4^{a}	4.8^{a}	18.3^{a}	5.15^{a}
14	0.5 g Luc+Grapeseed Oil	0.98^{b}	36.3^{b}	25.8^{b}	73.7^{e}	6.49^{b}
p-value		<0.001	<0.001	<0.001	<0.001	<0.001
SED		0.027	3.60	2.47	3.66	0.109
Mean		0.93	39.73	28.73	73.2	6.31

Luc = lucerne, GM = grape marc, DGM = defatted grape marc, PEG = polyethylene glycol SED: Standard Error of Differences

Summer Forages – Experiment 5. Dryland vs irrigated crops

The southeast Australian dairy industry is based on perennial ryegrass for its feed supply. Nevertheless, the variability in climate affects plant growth, leading to uncertainty in dryland pasture supply. Summer crops have emerged as an alternative forage as they have the ability to produce large quantities of forage when perennial pastures have low growth rates. Traditionally, brassica species, and in particular turnips, were the main forage used , although in recent years other brassica species with regrowth capability and forages such as chicory and plantain have gained interest as these offer greater flexibility in both sowing times and subsequent feeding options.

However, how summer crops affectruminal fermentation, including methane production is unclear. The aim of this study was to evaluate the *in vitro* fermentation characteristics and methane output of different forage crops grown under dryland or irrigated conditions.

The experiment evaluated 22 treatments, comprising 11 dryland and 11 irrigated summer active forage crops. Forage crops evaluated were Appin turnip (*Brassica rapa*) (bulb and leaf, Australian Purple Top (APT) turnip (bulb and leaf), Chicory (*Cichorium intybus* L. cv. Grouse) , Graza (a complex hybrid of *Raphanus sativus* L. cv Graza), Hunter (*Brassica campestris* L. X *Brassica napus* L. cv Hunter), Millet (*Echinochloa utilis* Ohwi&Yabuno) , Plantain (*Plantago lanceolata* L. cv. Tonic), Rangi (*Brassica napus* L. cv Rangi), and Winfred (*Brassica napus* L. cv Winfred) . Apart from the turnips where above (leaf) and below (bulb) components were tested, for all other forages only the above ground component was tested. The experiment was conducted over two *in vitro* experiments.

The nutritive characteristics of the forages are presented in Table 5.1. In general, irrigated forages had lower CP, ADF and NDF content and a higher IVDMD and estimated ME content. The only exceptions were chicory and plantain, where the IVDMD and estimated ME of the dryland forage was higher than for the irrigated forage.

Gas and methane production and other fermentation indices are presented in Tables 5.2, 5.3, 5.4 and Figure 5.1. After 24 h of fermentation, total gas production (Table 5. 2) was significantly different between irrigated and dryland in some of the forage crops ($P<0.05$). In the case of Appin bulb and leaf, chicory, Graza and Rangi, the irrigated treatments had a significantly higher ($P<0.05$) total gas production than the equivalent drylandtreatment.

Irrigated treatments produced higher methane that dryland, as evidenced in all the forage crops with the exception of Winfred.

Table 5.1. Nutrient concentrations in incubated forage crops

Crop	Leaf/ Bulb	Method	% CP	% ADF	% NDF	% IVDMD	Est ME (MJ/kg DM)	% WSC
APPIN	Bulb	Dryland	17.4	14.7	15.9	88.0	13.5	29.3
		Irrigated	8.8	11.8	13.2	91.1	14.0	35.5
	Leaf	Dryland	19.2	15.3	18.2	85.8	13.2	11.4
		Irrigated	10.7	13.6	17.8	88.4	13.6	16.8
APT	Bulb	Dryland	17.1	12.1	13.7	89.3	13.8	28.0
		Irrigated	9.6	11.9	14.5	90.8	14.0	32.5
	Leaf	Dryland	16.6	14.7	18.9	85.3	13.0	7.8
		Irrigated	9.8	15.6	20.3	86.3	13.2	7.3
Chicory	Leaf	Dryland	17.6	16.0	21.9	81.2	12.4	10.3
		Irrigated	12.9	18.8	22.5	79.8	12.1	12.2
Graza	Leaf	Dryland	21.7	14.1	20.6	84.3	12.9	5.8
		Irrigated	15.2	15.8	20.8	87.6	13.4	8.7
Hunter	Leaf	Dryland	16.6	12.9	17.0	86.4	13.3	10.2
		Irrigated	10.2	14.7	18.2	87.0	13.4	13.9
Millet	Leaf	Dryland	9.2	21.5	46.6	75.3	11.3	14.9
		Irrigated	7.8	28.5	55.0	71.5	10.7	10.1
Plantain	Leaf	Dryland	16.2	18.7	27.7	77.4	11.7	8.5
		Irrigated	9.2	24.5	33.4	73.1	11.0	5.9
Rangi	Leaf	Dryland	17.0	14.5	18.4	84.2	12.9	10.8
		Irrigated	11.1	13.6	19.2	86.5	13.3	16.5
Winfred	Leaf	Dryland	18.4	12.5	16.5	84.8	13.0	10.8
		Irrigated	16.6	14.3	19.8	86.6	13.3	14.9

DM: Dry Matter, CP: Crude Protein, ADF: Acid Detergent Fiber, NDF: Neutral Detergent Fiber, IVDMD: *In vitro* Dry Matter Digestibility, EST ME: Estimated Metabolisable Energy and WSC: Water Soluble Carbohydrates.

Table 5.2. Gas and methane production resulting from the *in vitro* fermentation of forage crops

Crop	Leaf/Bulb	Total Gas (ml)		CH_4 (ml/g)		CH_4 (%)		NH_3 (mg/dl)	
		Irrigated	Dryland	Irrigated	Dryland	Irrigated	Dryland	Irrigated	Dryland
APPIN	Bulb	120.77a	111.97b	49.74a	38.71b	41.28a	34.59b	15.21b	48.64a
APPIN	Leaf	119.45a	102.06b	49.67a	38.55b	41.60a	37.79b	17.82b	57.68a
APT	Bulb	116.4	117.55	46.66	42.37	40.10a	35.94b	18.86b	40.60a
APT	Leaf	98.35	100.87	44.39a	35.06b	45.17a	34.79b	23.92b	45.40a
Chicory	Leaf	101.77a	91.29b	42.88a	38.13b	42.18a	41.75	24.19b	37.92a
Graza	Leaf	113.39a	93.46b	46.87a	35.07b	41.33a	37.55b	34.48	42.67
Hunter	Leaf	117.3	110.65	47.93a	37.83b	40.82a	34.29b	19.16b	42.64a
Millet	Leaf	81.57	86.41	44.72	40.75	54.76a	47.03b	29.24	32.04
Plantain	Leaf	83.27	82.57	39.61	35.42	47.56a	42.93b	15.20b	32.12a
Rangi	Leaf	104.21a	99.59b	43.30a	35.94b	41.54a	36.07b	21.48b	36.80a
Winfred	Leaf	91.60	93.41	32.92	36.61	39.22a	36.06	31.72	40.00

CH_4: methane concentration. NH_3: ammonium concentration. Mean values with different letters within columns differ statistically, according to the Tukey test (P <0.05).

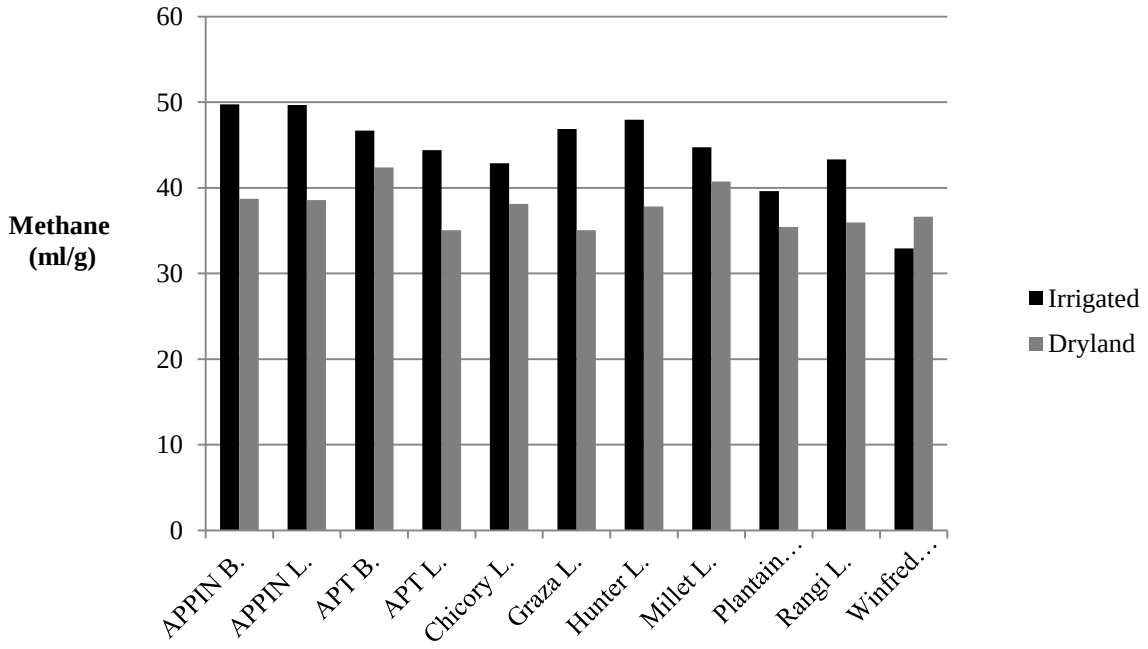

Fig. 5.1. Methane (ml/g) resulting from the *in vitro* fermentation of forage crops.

In all crops, with the exception of Graza, millet, and Winfred, ammonium concentration was higher ($p<0.05$) for dryland treatment crops than for those irrigated.

Table 5.3. Acetic, propionic, butyric acidsconcentrations for dryland and irrigated summer forage crops

Crop	Leaf/Bulb	Acetic (%)		Propionic (%)		Butyric (%)	
		Irrigated	Dryland	Irrigated	Dryland	Irrigated	Dryland
APPIN	Bulb	61.22	61.69	20.95	21.15	15.04a	12.97b
APPIN	Leaf	61.05	63.15	21.39a	19.07b	14.81a	12.88b
APT	Bulb	62.41	62.65	20.17	20.26	14.30	12.62
APT	Leaf	60.52	64.33	22.75a	18.88b	14.01	12.24
Chicory	Leaf	60.16b	64.37a	22.05	19.23	14.99a	12.07b
Graza	Leaf	62.00	64.90	20.51a	18.32b	14.64	12.39
Hunter	Leaf	63.04	62.38	20.99	20.23	13.07	13.11
Millet	Leaf	63.49	61.76	20.67	21.67	13.03	12.80
Plantain	Leaf	63.72	62.56	18.31b	22.02a	14.46a	11.87b
Rangi	Leaf	63.16	63.47	20.83	21.04	12.94	11.49
Winfred	Leaf	63.93	64.37	20.81	19.10	12.32	11.86

Mean values with different letters within columns differ statistically, according to the Tukey test ($P<0.05$).

There were minor differences in volatile fatty acid production between dryland and irrigated forages. In Appin leaf, chicory, and Graza the total VFA concentration was higher and A:P was lower for irrigated that dryland treatment. Final pH was significantly higher ($p<0.05$) for dryland treatment than irrigated in all of forage crops.

Table 5.4. Total fatty acid concentration, Acetate:propionate ratio and final pH for dryland and irrigated summer forage crops

Crop	Leaf/Bulb	Total VFA (mmol/L)		A:P		pH	
		Irrigated	Dryland	Irrigated	Dryland	Irrigated	Dryland
APPIN	Bulb	90.80	91.71	2.97	2.94	5.53b	5.98a
APPIN	Leaf	93.79a	86.61b	2.88b	3.31a	5.62b	6.12a
APT	Bulb	90.55	86.47	3.13	3.11	5.48b	5.87a
APT	Leaf	91.85	84.81	2.73	3.42	5.69b	6.07a
Chicory	Leaf	90.31a	74.25b	2.75b	3.37a	5.93b	6.14a
Graza	Leaf	92.05a	79.73b	3.04b	3.55a	5.71b	6.20a
Hunter	Leaf	88.06	88.42	3.04	3.12	5.59b	6.07a
Millet	Leaf	83.52	75.45	3.10	2.86	5.98b	6.09a
Plantain	Leaf	64.96	74.62	3.59a	2.85b	5.84b	6.12a
Rangi	Leaf	75.28	78.93	3.11	3.03	5.66b	6.07a
Winfred	Leaf	77.17	74.18	3.18	3.44	5.88b	6.07a

A:P: Acetic/ Propionic ratio. Mean values with different letters within columns differ statistically, according to the Tukey test ($P < 0.05$).

In vivo – Dairy cows

Experiment E1. Wheat dose

Dairy cows in Australia commonly graze pastures dominant in perennial ryegrass (*Lolium perenne*) and, on average, are also fed 6.0 kg DM/day of concentrate feed supplement, much of which is cereal grains. However, there has been little research published on methane production from dairy cows when wheat grain is included in a pasture diet. Thirty-two early lactation, multiparous, Holstein-Friesian cows were used in this experiment in spring. There were 4 dietary treatments in which cows were individually fed a basal diet of approximately freshly harvested perennial ryegrass. The cows offered the control diet (W0) were offered approximately 18 kg DM/cow/day of pasture. Cows in the other three treatments were also offered either 3.0 (W3), 6.0 (W6) or 9.0 kg DM/day of crushed wheat (W9) in place of an equal quantity of the basal diet, so that the net effect was that all cows ate daily, a total of approximately 18.0 kg DM. Methane emissions were measured on the last 5 days of the experiment by the SF_6 tracer technique. The composition of the dietary ingredients is shown in Table E1.1, The feed intakes, and milk yields are shown in Table E1.2, the methane production data are shown in Table E1.3, while ruminal fluid parameters are shown in Table E1.4. Mean milk yield from the cows fed the highest wheat dose was 21% greater than from those cows fed the pasture (control) diet. Since the dry matter intakes of the cows on both diets did not differ, the milk production response to wheat supplementation is likely due to the greater energy density of wheat compared to pasture. Cows fed the greatest dose of wheat had a 31% lower methane yield (g/kg DMI) and 42% lower methane intensity (g/kg milk) than cows fed the pasture diet. These reductions in methane yield and intensity appear to be due to both the proportion of grain in the diet and the type of grain fed.

Table E1.1 Composition of dietary ingredients (g/kg DM unless otherwise stated)

Parameter	Crushed wheat	Concentrate mix[1]	Pasture
DM (g/kg FW)	930	934	
Crude protein	118	242	184
Soluble protein (% of crude protein)	31	31	43
ADF	49	163	289
NDF	117	227	522
Lignin	13		32.5
NFC	749		170
Starch	658		2
Ash	20		100
TDN	845	830	710
Potassium	3.9	8.2	30.4
Calcium		3.3	4.2
Magnesium	1.3	3.4	2.8
Phosphorus	3.6	6.8	3.9
Sulfur	1.3	3.9	2.5
Crude fat	20	71	53

[1] The Concentrate mix was composed on a dry matter basis, of 45.5% cold-pressed canola meal, 45.5% cracked corn grain and 9.0% minerals.

Table E1.2. Influence of diet on feed intake, milk production and milk composition

Diet	W0	W3	W6	W9	SEM	*P* value
No. of cows	8	8	8	8		
Feed intake (kg DM/cow/d)						
Pasture	17.1	15.4	12.3	8.9		
Crushed wheat	0	3.0	5.9	8.9		
Concentrate mix[1]	2.1	2.0	2.0	2.0		
Total DMI	19.2	20.4	20.2	19.8		
Milk (L/cow/d)	30.4	32.8	34.5	36.7	0.95	< 0.001
ECM[2] (kg)	29.5	32.4	33.0	32.9	0.82	0.015
Fat%	3.93	3.94	3.69	3.17	0.141	0.001
Protein%	3.01	3.16	3.24	3.24	0.041	0.001
Lactose%	5.05	5.08	5.17	5.31	0.055	0.006
log10 SCC[3]	1.55	1.84	1.67	1.45	0.204	0.645
Production (kg/cow/d)						
Fat	1.18	1.30	1.27	1.17	0.045	0.168
Protein	0.91	1.04	1.12	1.19	0.025	< 0.001
Lactose	1.53	1.67	1.79	1.96	0.046	< 0.001
FP[4]	2.09	2.34	2.38	2.36	0.058	0.005

[1] The Concentrate mix was composed on a dry matter basis, of 45.5% cold-pressed canola meal, 45.5% cracked corn grain, 9.0% minerals. [2] ECM = energy corrected milk yield. [3] SSC = somatic cell count in cells/µ l. [4] FP = yield of milk fat plus milk protein. Means in the same row followed by different superscripts differ significantly (*P*< 0.05)

Table E1.3. Influence of diet on CH_4 emissions of cows during the last four days of the experiment

Diet	W0	W3	W6	W9	SEM	*P* value
Number of cows	8	7	8	8		
	Period 1					
Pasture (kg DM/cow/d)	16.9	15.6	12.2	8.8		
Crushed wheat	0	2.9	5.8	8.7		
Concentrate mix	2.1	2.1	2.1	2.1		
Total DMI	19.0	20.6	20.0	19.5		
CH_4 (g/cow/day)	436	443	413	311	18.3	<0.001
CH_4 (g/ kg DMI)	23.4	22.0	21.1	16.1	0.87	< 0.001
CH_4 (g/kg Milk)	15.1	14.0	12.5	8.7	0.74	< 0.001

Table E1.4. Influence of diet on concentrations in rumen fluid of ammonia, total volatile fatty acids (VFA), individual VFAs, and rumen protozoa

Diet	W0	W3	W6	W9	SEM	*P* value
Number of cows	8	8	8	8		
pH	6.54	6.51	6.66	6.30	0.089	0.051
Rumen fluid NH_3 (mg/L)	198	137	123	55	13.5	< 0.001
Rumen fluid Total VFAs (mM)	94	91	91	100	6.5	0.677
Individual VFAs (mM%)						
Acetic	64.5	64.6	62.8	54.6	0.99	< 0.001
Propionic	20.9	20.3	21.9	32.9	1.38	< 0.001
Is-Butyric	0.91	0.91	0.89	0.71	0.043	0.005
n-Butyric	11.2	11.7	11.5	8.2	0.644	0.001
Iso-Valeric	1.39	1.51	1.54	1.23	0.091	0.083
n-Valeric	1.05	0.95	1.26	2.00	0.127	< 0.001
Caproic	0.07	0.02	0.09	0.35	0.046	< 0.001
A:P ratio	3.15	3.13	2.99	1.73	0.194	< 0.001
D-lactate	11.9	26.1	27.4	38.6	4.83	0.004
Log10 (Protozoa)						
Entodinia	5.81	5.75	5.19	4.63	0.256	0.009
Epidinia	4.31	4.14	4.10	3.75	0.181	0.187
Isotricha	4.09	4.06	4.09	3.89	0.104	0.490
Daysytricha	4.48	4.49	4.07	3.87	0.111	<0.001
Other	3.96	3.88	3.80	3.84	0.071	0.492
Total	5.86	5.84	5.27	4.70	0.239	0.005

Means in the same row followed by different superscripts differ significantly (*P*< 0.05)

Experiment E2. Almond hulls and Citrus pulp

Almond hulls and citrus pulp are two common by-products that are fed to dairy cows. Almond hulls consist of the outer covering of the almond, but do not include the hard almond shell. Citrus pulp consists of the skin, pulp and seed residues post processing of citrus fruits for juice extraction. Although these two by-products are commonly fed to dairy cows, there is little published information on their feeding value for milk production and their effect on enteric methane (CH_4) emissions from ruminants. This experiment examined the effects of dietary supplementation with either almond hulls or citrus pulp on the milk yield, milk composition, and enteric CH_4 emissions in dairy cows.

Thirty two Holstein dairy cows in late lactation were offered one of three diets: a control (CON) diet, a diet containing almond hulls (ALH) and a diet containing ensiled citrus pulp (CIT). The cows on the CON diet consumed on a dry matter basis 14.2 kg/d of alfalfa cubes, 6.0 kg/d of crushed corn, 2.0 kg/d of cold pressed canola and 0.2 kg/d of mineral mix. Cows on the ALH diet consumed 10.5 kg/d of alfalfa cubes, 6.1 kg/d of crushed corn, 2.0 kg/d of cold pressed canola, 0.2 kg/d of mineral mix and 3.9 kg/d of almond hulls. Cows on the CIT diet consumed 11.0 kg/d of alfalfa cubes, 5.8 kg/d of crushed corn, 1.8 kg/d of cold pressed canola, 0.2 kg/d of mineral mix and 2.2 kg/d of ensiled citrus pulp. Individual cow feed intakes and milk yields were measured daily over this 28 day experiment and milk composition was measured on three days of each week. Individual cow CH_4 emissions were measured by the SF_6 tracer technique on days 24-28 of the experiment.

Results are shown in Tables E2.1, E2.2 and E2.3.The mean milk yield of cows fed the ALH diet was 24.6 kg/cow per d was less than the mean milk yield of cows fed the CON diet 27.4 kg/cow per d, while the mean milk yield of cows fed the CIT diet, 26.2 kg/cow per d was not different to the mean milk yield from cows fed the other two diets. Dietary treatment did not influence the concentrations of milk fat, protein or lactose, nor the daily fat yields, but the mean daily protein yield from cows fed the ALH diet was 0.78 kg/cow per d which was less than the protein yields from cows fed the CON diet (0.87 kg/d) or the CIT diet (0.85 kg/d). The mean CH_4 emissions were not influenced by dietary treatment and were 400, 430 and 414 g CH_4/cow per d for cows fed the CON, ALH and CIT diets. Similarly mean CH_4 yields were not influenced by diet and were 17.8, 19.1 and 19.0 g CH_4/kg DMI for cows fed the CON, ALH and CIT diets respectively. These findings indicate that although almond hulls and citrus pulp can be used as a low cost feed supplement to support milk production in dairy cows, they are unsuitable as feed supplements intended to inhibit enteric CH_4 emissions.

Table E2.1. Composition of main dietary ingredients (g/kg DM unless otherwise stated)

Parameter	Crushed corn	Cold pressed canola	Alfalfa cubes	Almond hulls	Ensiled citrus pulp
Composition (g/kgDM)[1]					
Crude protein	129	363	214	64	85
Soluble protein (% CP)	19	40	42	22	58
ADF	50	227	320	436	219
NDF	101	333	424	508	243
Lignin	20	-	65	-	-
NFC	717	184	272	330	566
Starch	626	-	10	-	-
Ash	21	72	107	-	-
TDN	870	765	615	555	690
Calcium	0.2	6.0	10.8	4.0	8.0
Magnesium	1.7	5.6	2.2	1.4	1.3
Sodium	0.1	0.6	0.4	0.2	0.3
Potassium	3.3	13.3	33.7	30.0	12.8
Chloride	-	-	7.2	-	-
DCAD (meq./100 gDM)					
Copper (mg/kg DM)	4.5	7.5	8.5	12.5	7.5
Sulfur	1.4	6.6	2.3	0.5	1.0
Crude fat	47	111	18	37	25

Table E2.2. Quantity of feeds eaten and milk yields from cows on each treatment

Parameter	CON[1]	ALH	CIT	SED	Contrast *P* value	
					CON vs ALH	CON vs CIT
Number of cows	12	9	9		-	-
Feed intake (kg DM/cow per d)						
Alfalfa cubes	14.19 [c]	10.46 [a]	11.04 [b]	0.175	< 0.001	< 0.001
Crushed corn grain	6.02 [b]	6.05 [b]	5.77 [a]	0.066	0.688	0.001
Cold pressed canola	1.95 [b]	1.96 [b]	1.84 [a]	0.029	0.694	0.001
Minerals	0.19	0.19	0.19	0.001	0.857	0.286
Almond hulls	0	3.92	0			
Ensiled citrus pulp	0	0	2.19			
Total	22.35 [b]	22.57 [b]	21.03 [a]	0.301	0.471	0.001
Milk (kg/cow per d)	27.4 [b]	24.6 [a]	26.2 [ab]	1.04	0.013	0.278
ECM (kg/cow per d)	26.4 [b]	24.6 [a]	25.4 [ab]	0.814	0.033	0.205
Fat %	3.81	4.14	3.76	0.234	0.164	0.857
Protein %	3.22	3.20	3.25	0.080	0.841	0.700
Lactose %	4.99	4.88	4.93	0.066	0.115	0.389
log10 SCC	2.19	1.89	2.07	0.232	0.211	0.611
Fat (kg/cow per d)	1.04	1.00	0.98	0.051	0.550	0.302
Protein (kg/cow per d)	0.87 [b]	0.78 [a]	0.85 [b]	0.030	0.005	0.543
Lactose (kg/cow per d)	1.36 [b]	1.19 [a]	1.29 [ab]	0.053	0.005	0.249

1. CON = control, ALH = almond hulls, CIT = ensiled citrus pulp

[a,b] Means in the same row followed by different superscripts differ significantly (*P*< 0.05)

Table 2.3. Influence of diet on methane emissions

Parameter	CON[1]	ALH	CIT	SED	Contrast *P* value	
					CON vs ALH	CON vs CIT
Number of cows	12	9	9			
CH_4 (g/cow/day)	400	430	414	30.1	0.325	0.635
CH_4 (g/ kg DMI)	17.8	19.1	19.0	1.26	0.330	0.338
CH_4 (g/L Milk)	14.8	17.7	16.6	1.73	0.104	0.304
Milk (L/d)	27.2 [b]	24.5 [a]	26.1 [ab]	1.10	0.022	0.332
DMI (kg/cow/d)	22.4 [b]	22.6 [b]	21.6 [a]	0.21	0.594	< 0.001

[a,b,c] Means in the same row followed by different superscripts differ significantly (*P*< 0.05)

1. CON = control, ALH = almond hulls, CIT = ensiled citrus pulp

Experiment E3.Red or white grape marc

Grape marc is the skins, seeds and stems remaining after grapes (*Vitis vinifera*) have been pressed to make wine and is increasingly being used as a source of nutrients (fibre and oil) for dairy cows. Grape marc has been shown to be a methane mitigant when consumed by dairy cows on a conserved forage diet in late summer. Thirty-two Holstein-Friesian cows were fed individually for 28 days to determine if red and/or white grape marc were a suitable replacement for fresh pasture, and would reduce methane when cows were fed fresh pasture. Methane emissions were measured by means of the SF_6 technique. Detailed results from the experiment are shown in Tables E3.1, E3.2, E3.3 and E3.4. Cows on all treatments had similar dry matter intakes and the nutritional characteristics of the red and white grape marcs were similar. Milk yield and methane yield (g CH_4/kg DMI) of cows fed grape marc were unaffected by type of grape marc and were 14% lower than for those fed pasture only. Feeding grape marc in place of spring pasture reduces methane emissions from dairy cows but this benefit is overshadowed by a reduction in milk production.

Table E3.1. Composition of dietary ingredients (g/kg DM unless otherwise stated)

Parameter	Pasture	Cold-pressed canola,	Corn	WGM	RGM
DM (g/kg FW)					
Crude protein	269	383	95	126	122
Soluble protein (% of crude protein)	34	-	22	-	-
ADF	271	280	37	417	423
NDF	483	335	98	477	476
Lignin	37	-	15	-	-
NFC	119	153	755	-	-
Starch	2	-	684	-	-
Ash	125	-	13.2	-	-
TDN	700	760	890	800	800
Sodium	2.3	0.4	0.02	0.1	0.1
Potassium	3.63	12.4	3.3	15.1	13
Calcium	5.6	6.0	0.1	4.5	3.7
Magnesium	2.3	5.3	1.1	1.0	0.8
Phosphorus	4.4	10.4	3.2	2.7	2.3
Chloride	15.1	-	-	-	-
Sulfur	3.7	-	1.0	-	-
Crude fat	61	112	51	118	112

Table E3.2. Influence of diet on feed intake, milk production and milk composition

Diet	CON	WGM	RGM	SEM	*P* value
No. of cows	12	10	10		
Feed intake (kg DM/cow/d)					
Pasture	13.67	9.74	9.84		
Cold-pressed canola	1.97	1.81	1.81		
Corn	2.98	2.83	2.83		
White grape marc	0	4.20	0		
Red grape marc	0	0	4.48		
Total DMI	18.62	18.58	18.96		
Milk (kg/cow/d)	29.1 [b]	26.0 [a]	26.9 [a]	0.44	0.001
ECM[2] (kg)	29.4 [b]	26.5 [a]	26.1 [a]	0.44	0.001
Fat%	4.23 [b]	4.30 [b]	3.96 [a]	0.081	0.034
Protein%	3.11	3.06	3.01	0.030	0.085
Lactose%	5.09	5.03	5.05	0.030	0.340
log10 SCC[3]	1.65	1.68	2.02	0.180	0.336
Production (kg/cow/d)					
Fat	1.22 [b]	1.1 1 [a]	1.05 [a]	0.026	0.001
Protein	0.902 [b]	0.794 [a]	0.806 a	0.013	0.001
Lactose	1.48 [b]	1.32 [a]	1.34 [a]	0.024	0.001
FP[4]	2.12 [b]	1.91 [a]	1.85 [a]	0.033	0.001

[1] The Concentrate mix was composed on a dry matter basis, of 45.5% cold-pressed canola meal, 45.5% cracked corn grain, 9.0% minerals. [2] ECM = energy corrected milk yield. [3] SSC = somatic cell count in cells/µ l. [4] FP = yield of milk fat plus milk protein. Means in the same row followed by different superscripts differ significantly ($P < 0.05$)

Table E3.3. Influence of diet on concentrations in rumen fluid of ammonia, total volatile fatty acids (VFA), individual VFAs, and rumen protozoa

Diet	CON	WGM	RGM	SEM	*P* value
Number of cows	12	10	10		
pH	6.77	6.81	6.78	0.059	0.896
Rumen fluid NH_3 (mg/L)	280 [b]	224 [a]	249 [ab]	11.5	0.011
Rumen fluid	85.9	83.4	85.9	2.38	0.728
Total VFAs (mM)					
Individual VFAs (mM%)					
Acetic	69.0 [a]	71.0 [b]	69.1 [a]	0.367	0.002
Propionic	17.7 [b]	15.5 [a]	17.2 [b]	0.31	0.001
Iso-Butyric	1.30 [b]	1.13 [a]	1.24 [b]	0.030	0.003
n-Butyric	9.07	9.38	9.66	0.223	0.225
Iso-Valeric	1.84	1.70	1.66	0.063	0.160
n-Valeric	1.02 [b]	0.92 [a]	0.94 [ab]	0.032	0.106
Caproic	0.11 [a]	0.36 [b]	0.14 [a]	0.032	0.001
A:P ratio	3.90 [a]	4.59 [b]	4.04 [a]	0.094	0.001
Log10 (Protozoa)					
Entodinia	4.72	4.49	4.97	0.130	0.075
Epidinia	2.37	2.47	2.81	0.200	0.233
Total	4.82	4.63	5.09	0.128	0.073

Means in the same row followed by different superscripts differ significantly ($P< 0.05$)
= yield of milk fat plus milk protein. Means in the same row followed by different superscripts differ significantly ($P< 0.05$)

Table E3.4. Effect of white grape marc (WGM) and red grape marc (RGM) on methane emissions from dairy cows

Variate	**Control**	**WGM**	**RGM**	**SED**	**P-value**
n	11	10	10	-	-
Total DMI (kg/cow per d)	18.6	18.9	18.8	0.26	0.801
Methane (g/cow per d)	383 [b]	327 [a]	325 [a]	12.9	0.001
Methane (g/kg milk)	13.7	12.9	12.3	0.49	0.199
Methane yield (g/kg DMI)	20.6[b]	17.4[a]	17.4[a]	0.68	<0.001
CoV of CH_4 yield	6.6	9.8	8.5	-	-

Means in the same row followed by different superscripts differ significantly ($P< 0.01$)

Experiment E4. Forage brassica (rape) or forage chicory on their milk production and methane emissions.

Forage brassica (FBR) and chicory (CHC) are alternatives to summer pasture as feeds for dairy cows, but there is little information about their effects on milk production and methane emissions. Thirty-two Holstein Friesian cows were fed for 10 days on a diet dominant in lucerne hay (CON) or diets in which FBR or CHC were substituted for some of the lucerne. Results from this experiment are shown in Tables E4.1 – E4.4. Cows offered the FBR diet produced more milk (27.4 kg/day) than cows offered the CON diet (23.7 kg/day), even though the dry matter intake was not different for cows in both groups (20.6 kg/day). In contrast, cows offered the CHC diet produced less milk (20.5 kg/day) than cows in the other two groups, reflecting the lower dry matter intake by cows offered the CHC diet (17.6 kg/day). Methane yield (g CH_4/ kg DMI) was significantly greater on the CHC diet (26.1) than on either the CON (21.0) or FBR (20.5) diets. The ratio of acetate to propionate measured in a 'spot sample' of ruminal fluid was proportional to methane yield. A meta-analysis showed this to be the case for previously published experiments as well. Diet type was associated with differences in the proportions of only a few specific milk fatty acids, and differences in proportions of specific fatty acids were not related to methane emissions.

Table E4.1. Composition of main dietary ingredients (g/kg DM, unless stated otherwise)

Parameter	Maize grain	Lucerne hay	Forage brassica	Chicory
Composition				
Crude protein	251	187	203	142
Soluble protein (% CP)	57	46	64	50
Acid detergent fibre	186	335	140	290
Neutral detergent fibre	255	411	202	381
Lignin	54	70	25	37
Non-fibre carbohydrate	366	321	461	353
Starch	209	2	76	12
Ash	53	79	113	118
TDN	820	620	740	640
Calcium	2.8	11.3	17.2	9.5
Magnesium	3.0	2.9	4.0	3.2
Sodium	0.1	0.7	2.3	3.1
Potassium	6.9	16.7	20.8	35.8
Chloride	1.3	6.6	10.1	13.4
Copper (mg/kg DM)	4	6	5	17
Sulphur	2.7	2.3	7.7	3.0
Crude fat	104	25	49	40
Metabolisable energy (MJ/kg)	14.3	10.1	12.3	10.0

Table E4.2. Influence of diet on feed intake (kg/cow.day), energy intake (MJ/cow.day, milk production (kg/cow.day) and milk composition (g/kg)

Diet	CON[1]	FBR	CHC	SEM	Contrast *P* value		
					CON vs FBR	CON vs CHC	FBR vs CHC
n	12	10	10				
Feed intake	20.8 [b]	20.6 [b]	17.7 [a]	0.437	0.610	<0.001	<0.001
Lucerne	15.4	6.4	6.4				
Cold-pressed canola	2.1	2.1	2.1				
Maize	3.1	3.1	3.1				
Minerals	0.2	0.2	0.2				
Chicory	0	0	5.9				
Brassica	0	8.8	0				
Energy intake	229 [b]	246 [c]	197 [a]	4.76	0.001	<0.001	<0.001
Grain proportion	0.25 [a]	0.25 [a]	0.29 [b]	0.007	0.635	<0.001	<0.001
Milk Production	23.9 [b]	27.5 [c]	20.4 [a]	5.44	0.001	0.001	0.001
ECM	22.7 [b]	25.4 [c]	19.3 [a]	5.51	0.001	0.001	0.001
Fat	0.883 [b]	0.950 [b]	0.754 [a]	0.027	0.073	0.001	0.001
Protein	0.783 [b]	0.836 [c]	0.592 [a]	0.019	0.001	0.001	0.001
Lactose	1.20 [b]	1.36 [c]	0.99 [a]	0.030	0.001	0.001	0.001
Fat plus Protein	1.61 [b]	1.79 [c]	1.35 [a]	0.043	0.006	0.001	0.001
Milk composition							
Fat	37.1	35.3	37.4	0.76	0.080	0.805	0.056
Protein	30.8 [b]	30.6 [b]	29.7 [a]	0.25	0.569	0.003	0.017
Lactose	50.0	49.4	49.5	0.23	0.076	0.153	0.721
log10 SCC	1.94	2.05	1.87	0.077	0.307	0.489	0.103

[1] CON = control, FBR = forage brassica, CHC = chicory, ECM = energy corrected milk

Means in the same row followed by different superscripts differ significantly (*P*< 0.05)

Table E4.3. Effect of diet on methane emissions from dairy cows fed a control (CON), forage brassica (FBR) or chicory (CHC) diet

Variate	CON	FBR	CHC	SEM	Contrast P value		
					CON vs FBR	CON vs CHC	FBR vs CHC
n	12	10	10				
Total DMI (kg/day)	20.8 [b]	20.6 [b]	17.7 [a]	0.32	0.610	0.001	0.001
Methane							
Emission (g/day)	436	421	461	18.2	0.538	0.327	0.131
Yield (g/kg DMI)	21.0 [a]	20.5 [a]	26.1 [b]	0.85	0.661	0.001	0.001
Intensity (g/kg milk)	18.6 [b]	15.7 [a]	22.4 [c]	1.04	0.048	0.013	0.001

Means in the same row followed by different superscripts differ significantly (P< 0.01)

Table E4.4. Influence of diet on pH and concentrations of ammonia, total volatile fatty acids (VFA), individual VFAs, and protozoa in ruminal fluid from dairy cows fed a control (CON), forage brassica (FBR) or chicory (CHC) diet

Diet	CON	FBR	CHC	SEM	Contrast *P* value		
					CON vs FBR	CON vs CHC	FBR vs CHC
n	12	10	10				
pH	6.61 [a]	6.60 [a]	6.95 [b]	0.105	0.985	0.022	0.027
NH_3 (mg/L)	221.2 [b]	172.4 [a]	202.6 [ab]	12.702	0.008	0.287	0.103
Total VFAs (mM)	109.6	116.1	91.0				
Individual VFAs (mol/100mol)							
Acetic	66.00 [b]	62.57 [a]	68.55 [c]	0.430	<0.001	<0.001	<0.001
Propionic	20.04 [b]	22.44 [c]	16.17 [a]	0.713	0.019	<0.001	<0.001
Iso-Butyric	0.905 [b]	0.711 [a]	1.029 [c]	0.037	0.001	0.019	0.000
n-Butyric	10.02 [a]	11.65 [b]	11.39 [b]	0.350	0.002	0.007	0.599
Iso-Valeric	0.977 [b]	0.767 [a]	1.249 [c]	0.041	0.001	<0.001	<0.001
n-Valeric	1.960 [c]	1.774 [b]	1.519 [a]	0.058	0.025	<0.001	0.004
Caproic	0.092	0.078	0.084	0.008	0.181	0.444	0.574
A:P ratio	3.32 [b]	2.85 [a]	4.27 [c]	0.134	0.015	<0.001	<0.001
Protozoa (Log_{10}(x+1000))							
Entodinia	5.045	5.213	5.165	0.148	0.409	0.553	0.822
Epidinia	4.143	4.423	4.279	0.169	0.231	0.557	0.552
Daysytricha	3.362	3.360	3.488	0.166	0.991	0.581	0.589
Total	5.125	5.292	5.253	0.140	0.387	0.507	0.845

Means in the same row followed by different superscripts differ significantly (P< 0.05)

Experiment E5. Different methods of offering starch based grain to lactating animals on their milk production and emissions of methane.

In two previous experiments at Ellinbank, the supplementary feeding of crushed wheat to dairy cows resulted in reductions in methane emissions of between 35 – 45%. No information is available in the scientific literature on how the manner or timing of offering a wheat diet to lactating dairy cows might influence milk production and methane emissions. Thirty-two mid lactation rumen-fistulated Holstein Friesian cows were fed for 35 days on one of four diets. Diets were a corn based control diet (CON) offered twice per day, a wheat based diet (W2) offered twice per day, a wheat based diet (W6) offered six times per day and a wheat based diet (W2B) offered twice per day with buffers. Methane emissions from individual cows were measured over the last five days of the experiment by the SF_6 technique. Results from this experiment are shown in Tables E5.1 – E5.3.Total dry matter intakes on all diets were similar. Milk productions on the W2B and W6 diets were greater than on the control diet, while production of milk fat was unaffected by diet, but production of milk protein from all diets containing wheat was greater than from the CON diet. Diet type had little effect on methane production, methane yield or methane intensity.

Table E5.1. Composition (g/kgDM) unless otherwise noted) of main dietary ingredients

Parameter	Alfalfa hay	Crushed wheat	Crushed corn	Cold pressed canola	Mineral mix
Crude protein	171	128	91	379	
Soluble protein (% CP)	41.8	32.8	16	16	
ADF	378	32	21	229	
NDF	114	514	507	535	
Lignin	99	11.2	7.2	90	
NFC	260	748	799	128	
Starch	13.5	625	708	5.5	
Ash	77.2	16.6	12.2	64.9	
TDN	558	863	902	768	
Fat	22.0	20.5	40.8	98.8	
Calcium	11.3	0.6	0.1	5.6	134
Magnesium	2.7	1.2	1.1	5.1	110
Phosphorous	2.5	3.3	2.7	10.4	60
Sodium	2.8	0.03	0.01	0.13	
Potassium	19.8	4.6	3.0	13.6	
Chloride	5.60	1.03	1.34	0.90	
Copper (mg/kg DM)	6.5	3.0	2.0	5.3	1.2
Sulphur	2.2	1.6	1.3	6.9	

Table E5.2. Influence of manner of feeding wheat on milk yield, milk composition and yields of milk components in the two week experimental period before cows entered the respiration chambers

Item	CON	W2	W2B	W6	SEM	Rx
Number of cows	8	8	8	8		
Intake (DMI, kg/d)						
Corn	8.78	0	0	0		
Wheat	0	9.00	8.92	8.91		
Canola	0.97	0.99	0.98	0.98		
Minerals	0.22	0.22	0.22	0.21		
Buffer	0	0	0.28	0		
Lucerne	8.70	8.76	8.76	8.77		
Total	18.70	18.97	19.15	18.79		
Milk yield, kg/d	20.5 [a]	21.4 [ab]	22.5 [b]	22.3 [b]	0.50	0.032
ECM, kg/d	20.6 [a]	22.0 [ab]	22.4 [b]	22.6 [b]	0.51	0.05
Milk fat						
g/kg	40.6 [a]	41.7 [a]	39.81 [a]	40.4 [a]	1.00	0.596
kg/d	0.829 [a]	0.895 [a]	0.893 [a]	0.896 [a]	0.026	0.211
Milk protein						
g/kg	33.1 [a]	33.8 [ab]	33.2 [a]	34.6 [b]	0.03	0.012
kg/d	0.673 [a]	0.723 [b]	0.741 [b]	0.766 [b]	0.017	0.005
Milk lactose						
g/kg	49.7 [a]	49.3 [a]	49.1 [a]	49.3 [a]	0.04	0.813
kg/d	1.019 [a]	1.057 [ab]	1.111 [b]	1.099 [ab]	0.027	0.67
Log_{10}SCC	2.15 [a]	1.86 [a]	1.99 [a]	2.35 [a]	0.180	0.275
Methane						
(g/cow/day)	461 [a]	527 [b]	517 [b]	453 [a]	19.0	0.018
(g/ kg DMI)	24.4 [ab]	27.8 [c]	27.0 [bc]	24.1 [a]	0.98	0.029
(g/kg milk)	23.8 [a]	23.8 [a]	23.3 [a]	21.6 [a]	1.99	0.836

[a,b] Means in the same row followed by different superscripts differ significantly ($P< 0.05$)
[1] CON = Control diet; W2 = diet with wheat fed twice daily; W2B = diet with wheat and buffer fed twice daily; W6 = diet with wheat fed six times daily

TableE5.3. Results of bolus pH measurements during the last two weeks of the experiment

	Treatment					
Parameter	CRN2	WT2B	WHT2	WHT6	SEM	P-Values
Mean__pH	6.26 [a]	6.32 [a]	6.34 [a]	6.36 [a]	0.072	0.763
Min__pH	5.75 [a]	5.83 [a]	5.87 [a]	6.00 [a]	0.083	0.200
Max_pH	6.77 [a]	6.89 [a]	6.92 [a]	6.79 [a]	0.059	0.202
pH_Range	1.02 [b]	1.06 [b]	1.05 [b]	0.79 [a]	0.058	0.009
log_Durn_below_6_min	2.20 [b]	1.93 [ab]	1.97 [ab]	1.24 [a]	0.319	0.196
log_Area_below_6_pHmin	1.44 [a]	1.12 [a]	1.06 [a]	0.73 [a]	0.282	0.381

[a,b] Means in the same row followed by different superscripts differ significantly ($P < 0.05$)

In vivo – Sheep experiments

Experiment D1 - BioProtect

Mean live weights and dry matter intake (DMI) of the ration ingredients are shown in Table D1.1. Treatment had a significant effect on the average daily gain (ADG) of the animals with the animals fed maize and forage, gaining significantly (P<0.001) more weight per day over the experimental period. There were no significant differences between the average daily gain (ADG) of the wheat and the BioProtect treated animals (0.046 and 0.021 kg/day, respectively), both of which however, showed a significantly lower (P<0.001) ADG than both the maize and the forage treatment groups, between which there was no difference (0.117 and 0.111 kg/day, respectively). Total DMI did not differ between the three grain diets, wheat, BioProtect and maize (655.6, 667.3 and 610.5 g/day, respectively). Those on the forage diet however, consumed significantly more (P<0.001, 1193.2 g/day).

Table D1.1. Mean initial weight, final weight, intake and average daily gain over the experimental period for the four treatments

	Treatment					
	Wheat	BioProtect	Maize	Forage	sed	P-Value
Live weights (kg)						
Initial weight	32.6	31.0	30.9	33.7	1.057	<0.001
Final weight	33.5	31.5	33.4	36.0	0.595	<0.001
Average daily gain (kg/day)	0.046	0.021	0.117	0.111	0.0198	<0.001
DM intake (g/day)						
Total DMI	655.6	667.3	610.5	1193.2	84.45	<0.001
Lucerne/oaten chaff				1287.9		
Wheat	347.3			375.0		
BioProtect wheat		353.4		381.7		
Maize			328.0	354.1		

All CH_4 production parameters for the two sampling periods (AM/PM) are shown in Table D1.2. Diet treatment had a significant effect (P=0.024) on the amount of CH_4 produced (mL/min), such that the animals on the forage diet produced the most methane followed by those on the maize diet (7.14 and 5.10 mL/min respectively), with both producing greater levels of CH_4 than the wheat and BioProtect diets (1.77 and 2.61 mL/min respectively, sed. 1.638). There was no significant difference between CH_4 production from the wheat and the BioProtect diets. The time of measurement significantly (P<0.001) affected the overall average CH_4 production, which was lower in the pre-feed measures (AM) compared to the post-feed measurements (PM) (2.36 and 5.95 ml/min respectively, sed. 0.690). Overall there was no relationship between treatment and sample time for CH_4 production. The maximum CH_4 peak was not altered by the individual effects of treatment (P=0.196) or time (P=0.812), although there was an interaction between treatment and time (Table 3), with the maximum peak produced by sheep fed the BioProtect diet in the PM measurement period and the lowest produced by the wheat treatment in the AM period. Treatment did not alter the time between CH_4 peaks (P=0.728), while the peaks were less frequent in the AM compared to the PM measurement period (4.9 vs. 3.4 mins respectively, sed. 0.572, P<0.001). There was an interaction between treatment and time such that the time between peaks was greater in the am and longer for sheep fed BioProtect or forage diets compared to those fed maize or wheat (P=0.007, Table D1.2).

The time of measurements altered (P<0.001) the number of CH_4 peaks, such that overall a greater number of peaks were seen in the PM measurements compared to the AM (5.8 and 3.5 respectively, sed. 0.512). The number of CH_4 peaks were also influenced by treatment (P=0.024) whereby the least number of peaks were recorded in sheep fed wheat treated with BioProtect (3.1) when compared to all other treatments (wheat 4.6, forage 5.4, maize 5.5, sed. 0.929). There was an

interaction between treatment and measurement time (P<0.001) to influence the number of peaks, with these differences being driven by the wheat treatment in which the PM measurements were significantly higher than the AM (Table 3).

Table D1.2. Average methane production and number of methane peaks during the 15 min sampling period for the four diets before the morning feed (AM) and approximately four hours post feeding (PM)

	Time	Treatment				Sed.	P-value		
		Wheat	BioProtect	Maize	Forage		Trt.	Time	Trt x time
CH_4 (mL/min)	AM	-0.33	1.51	3.49	4.74	1.825	0.024	<0.001	0.624
	PM	3.87	3.71	6.70	9.54				
Maximum peak (CH_4 ppm)	AM	607	1128	1584	1274	366.1	0.196	0.812	0.026
	PM	903	1648	1271	1142				
Time between peaks (min)	AM	4.10	5.07	6.77	3.45	1.406	0.728	<0.001	0.007
	PM	2.97	4.21	2.57	3.98				
Number of peaks	AM	2.62	3.00	3.15	5.18		0.024	<0.001	<0.001
	PM	6.62	3.12	7.83	5.68	1.150			

Experiment D2 – BioProtect dose response

Dry matter intake and live weight response are presented in Table D2.1. There was a significant effect of treatment on initial and final live weights such that sheep fed the maize diet started and finished the experiment at a higher weight than the sheep fed the other treatments. There was no difference in live weight between sheep fed the forage, wheat or BioProtect treated wheat diets.

The total DMI was significantly greater for sheep fed the forage only diet (P<0.001), and sheep fed the wheat diet ate significantly more DM (~50g/day) than sheep fed the BioProtect treated wheat. There was no significant difference in DMI between the sheep fed wheat treated with 8 and 16 L of BioProtect.

Rectal temperatures and respiration rates were significantly greater in the PM (post-feeding) measurement period (Table D2.2, P<0.001). There was no significant effect of dietary treatment on any physiological measures obtained, nor were there any significant interactions between treatment and time (Table D2.2).

Table D2.1. Mean initial weight, final weight and intake over the experimental period for sheep fed either forage (oaten/lucerne chaff) or grain and forage diets of wheat, BioProtect treated wheat (8L and 16L/t wheat) or maize plus forage (50% oaten and lucerne chaff, n=12 sheep per treatment)

	Treatment					
	Forage	Wheat	8 L BioProtect	16 L BioProtect	Sed.	P-Value
Live weights						
Starting	30.5	31.6	31.2	31.2	1.123	<0.001
Final	33.4	32.4	31.3	30.4	1.195	<0.001
DM intake (g/day)						
Total DMI	848.8	780	729.3	730.4	12.64	<0.001
Forage	771.5				14.57	
Wheat		306.7				
BioProtect Wheat			293.2	267.7		
Maize						

Table D2.2. Physiological measurements obtained pre (AM) and post (PM) feeding prior to measurements of gas exchange in sheep fed either forage (oaten/lucerne chaff) or grain and forage diets of wheat, BioProtect treated wheat (8L and 16L t wheat) or maize plus forage (50% oaten and lucerne chaff, n=12 sheep per treatment)

		Treatment					P-values		
	Time	Forage	Wheat	8 L BioProtect	16 L BioProtect	Sed.	Trt.	Time	Trt. x time
Rectal temp. (°C)	AM	38.5	38.7	38.5	38.4	0.149	0.149	<0.001	0.454
	PM	39.2	39.2	39.0	39.1				
Respiration rate (breaths min^{-1})	AM	34	34	35	38	3.94	0.204	<0.001	0.410
	PM	39	48	44	48				
Heart rate (beats min^{-1})	AM	115	124	120	109	11.6	0.596	0.346	0.423
	PM	124	112	106	106				

Table D2.3. Average (over the 20 min measurement period) methane and spirometry obtainedpre (AM) and post (PM) feeding in sheep fed either forage (oaten/lucerne chaff) or grain and forage diets of wheat, BioProtect treated wheat (8L and 16L t wheat) or maize plus forage (50% oaten and lucerne chaff, n=12 sheep per treatment)

		Treatment					P-values		
	Time	Forage	Wheat	8L BioProtect	16L BioProtect	sed.	Trt.	Time	Trt x time
CH_4 STPD (mL min^{-1})	AM	1.40	1.43	1.97	0.87	0.6127	0.085	<0.001	0.084
	PM	4.19	3.68	2.76	2.28				
CH_4 Increment STPD (mL min^{-1})	*	2.77	2.22	0.97	1.50	0.677	0.057	*	*
CH_4 peak (ppm)	AM	2750	2254	1418	2561	672.1	0.553	0.033	0.383
	PM	2687	3103	3095	3190				
CH_4 peak Increment change (ppm)	*	-41.9	871.4	1699.1	564.3	993.7	0.366	*	*
Ave time between peaks (min)	AM	2.0	3.8	3.7	1.8	1.07	0.300	0.336	0.043
	PM	2.7	3.1	2.7	5.0				
Number of peaks	AM	3.7	2.5	2.5	1.5	1.07	0.014	<0.001	0.628
	PM	8.6	5.8	6.3	5.0				
Respiratory exchange ratio (RER)	AM	0.74	0.77	0.78	0.78	0.019	0.116	<0.001	0.701
	PM	0.83	0.87	0.84	0.87				
Volume CO2 STPD (mL)	AM	47.4	53.1	76.9	39.7	13.32	0.144	0.374	0.153
	PM	65.2	68.9	55.5	50.2				
Volume O2 STPD (mL)	AM	64.0	69.7	100.0	50.3	16.85	0.131	0.867	0.153
	PM	78.3	78.0	64.6	57.4				

Measures of spirometry and methane output are presented in Table D2.3. Average methane production tended to be lower (P=0.085) in sheep fed wheat treated with 16 L BioProtect compared to those fed forage, wheat or wheat treated with 8 L BioProtect (1.57, 2.80, 2.56 and 2.37 mL min^{-1} respectively). Methane production was greater (P < 0.001) in the PM (post-feeding) compared to the AM measurement periods (1.42 vs. 3.23 mL min-1 for AM and PM respectively). The increment of methane increase (PM – AM) tended to be lower in sheep fed wheat treated with 8 L BioProtect, which was also observed (albeit to a lesser degree) in those fed 16 L treatment. The increment of change in peak methane concentration was not influenced by treatment (P = 0.366). There were no individual effect of diet or time on the time between methane peaks, while there was an interaction between diet and time such that the time between peaks was lower in the PM period for sheep fed 8 L BioProtect and wheat, but greater in the PM period for those fed 16 L BioProtect or forage (Table D2.3). The number of methane peaks recorded was greater (P=0.014) in forage fed sheep and lower in sheep fed wheat treated with 16 L BioProtect (6.15, 4.15, 4.43 and 3.28 peaks for forage, wheat, 8 L and 16 L BioProtect respectively, sed. 0.908). The number of methane peaks recorded was also greater in the PM compared to the AM measurement period (6.44 vs. 2.56 peaks, sed. 0.441, P<0.001), although there was no interaction between time and diet type.

Measures of spirometry were also obtained, demonstrating that the respiratory exchange ratio (RER) tended to be lower (P=0.078) in sheep fed the forage diet (0.784, 0.820, 0.811 and 0.827 for forage, wheat, 8 L and 16 L BioProtect respectively). The RER was lower (P<0.001) in the AM compared to the PM measurement period (0.768 vs. 0.853 for AM and PM respectively) and there tended to be an interaction between treatment and time such that the RER was consistently lower in the forage fed sheep but greater in the PM period.

Experiment D3 – Forage type

Liveweight did not differ at the beginning or by the end of the experiment between treatments (Table D3.1). There was a significant treatment by week interaction such that DMI was significantly greater for sheep fed lucerne hay compared to those fed pasture and vetch hay.

Table D3.1. Liveweight and feed intake (DM) of sheep fed either lucerne, vetch or pasture hay (n = 12 per treatment)

	Treatment				
	Lucerne	Vetch	Pasture	Sed.	P-Value
Live weights (kg)					
Initial weight	36.1	35.8	36.4	1.50	0.956
Final weight	36.1	35.6	36.9	1.74	0.717
DM intake (g/day)	756	711	702	22.1	0.047

Observed respiration rates were not influenced by feed treatment, but were greater (P <0.001) in the pm compared to the am measures (32.9 vs. 45.9 breaths min-1 for am and pm respectively). The same pattern was observed such that there was no variation due to feed treatment, but both heart rate (93.4 vs. 124.5 beats min^{-1} for am and pm measures respectively (P < 0.001) and rectal temperature (38.5 vs. 39.2 °C for am and pm measures respectively (P < 0.001) were greater in the pm compared to the am measures. There were no significant interactions between treatment and time for any of the physiological measures recorded (Table D3.2).

Exhaled methane concentrations measured as CH_4 STPD (mL min^{-1}) were greater in the pm compared to the am period (0.6 vs. 3.0 for am and pm respectively P < 0.001) and were greater in sheep fed vetch hay compared to those fed pasture or lucerne (2.3 vs. 1.4 vs. 1.7 for vetch, pasture or lucerne respectively P = 0.003). The increment of change in methane concentration between the am and pm measures was not influenced by diet type (2.70 vs. 1.98 vs. 2.68 for lucerne, pasture and vetch hay respectively (P = 0.195). Similarly, the average CH_4 peak was greater in sheep fed vetch compared to those fed pasture or lucerne (1533 vs. 1127 vs. 1153 for vetch, pasture or lucernerespectively (P = 0.007). While there was no individual effect of measurement time on the average CH_4 peak, there was an interaction (P = 0.009) between treatment and time such that sheep fed lucerne had greater peaks in the pm compared to the am period (918 vs. 1388 ppm for am and pm respectively) while the opposite relationship was present for pasture (1210 vs. 1045 ppm for am and pm respectively) and vetch (1695 vs. 1372 ppm for am and pm respectively). The time between methane peaks was greater in the am compared to the pm measures (6.9 vs. 2.0 min for am and pm respectively (P < 0.001), in conjunction with an increase in the number of methane peaks occurring in the pm compared to the am measures (2.0 vs. 10.5 for am and pm respectively (P <0.001). There was a tendency for the number of methane peaks to be greater in sheep fed pasture compared those fed lucerne or vetch (6.1 vs. 7.1 vs. 5.5 for vetch, pasture or lucernerespectively (P = 0.071). The respiratory exchange ratio (RER) was greater in the pm compared to the am measures (0.82 vs. 0.88 for am and pm respectively (P = 0.006), although treatment did not influence RER measures. The respiration rate of sheep fed vetch was greater than that measured in sheep fed lucerne or pasture (95.2 vs. 71.9 vs. 78.1 breaths min^{-1} for vetch, pasture or lucernerespectively (P = 0.035). Respiration rates were greater in the pm compared to the am measurement period (57.1 vs. 106.3 for am and pm respectively (P< 0001). The volume of O_2 tended to be greater in sheep fed vetch compared to those fed lucerne or pasture (95.2 vs. 71.9 vs. 78.1 for vetch, pasture or lucerne respectively (P = 0.086), as was also observed for the volume of CO_2 (77.9 vs. 52.5 vs. 57.3 for vetch, pasture or lucerne respectively (P = 0.069). Both O_2 (57.1 vs. 106.3 for am and pm respectively (P = 0.009) and CO_2 (51.7 vs. 73.5 for am and pm respectively (P = 0.004) were greater in the pm compared to the am measures, although there were no interactions between treatment and measurement time.

Table D3.2. Physiological, spirometry and gas exchange responses in sheep fed Lucerne, pasture or vetch hay (n = 12 per group) measured pre (am) and 4 hours post (pm) feeding

	Treatment							P-Values		
	Lucerne		Pasture		Vetch					
	Am	Pm	Am	Pm	Am	Pm	Sed.	Treatment	Time	Trt. X time
Respiration rate (breaths min^{-1})	32.9	49.0	31.4	43.1	34.4	45.5	5.01	0.559	<0.001	0.749
Heart rate (beats min^{-1})	96.5	119.5	102.6	136.1	81.1	117.9	12.55	0.211	<0.001	0.609
Rectal Temperature (°C)	38.4	39.3	38.4	39.2	38.5	39.2	5.25	0.969	<0.001	0.804
CH_4 mL/min STPD	0.40	3.03	0.49	2.38	1.02	3.55	0.294	0.003	<0.001	0.129
CH_4 mL/min STPD Increment	2.70		1.98		2.68		0.448	0.195		
Ave CH_4 peak (ppm)	918	1388	1210	1045	1695	1372	195.7	0.007	0.926	0.009
Time between peaks (min)	7.76	2.24	5.71	1.91	7.14	1.73	1.070	0.862	<0.001	0.413
Number of peaks	1.25	9.83	2.90	11.29	1.79	10.49	0.876	0.071	<0.001	0.960
Respiratory exchange ratio (RER)	0.79	0.86	0.82	0.88	0.85	0.88	0.030	0.166	0.006	0.769
Respiratory rate (breaths min^{-1})	54.6	101.7	50.6	93.1	66.2	124.1	9.71	0.035	<0.001	0.367
V O_2 mL STPD	47.2	87.2	49.6	76.6	87.1	91.9	15.90	0.086	0.009	0.240
V CO_2 mL STPD	39.5	75.1	40.8	64.2	74.7	81.1	13.66	0.069	0.004	0.236

Experiment D4 – Grape marc

Starting liveweight did not differ due to treatment group (P = 0.999, Table D4.1), which was also the case for the final liveweight (P = 0.780). Liveweight fluctuated with experimental week such that they were similar at the beginning and the end of the experiment (43.3, 44.8, 44.3, 43.7 and 43.1 kg for weeks 0 to 4 respectively (P< 0.001). Mean DMI for the entire experiment did not differ due to treatment group (P = 0.425). Dry matter intake generally increased with week throughout the experiment (883.5, 936.9, 911.1, 923.5 and 971.3 g/day for weeks 0 to 4 respectively (P< 0.001). There was a significant interaction between DM intake and experimental week such that intake generally increased with week and was lowest in sheep fed crimped grape marc at 30% and highest in those fed control or spent grape marc at 10% in the final week (data not shown, P<0.001).

Table D4.1. Liveweight and dry matter (DM) intake in sheep fed control (Lucerne and oaten chaff) diets or crimped or ensiled grape marc fed at 10, 20 or 30% (n = 7 per group)

	Control	Spent Grape marc			Crimped Grape marc				
		10%	20%	30%	10%	20%	30%	Sed.	P-value
Starting liveweight	43.87	43.37	43.46	43.4	43.26	43	42.99	1.732	0.999
Final liveweight	44.34	43.87	43.69	42.64	42.73	42.57	41.77	1.745	0.780
DM intake (g/day)	938.1	955.3	934.7	911.4	901.6	937.3	898.5	33.69	0.425

Table D4.2. Physiological, spirometry and gas exchange responses in sheep fed control (lucerne and oaten chaff) diets or crimped or ensiled grape marc fed at 10, 20 or 30% (n = 7 per group) measured pre (am) and 4 hours post (pm) feeding

			Crimped			Ensiled				P-Values		
		Control	10%	20%	30%	10%	20%	30%	Sed.	Trt.	Time	Trt. x time
Rectal temperature (°C)	AM	38.9	38.8	38.9	39.0	38.9	38.8	38.6	0.19	0.752	<0.001	0.588
	PM	39.3	39.4	39.4	39.5	39.5	39.3	39.5				
Respiration rate (breaths min^{-1})	AM	78.0	54.0	51.0	46.0	56.0	46.0	44.0	13.58	0.416	<0.001	0.791
	PM	76.0	72.0	74.0	70.0	71.0	68.0	65.0				
Heart rate (beats min^{-1})	AM	100.0	112.0	99.0	96.0	87.0	88.0	74.0	12.40	0.207	<0.001	0.128
	PM	106.0	111.0	107.0	134.0	117.0	116.0	106.0				
Methane (mL min^{-1})	AM	1.51	0.75	1.07	1.69	1.57	1.40	0.95	0.944	0.551	<0.001	0.915
	PM	6.26	5.29	5.73	6.24	5.94	4.43	5.32				
CH4 mL/min STPD Increment	*	4.79	3.51	5.07	5.57	4.78	3.46	3.73	1.058	0.308	*	*
Ave. methane Peak (ppm)	AM	1131	1014	1071	1262	1031	1309	1225	246.9	0.783	0.002	0.344
	PM	1572	1513	1492	1683	1359	1061	1475				
Ave methane peak increment change (ppm)	*	468	235	512	585	419	-123	83.4	261.5	0.091	*	*
Time between peaks (min)	AM	3.42	4.59	3.44	4.18	3.91	8.16	4.80	0.876	0.082	<0.001	0.005
	PM	1.46	1.39	1.54	1.46	1.53	1.54	1.29				
Number of peaks	AM	4.7	4.6	3.6	4.4	5.4	3.2	4.0	1.407	0.960	<0.001	0.635
	PM	13.6	13.1	13.4	14.4	13.1	13.9	14.7				
Respiration Rate (Breaths min^{-1})	AM	71.9	62.7	57.7	68.4	96.9	90.2	62.1	13.96	0.125	<0.001	0.326
	PM	99.3	102.3	95.7	89.3	112.4	113.7	90.9				
VO_2 STPD (mL)	AM	51.8	46.3	41.9	43.5	50.3	59.0	55.5	10.84	0.246	<0.001	0.980
	PM	95.2	87.6	77.0	78.4	91.8	91.7	91.0				
VCO_2 STPD (mL)	AM	48.4	47.4	38.3	37.6	45.3	55.3	50.9	9.61	0.090	<0.001	0.960
	PM	88.4	77.0	68.5	70.0	81.2	87.5	81.3				
RER	AM	0.959	0.982	0.884	0.841	0.906	0.903	0.909	0.061	0.106	0.707	0.983
	PM	0.936	0.978	0.877	0.874	0.897	0.941	0.949				

Neither rectal temperature, respiration rate or heart rate was influenced by dietary treatment (Table 4.2, $P < 0.2$), although all physiological data were increased in the pm compared to the am measurement periods (53.7 vs. 71.1 breaths min^{-1}, sed. 4.75; 38.8 vs. 39.4°C, sed. 0.060; and 94.0 vs. 114.2 beats min^{-1}, sed. 4.42 for am and pm respiration rate, rectal temperature and heart rate respectively, $P < 0.001$).

The mean methane concentration (STPD) expired throughout the gas measurement period was not influenced by treatment group, although the concentration was greater in the pm compared to the am measurement period (1.28 vs. 5.60 mL min^{-1} for am and pm respectively $P < 0.001$). This was also the case with the maximum methane peak, with no noted effect of treatment on methane peak ($P = 0.783$), while peaks were greater in the pm compared to the am measurement period (1149 vs. 1451 ppm for am and pm respectively ($P = 0.002$). There were no interactions between treatment and time for measures of total methane or methane peak. The increment of change between the am and pm average methane concentration was not influenced by treatment, although the increment of change for the peak methane concentration was reduced in sheep fed ensiled grape marc at 20% due to the peak volume of methane being greater in the am compared to the pm measurement period for this treatment. The time between methane peaks tended to be greater in sheep fed ensiled grape marc at 20% and (to a lesser degree) 30% of the diet (2.44, 2.99, 2.49, 2.82, 2.72, 4.85 and 3.05 min for control followed by crimped then ensiled at 10, 20 and 30% respectively ($P = 0.082$). The frequency of the peaks was greater in the pm compared to the am measures, as highlighted by the reduced time between peaks (4.64 vs. 1.46 min for am and pm measures respectively ($P< 0.001$). There was an interaction between treatment and time such that the time between peaks was greater in the am compared to the pm period for all treatments and was relatively similar for all treatments in the pm period but was lowest in the sheep fed crimped grape marc at 20% and greatest in those fed ensiled grape marc at 20% in the am period. The number of methane peaks produced was not influenced by treatment or a treatment by measurement time interaction, although the number of peaks was greater in the pm compared to the am measures (4.28 vs. 13.76 peaks for am and pm respectively ($P < 0.001$). Similarly, the respiration rate recorded during the gas production measures was not influenced by treatment or an interaction between treatment and measurement time, while the respiration rate was greater in the pm compared to the am measurement period (72.8 vs. 100.5 breaths $min^{-1}P < 0.001$). Neither the volume of O_2 or CO_2 expired during the measurement period was influenced by treatment or a treatment by time interaction, although both values were greater in the pm compared to the am measures (49.4 vs. 87.3, sed. 3.87; and 45.5 vs. 79.2 mL for am and pm measures of O_2 and CO_2 respectively, $P < 0.001$).

DISCUSSION

In vitro studies

This project has undertaken a range of *in vitro* studies that have investigated pre-treatment of grains, forages and grape marc as options to reduce methane production. The *in vitro* technique is widely acknowledged as an effective screening tool to examine a wide range of treatments in a more cost effective manner than undertaking *in vivo* experimentation. As such it is hard to determine if data generated from *in vitro* studies could be directly applied by end users. However, in the context of this project, the use of *in vitro* techniques was warranted, given the varying range of potential forages and supplements that could be used in the livestock industries.

Of benefit, has been the close collaboration with AWRI in determining the potential efficacy of grape marc as a methane mitigant for the livestock industries. Use of the *in vitro* system enables early screening of 20 grape marc products, followed up by more detailed studies on the more promising options. Furthermore, the data generated through *in vitro* can now be correlated with detailed biochemical analyses of the grape marc products to elucidate the underlying metabolites that may in fact be inferring the mitigant activity in grape marc. Initially it was considered that either the tannin or fat content was responsible for the mitigant nature of grape marc, however one of the latter experiments conducted in this project has raised question as to whether this is in fact the mode of action. The addition of polyethylene glycol (PEG) to grape marc did not result in an increase in methane production compared to a grape marc only treatment. This observation suggests that any tannin present in the grape marc did not have an inhibitory effect on methanogenesis. In addition, a defatted grape marc produced a similar amount of methane as anon-defattedgrape marc.

Utilisation of the *in vitro* methodology has been of considerable benefit in this project meeting all deliverables, with the technique enabling the project to screen a range of mitigant options prior to proceeding to *in vivo* studies. Of note, has been the detection of an error in the way in which total gas production was being measured and this in turn impacted on calculating the proportion of methane present in total gas produced. This error has been rectified and data is currently being re-analysed from earlier experiments.

In vivo studies

A series of five experiments were undertaken using lactating dairy cattle at the Ellinbank research centre. These experiments used both the SF_6 technique and calorimeters to determine methane emissions and intensity. Significant advances have been made in the use of the SF_6technique. Research in this project has identified the source of measurement errors within the SF_6 tracer technique and has led to development and validation of a modified method (Deighton et al. 2014b). Implementation of the tracer gas technique using these modifications enabled measurement of CH_4 emissions by the SF_6 technique to be highly concordant with measurements made using calorimetric chambers (Deighton et al. 2013b). Another experiment in this project has demonstrated the high degree of accuracy of the modified SF_6technique (Deighton et al. 2014b). In this experiment, lactating dairy cows were fed on freshly harvested perennial ryegrass and mean CH_4 yield (g/kg DMI) was 21.9 ± 1.65 when measured by calorimetric chamber and 22.3 ± 1.44 when measured by the SF_6 technique. The between-cow coefficient of variation was 7.5% when CH_4 yield was measured in chambers and 6.5% when measured by the SF_6 technique. Recently, Moate et al. (2015) have shown that Michaelis-Menten kinetics accurately predict the rate of SF_6 release from permeation tubes used to estimate methane emissions from ruminants. The important implications of this latest research are that when Michaelis-Menten kinetics are used to predict SF_6 release rate from permeation tubes, this will increase the accuracy of the estimation of methane emissions from ruminants and extend for up to one year after deployment of the tubes, the period during which methane emissions can be accurately measured. In association with these advances one of the project team (Matthew Deighton) was awarded his PhD. We now consider that these improvements in the SF_6method will enable this method to be used interchangeably with calorimeters. This development should enable more animals to be tested and in particular those under grazing regimes, although questions remain on accurately measuring forage intake under grazing conditions

This project has further quantified the methane inhibitory effect of feeding a fatty feed supplement (grape marc) to dairy cows and shown that methane emissions are reduced by approximately 3.5% for every 1% increase in dietary fat concentration. This information has been added to the existing database on fats and used to develop a carbon farming methodology by the Australian federal government (ComLaw 2013).

This project has quantified the methane emissions when Australian dairy cows are fed diets containing 70% or more forage and shown that for such diets the methane yield is 21.1 g CH4/kg DMI. This finding should be used to update the Australian Inventory of greenhouse Gases. If this methane yield is incorporated into the Australian GG inventory, it is estimated that this will result in a 10% decrease in the amount of methane deemed to have been produced by the Australian dairy Industry.

The feeding of wheat to dairy cows in an earlier experiment resulted in a 35% reduction in methane emissions, while in a second experiment, the feeding of wheat to dairy cows did not reduce methane emissions. These conflicting findings indicate that while the feeding of wheat to cows has the potential to cause substantial reductions in methane emissions, we still do not understand the mechanisms involved and the dietary circumstances under which the feeding of wheat stimulates a reduction in methane emissions.

A further series of four experiments were undertaken using sheep at the Dookie campus of the University of Melbourne. The collective group of experiments demonstrated that it is possible to measure real time methane emissions in sheep using an open circuit respiration system combined with a face mask, although only for a short 'point in time' measurement period. However, the limitations to this system are the low throughput and the limited time for which the sheep can be assessed as the face masks are restricting and cannot be worn for extensive periods of time. The measurements obtained using this method cannot be extrapolated to predict emissions for an entire day, and it is therefore plausible that the measurement period selected in each experiment may have neglected to capture any variation in methane production occurring due to feed treatment (a similar issue for many other point in time measures). Furthermore, the low number of animals sampled in these experiments has likely contributed to the limited responses observed and an increased number of animals or further replications may change the responses observed in these experiments.

Taken together, this set of experiments highlight that the volume (and pattern) of methane production in sheep is influenced by the type of diet fed, although some of the feeds hypothesised to reduce methane emissions were not as successful as anticipated. This information is important to Australian producers as it further validates that methane production can be altered using dietary manipulations.

The work undertaken in this project supports published data that highlight the natural variation in methane production that exists between animals, which is likely related to genetics and the microbiome of the rumen. Further, availability and costs of forage, feed treatments or supplementary feeds will limit the adoption of any new feeding regime by commercial producers. Providing feed is a major cost of any enterprise and careful cost analysis and system fit considerations need to be undertaken before any new practice will be adopted. Therefore, further research into all of the feeds and treatments utilized in this project is required to confirm that these products are in fact creating a real change and are not impacting production in the long term.

Primarily, it is imperative that an increased number of animals are observed over a longer period of time to determine if observed responses are (i) consistent and (ii) maintained.

Summary of significance of findings for Australian agriculture

This project has identified and quantified feed supplements, grain treatments and novel forages that offer improved strategies for enhancing milk and meat production while simultaneously reducing methane intensity and possibly greenhouse gas emissions. The information and knowledge generated from this project will be invaluable to the Australian dairy and livestock industries and dairy and livestock farmers in the temperate zone of Australia who will benefit in the long term from strategies to improve production while meeting targets for managing methane emissions. For example, research on the commonly fed supplement wheat, has raised the possibility of using this

supplement at different feeding rates to significantly reduce methane emissions and intensity from dairy cows while improving milk production.

The research team have also contributed to the research underpinning development of the ERF methodology for feeding fat/oil to dairy cows. Their research has confirmed that there is approximately a 3.5% fall in methane emissions for every 1% increase in fat concentration in the diet.

This project has accurately measured methane emissions from a large number dairy cows consuming diets containing 70% or more forage and clearly showed methane yield to be relatively constant at 21.1 g per kg of dry matter intake. This value is approximately 10% lower than the value currently used in the Australian Greenhouse Gas Inventory for dairy cows at pasture and clearly indicates lower methane emissions from animals consuming forage based diets.

4. Future research needs

Currently, there are only a few practical and cost effective strategies that can be used on Australian farms to achieve further reductions in total methane emissions. Those currently available include the feeding of lipid rich feed supplements such as; brewers grains, cold pressed canola, cottonseed, hominy meal, grape marc, and the feeding of wheat. Other promising strategies are in early stages of development but offer the possibility of long-term mitigation. These include; genetic selection of animals that are efficient at feed conversion to milk and meat, genetic selection of low-methane emitting animals, vaccines to reduce ruminal methanogens and intra-ruminal administration of specific chemical inhibitors of methanogens. However, well-resourced research on methane mitigation in Australia has been undertaken for less than 15 years and it is likely that further research will be required for significant, sustainable and cost-effective solutions to be developed.

The following areas should have high priority in future methane mitigation research:

(1) The quantification of methane mitigation resulting from feeding wheat

(2) The elucidation of the mechanisms by which the feeding of fatty feed supplements and the feeding of wheat reduce the methane emissions of ruminants

(3) The elucidation of how rumen microbiology influences enteric methane production

(4) The development of low-cost methods for measuring enteric methane production

(5) Large scale screening of animals to identify low methane emitting animals

(6) Research to enhance the productivity of from livestock systems so as to further reduce their methane intensity

5. Publications

Peer reviewed Journal publications

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Deighton MH, Williams SRO., Hannah MC, Eckard RJ, Boland TM, **Moate PJ** (2014) A modified sulphur hexafluoride tracer technique enables accurate determination of enteric methane emissions from ruminants. Animal Feed Science and Technology 197, 47-63.

Durmic Z, **Moate PJ,** Eckard R, Revell DK, **Williams SRO,** Vercoe PE (2013) *In vitro* screening of selected feed additives, plant essential oils and plant extracts for rumen methane mitigation. Journal of the Science of Food and Agriculture 94, 1191-1196. DOI: 10.1002/jsfa.6396.

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Appendix I: Abstracts from published peer reviewed scientific papers

Aprianita, A, Donkor ON, **Moate, PJ, Williams, SRO**, Auldist MJ, Greenwood, JS, Hannah, MC, Wales, WJ, Vasiljevic T. (2014) Effects of dietary cottonseed oil and tannin supplements on protein and fatty acid composition of bovine milk. Journal of Dairy Research 81, 183-193.

Abstract
This experiment was conducted to determine the effects of diets supplemented with cottonseed oil, Acacia mearnsii-condensed tannin extract, and a combination of both on composition of bovine milk. Treatment diets included addition of cottonseed oil (800 g/d; CSO), condensed tannin from Acacia mearnsii (400 g/d; TAN) or a combination of cottonseed oil (800 g/d) and condensed tannin (400 g/d; CPT) with a diet consisting of 6·0 kg dry matter (DM) of concentrates and alfalfa hay *ad libitum*, which also served as the control diet (CON). Relative to the CON diet, feeding CSO and CPT diets had a minor impact on feed intake and yield of lactose in milk. These diets increased yields of milk and protein in milk. In contrast to the TAN diet, the CSO and CPT diets significantly decreased milk fat concentration and altered milk fatty acid composition by decreasing the proportion of saturated fatty acids but increasing proportions of monounsaturated and polyunsaturated fatty acids.
The CPT diet had a similar effect to the CSO diet in modifying fatty acid profile. Overall, reduction in milk fat concentration and changes in milk fatty acid profile were probably due to supplementation of linoleic acid-rich cottonseed oil. The TAN diet had no effect on feed intake, milk yield and milk protein concentration. However, a reduction in the yields of protein and lactose occurred when cows were fed this diet. Supplemented tannin had no significant effect on fat concentration and changes in fatty acid profile in milk. All supplemented diets did not affect protein concentration or composition, nitrogen concentration, or casein to total protein ratio of the resulting milk.

Beecher M, Buckley F, Waters SM, Boland TM, Enriquez-Hidalgo D, **Deighton MH**, O'Donovan M, Lewis E. (2014) Gastrointestinal tract size, total-tract digestibility, and rumen microflora in different dairy cows genotypes. Journal of Dairy Science 97, 3906 – 3917.

Abstract
The superior milk production efficiency of Jersey (JE) and Jersey × Holstein-Friesian (JE × HF) cows compared with Holstein-Friesian (HF) has been widely published. The biological differences among dairy cow genotypes, which could contribute to the milk production efficiency differences, have not been as widely studied however. A series of component studies were conducted using cows sourced from a longer-term genotype comparison study (JE, JE × HF, and HF).The objectives were to (1) determine if differences exist among genotypes regarding gastrointestinal tract (GIT) weight, (2) assess and quantify whether the genotypes tested differ in their ability to digest perennial ryegrass, and (3) examine the relative abundance of specific rumen microbial populations potentially relating to feed digestibility. Over 3 yr., the GIT weight was obtained from 33 HF, 35 JE, and 27 JE × HF nonlactatingcowspostslaughter. During the dry period the cows were offereda perennial ryegrass silage diet at maintenancelevel. The unadjusted GIT weight was heavier for theHF than for JE and JE × HF. When expressed as a proportion of body weight (BW), JE and JE × HF had a heavier GIT weight than HF. *In vivo* digestibility was evaluated on 16 each of JE, JE × HF, and HF lactating dairy cows. Cows were individually stalled, allowing forthe total collection of faeces and were offered freshly cutgrass twice daily. During this time, daily milk yield, BW and dry matter intake (DMI) were greater forHF and JE × HF than for JE; milk fat and proteinconcentration ranked oppositely. Daily milk solids yield did not differ among the 3 genotypes. Intake capacity, expressed as DMI per BW, tended to be different among treatments, with JE having the greatest DMIper BW, HF the lowest, and JE × HF being intermediate. Production efficiency, expressed as milk solids perDMI, was higher for JE than HF and JE × HF. Digestive efficiency, expressed as digestibility of dry matter, organic matter, N, neutral detergent fibre, and acid detergent fibre, was higher for JE than HF. In grazing cows (n = 15 per genotype) samples of rumen fluid, collected using a transesophageal sampling device, wereanalyzed to determine the relative abundance of rumen microbial populations of cellulolytic bacteria, protozoa, and fungi. These are critically important for fermentationof feed into short-chain fatty acids. A decrease was observed in the relative abundance of *Ruminococcusflavefaciens* in the JE rumen compared with HF andJE × HF. We can deduce from this study that the

JE genotype has greater digestibility and a different rumen microbial population than HF. Jersey and JE× HF cows had a proportionally greater GIT weight than HF. These differences are likely to contribute tothe production efficiency differences among genotypes previously reported.

Deighton MH, Williams SRO, Lassey KR, Hannah MC, Boland TM, Eckard RJ, **Moate PJ** (2013). Temperature, but not submersion or orientation, influences the rate of sulphur hexafluoride release from permeation tubes used for estimation of ruminant methane emissions. Animal Feed Science and Technology 194, 71-80

Abstract
Predictable release of sulphur hexafluoride (SF_6) tracer gas from permeation tubes into thereticulo-rumen is necessary to estimate methane emissions from ruminants using the SF_6tracer technique. Any discrepancy between the laboratory determined rate of $_{SF6}$releasefrom permeation tubes and the actual rate of release in the reticulo-rumen would bias calculated methane emissions. The purpose of this investigation was to determine the effect of temperature, submersion and orientation on the rate of $_{SF6}$release from permeation tubes. Four experiments were undertaken. Experiment 1 determined that release of SF_6 increased by 2.5 ± 0.14% per degree Celsius increase in temperature between 37 and 41◦C($P < 0.001$). Experiment 2 determined that the Arrhenius equation can be used to describe the temperature dependence of SF_6release rate from permeation tubes between 0 and70◦C, consistent with a change in release rate of 2.3 ± 0.08% per degree Celsius change in temperature. Experiment 3 determined that submersion of permeation tubes in water did not affect the rate of SF_6release ($P = 0.13$). Experiment 4 determined that SF_6release rate was not influenced by permeation tube orientation ($P = 0.42$). In addition we determined the activation energy of permeation, Ep, describing the overall temperature dependence of SF_6permeation flux from permeation tubes, to be 18,424 ± 680 J/mol. This research implies that the short-term release rate of $SF_6$from permeation tubes within the reticulo-rumen will vary in response to temperature change due to animal, diet and/or environmental factors. A short term decrease in temperature of reticulo-rumen contents, induced by drinking cold water, is expected to have a larger influence on the accuracy of estimated methane emission derived from time-averaged sampling periods less than 24 h. Use of the SF_6technique to detect differences in enteric methane emissions due to diet or between animal species maybe confounded by diet or genetic effects on body temperature. Unless the effect of temperature is managed through careful implementation of the technique, substantial errors could be caused as illustrated by the following example: a +2◦C error in calibration temperature (41◦C), and a −2◦C discrepancy between the actual (37◦C) and assumed reticulo-rumen temperature (39◦C), could bias estimated methane emissions by approximately +10%.

Deighton MH, Williams SRO., Hannah MC, Eckerd RJ, Boland TM, **Moate PJ** (2014) A modified sulphur hexafluoride tracer technique enables accurate determination of enteric methane emissions from ruminants. Animal Feed Science and Technology 197, 47-63.

Abstract
The sulphur hexafluoride ($_{SF6}$) tracer technique enables determination of enteric methane emissions from large numbers of individual ruminant animals. The objective of this research was to identify and correct substantial errors within the $_{SF6}$technique. Six experiments were undertaken using respiration chamber, laboratory or SF_6techniques. Experiment 1 used respiration chambers to demonstrate that the daily pattern of methane emissions from dairy cows was related to their pattern of feed intake. In contrast, the daily emission of $SF_6$from these cows was constant and independent of the pattern of methane emission. This finding supports the contention that in order to accurately determine daily methane emissions using the SF_6technique, it is necessary that gases are collected continuously at a constant rate for 24 h. Since development of the SF_6technique in 1993, it has been pro-pounded that capillary-tube flow restrictors achieved a constant rate of sample collection into evacuated gas collection canisters. Laboratory experiments 2, 3, 4 and 5 demonstrated that, when capillary-tube flow restrictors are used, the rate of sample collection declined and caused a bias of up to 15.6% in calculated methane emissions. This error was caused by an interaction between the declining sample collection rate and the pattern of an animal's methane emission over 24 h. In contrast, orifice plate flow restrictors maintained a constant sample collection rate at canister pressures <0.31 atm and thereby minimised the decline in sample collection rate. Experiment 5 also demonstrated that sample collection using orifice plate flow restrictors, combined with initial (<0.03 atm) and final (<0.49 atm)

canister pressures, substantially reduced measurement error. Accuracy of the modified $_{SF6}$technique, incorporating orifice plate flow restrictors for 24 h sample collection, was validated in Experiment 6. The mean (S.D.) methane yield (g CH4/kg DMI) of eight cows did not differ (P = 0.135) when determined using the modified SF_6technique 22.3 (1.44) or chambers 21.9 (1.65). In addition, the between-animal coefficient of variation for methane yield determined using the modified $_{SF6}$technique (6.5%) was similar to that determined using chambers (7.5%). Consequently the modified $_{SF6}$technique enables the statistical power of experiments to be increased or their size decreased. We conclude that the modified $_{SF6}$technique reduced error associated with $_{SF6}$release, sample collection and analysis. It is recommended that the modified $_{SF6}$technique should be used in preference to the original$_{SF6}$technique for determination of enteric methane emissions from ruminants.

Durmic Z, **Moate PJ,** Eckard R, Revell DK, **Williams SRO,** Vercoe PE (2013) *In vitro* screening of selected feed additives, plant essential oils and plant extracts for rumen methane mitigation. Journal of the Science of Food and Agriculture 94, 1191-1196.

Abstract
BACKGROUND: Ruminants produce large quantities of methane in their rumen as a by-product of microbial digestion of feed. Antibiotics are added to ruminant feed to reduce wasteful production of methane; however, this practice has some downsides. A search for safer and natural feed additives with anti-methanogenic properties is underway. The objective of this research was to examine selected feed additives, plant essential oils and plant extracts for their anti-methanogenic potential in the rumen using an *in vitro*batch fermentation system.
RESULTS:Asignificant reduction (P<0.05) in methane production was observed with nine feed additives (up to40%reduction), all eight essential oils (up to75%reduction) and two plant extracts (14%reduction) when compared to their respective controls. Amongst these, only an algal meal high in docosahexaenoic acid, preparations of Nannochloropsis oculata, calcareous marine algae, yeast metabolites and two tannins did not inhibit microbial gas and volatile acid production.
CONCLUSIONS: The current study identified some potent dietary ingredients or plant compounds that can assist in developing novel feed additives for methane mitigation from the rumen.

Enriquez-Hidalgo D, Gilliland T, **Deighton MH,** O'Donovan M, Hennessy D (2014) Milk production and enteric methane emissions by dairy cows grazing fertilized perennial ryegrass pasture with or without inclusion of white clover. Journal of Dairy Science 97, 1400-1412.

Abstract
An experiment was undertaken to investigate the effect of white clover inclusion in grass swards (GWc) compared with grass-only (GO) swards receiving high nitrogen fertilization and subjected to frequent and tight grazing on herbage and dairy cow productivity and enteric methane (CH4) emissions. Thirty cows were allocated to graze either a GO or GWc sward (n = 15) from April 17 to October 31, 2011. Fresh herbage [16kg of dry matter (DM)/cow] and 1 kg of concentrate/cow were offered daily. Herbage DM intake (DMI) was estimated on 3 occasions (May, July, and September)during which 17 kg of DM/cow per day was offered(and concentrate supplementation was withdrawn). In September, an additional 5 cows were added to each sward treatment (n = 20) and individual CH4 emissions were estimated using the sulfur hexafluoride (SF_6) technique. Annual clover proportion (±SE) in the GWc swards was 0.20 ± 0.011. Swards had similarpregrazing herbage mass (1,800 ± 96 kg of DM/ha)and herbage production (13,110 ± 80 kg of DM/ha).The GWc swards tended to have lower DM and NDFcontents but greater CP content than GO swards, but only significant differences were observed in the last part of the grazing season. Cows had similar milk and milk solids yields (19.4 ± 0.59 and 1.49 ± 0.049 kg/respectively) and similar milk composition. Cows also had similar DMI in the 3 measurement periods (16.0± 0.70 kg DM/cow per d). Similar sward and animal performance was observed during the CH4 estimation period, but GWc swards had 7.4% less NDF than GO swards. Cows had similar daily and per-unit-of-output
CH4 emissions (357.1 ± 13.6 g of CH4/cow per day, 26.3± 1.14 g of CH4/kg of milk, and 312.3 ± 11.5 g of CH4/kg of milk solids) but cows grazing GWc swards had11.9% lower CH4 emissions per unit of feed intake than cows grazing GO swards due to the numerically lower CH4 per cow per day and a tendency for the GWc cows to have greater DMI compared with the GO cows. As a conclusion, under the conditions of this study, sward clover content in the GWc swards was not sufficient to improve overall sward herbage production and quality, or dairy cow productivity. Although GWc cows had a

tendency to consume more and emitted less CH4 per unit of feed intake than GO cows, no difference was observed in daily or per-unit-of-output CH4 emissions.

Fitzsimons C, Kenny DA, **Deighton MH**, Fahey AG, McGee M (2013) Methane emissions, body composition traits, and rumen fermentation variables of beef heifers differing in phenotypic residual feed intake. Journal of Animal Science 91, 5789-5800.

Abstract
This study examined the relationship of residual feed intake (RFI) and performance with methane emissions, rumen fermentation, and digestion in beef heifers. Individual DMI and growth performance were measured for 22 Simmental heifers (mean initial BW 449 kg, SD = 46.2 kg) offered grass silage ad libitum for 120 d. Ultrasonically scanned muscle and fat depth, BCS, muscularity score, skeletal measurements, blood variables, rumen fermentation (via stomach tube), and total tract digestibility (indigestible marker) were measured. Methane production was estimated using thesulfur hexafluoride tracer gas technique over two 5-dperiods beginning on d 20 and 75 of the RFI measurement period. Phenotypic RFI was calculated as actualDMI minus expected DMI. The residuals of the regression of DMI on ADG and midtest metabolic body weight, using all heifers, were used to compute individual RFIcoefficients. Heifers were ranked by RFI and assigned to low (efficient), medium, or high (inefficient) groupings. Overall ADG and DMI were 0.58 kg (SD = 0.18) and 7.40 kg (SD = 0.72), respectively. High-RFI heifers consumed 9 and 15% more ($P < 0.05$) than mediumandlow-RFI groups, respectively. Body weight, growth, skeletal, and composition traits did not differ ($P > 0.05$) between low- and high-RFI groups. High-RFI heifers had higher concentrations of plasma glucose (6%) andurea (13%) and lower concentrations of plasma creatinine (9%) than low-RFI heifers ($P < 0.05$). Rumen pHand apparent *in vivo* digestibility did not differ ($P > 0.05$) between RFI groups, although acetate:propionate ratio was lowest ($P = 0.07$) for low-RFI (3.5) and highest for high-RFI (4.6) heifers. Methane production expressed as grams per day or grams per kilogram metabolic bodyweight was greater ($P < 0.05$) for high (297 g/d and 2.9g/kg BW0.75) compared with low (260 g/d and 2.5 g/kgBW0.75) RFI heifers, with medium (275 g/d and 2.7 g/kg BW0.75) RFI heifers being intermediate. Regression analysis indicated that a 1 kg DM/d increase in RFI was associated with a 23 g/d increase ($P = 0.09$) in methane emissions. Results suggest that improved RFI will reduce methane emissions without affecting productivity of growing beef cattle.

Meale SJ, Chaves AV, Hannah MC, **Williams SRO,** Hume DE, Mace WJ, **Moate PJ** (2013) Comparison of wild type, AR1 and AR37 endophyte infected perennial ryegrass on *in vitro* methanogenesis. Animal Feed Science and Technology 180, 10-17.

Abstract
Perennial ryegrass (Lolium perenne) is a major pasture species grazed by cattle and sheep in the temperate regions of Australia. The principal aim our study was to examine effects of ryegrass endophytic fungi on *in vitro* ruminal methanogenesis. Samples of perennial ryegrass pasture infected with either wild-type endophyte or the novel endophytes,AR1 or AR37, were seasonally collected, over 3 years. Samples were collected during spring, a period which usually coincides with low alkaloid concentrations, and in late summer–autumn, a period in which alkaloid concentrations generally peak. The pasture samples were freeze–dried, ground and measured for concentrations of lolitrem B, ergovaline,peramine and total epoxy janthitrems. Samples of freeze–dried ground pasture were incubated in triplicate for 48 h *in vitro* in cow rumen fluid. Gas production was measured at9, 24 and 48 h post inoculation, the same time at which gas headspace was sampled for CH4concentration. Culture pH and *in vitro* dry matter disappearance were measured at 48 h.In ryegrass with wild-type endophyte, mean (±SD) concentrations (mg/kg DM) of lolitremB were 0.02±0.041 in spring and 1.2±0.89 in summer–autumn while, for ergovaline, the respective concentrations were 0.1±0.04 and 0.5±0.26 and for peramine 6.8±2.27 and
12.5±2.78. For ryegrass infected with AR1, concentrations of peramine were 6.7±2.17 in spring and 12.7±2.97 in summer–autumn. For ryegrass infected with AR37, concentrations of total epoxy-janthitrems were 0.05±0.060 in spring and 10.8±5.89 in summer–autumn.Lolitrem B and ergovaline were not detected in samples of ryegrass infected with AR1or AR37 endophytes. Peramine was not detected in ryegrass infected with AR37 endophyteand epoxy-janthitrems were not detected in ryegrass infected with wild-type or AR1endophytes. Neither endophytic strain nor concentration of alkaloid had any effect on CH4production expressed as ml/g DM. However, CH4 production

expressed as ml/g digestedDM was lower from samples collected in spring than from samples collected in summer/autumn
(P=0.001). Endophyte strain and sampling period had minor effects on other indices of rumen fermentation. Compared to ryegrass infected with wild type endophyte, the novelendophyte strains AR1 and AR37 do not inhibit CH4 production *in vitro*, and are unlikely to have any useful role in CH4 abatement strategies.

Moate PJ, Deighton MH, Ribaux BE, Hannah MC, Wales WJ, **Williams SRO** (2014) Michaelis-Menten kinetics predict the rate of SF_6 release from permeation tubes used to estimate methane emissions from ruminants. Animal Feed Science and Technology 200, 47 – 56.

Abstract
The sulphur hexafluoride (SF_6) technique used to estimate methane emissions from ruminants involves placement of a permeation tube into the reticulo-rumen of animals. The permeation tube releases a trace amount of SF_6across a polytetrafluoroethylene membrane. The animals emit in exhaled breath enteric methane along with a trace amount of theSF_6gas enabling the rate of methane emission to be calculated from the ratio of methane toSF_6. The SF_6release rate from individual permeation tubes is generally determined over a60–90 day period immediately prior to their dosing into the reticulo-rumen of experimental animals. However, the release rate of $SF_6$from permeation tubes slowly declines overtime. This decline can result in substantial error in calculated methane emissions unless gas measurements are made within 30 days of tube dosing or a correction is applied to thepre-determined rate of SF_6release. The research presented here shows that the declining rate of SF_6release can be described by Michaelis–Menten kinetics for up to 800 days, thatMichaelis–Menten parameters can be predicted from the initial mass and loss rate ofSF_6, and that Michaelis–Menten parameters thus determined can be used to accurately predict the future release rate of $SF_6$from permeation tubes for at least 1 year. The two important implications of this research are that application of Michaelis–Menten kinetics to predictSF_6release rates can: (1) increase the accuracy of estimation of methane emissions from ruminants, and (2) extend the period during which estimates of methane emissions can be accurately estimated by the SF_6 technique to 1 year after insertion of permeation tubes into the reticulo-rumen.

Moate PJ, Williams SRO, Grainger C, Hannah MC, Ponnampalam EN, Eckard RJ (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 CH4emissions 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 onCH4 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 contained303 g/kg DM of cracked wheat grain and 70 g/kg DM of solvent extracted canola meal andthe 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 CH4/L milk. Combining data from the fat supplemented diets enabled comparison of CH4 emissions from the CON diet with CH4 emissions from the fat supplementeddiets. 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 by0.79 g CH4/kg DM intake or~3.5% thereby allowing estimation of the magnitude of enteric CH4 abatement based on dietary fat supplementation.

Moate PJ, Williams SRO, Deighton MH, Pryce JE, Hayes BJ, **Jacobs JL**, Eckard RJ, Hannah MC, Wales WJ. (2014) Mitigation of enteric methane emissions from the Australian dairy industry. Proceedings of the 6th Australasian Dairy Science Symposium Pages 121 – 140.

Abstract
Dairy products are the single largest commodity exported from south east Australia. However, dairy farming contributes 12% of Australia's national greenhouse gas emissions. During the last nine years, the Australian Federal Government and the Victorian State Government have funded considerable research towards mitigating greenhouse gas emissions from the agricultural sector. This review examines the findings of that research which pertains to the Australian dairy industry. Calorimeter measurements of 220 cows show a linear increase in enteric methane to increasing feed intake, over a range of forage based diets, with an average enteric methane yield of 21.4 g CH4/kg DMI (n = 220, R2 = 0.70). Adoption of this empirical methane yield, rather than the equation currently used in the Australian greenhouse gas inventory would reduce the methane emissions attributed to the Australian dairy industry by approximately 10%. Analysis of additional data showed that enteric methane yield declined linearly by 0.093 ± 0.0174 CH4 (g CH4/kg DMI) per gram increase in dietary lipid concentration. In addition, enteric methane yield also decreased markedly as the proportion of wheat in the diet increased. It is estimated that in 1980, the Australian Dairy industry produced approximately 185,000 tonnes of enteric methane and enteric methane intensity was approximately 34.1 g CH4/L milk. In 2010, the estimated production of enteric methane was 182,000 tonnes, but the enteric methane intensity had declined by approximately 40% to 20.2 g CH4/L milk. This remarkable decline in enteric methane intensity was mainly achieved by increased per-cow milk production, brought about by the on-farm adoption of research findings related to the feeding and breeding of dairy cows. Options currently available to further reduce enteric methane emissions from Australian dairy cows include the feeding of lipid-rich feed supplements such as cottonseed, brewers grains, cold pressed canola, hominy meal, and grape marc, as well as the feeding of higher rates of wheat. Future technologies that hold promise for reducing enteric methane emissions from the Australian dairy industry include genetic selection of cows with improved efficiency of feed conversion to milk or low methane intensity, vaccines to reduce ruminal methanogens and chemical inhibitors of methanogenesis.

Moate PJ, Williams SRO, Hannah MC, Eckard RJ, Auldist MJ, **Ribaux BE, Jacobs JL,** Wales WJ (2013) Effects of feeding algal meal high in docosahexanoic acid on feed intake, milk production and methane emissions of dairy cows. Journal of Dairy Science 96, 3177-3188.

Abstract
This study examined effects on milk yield and composition, milk fatty acid concentrations and methane
(CH4) emissions when dairy cows were offered diets containing different amounts of algal meal. The algal meal contained 20% docosahexaenoic acid (DHA) and cows were offered either 0, 125, 250, or 375 g/cow per d of algal meal corresponding to 0, 25, 50, or 75 g of DHA/cow per d. Thirty-two Holstein cows in mid lactation were allocated to 4 treatment groups, and cows in all groups were individually offered 5.9 kg of dry matter (DM) per day of concentrates [683 g/kg of cracked wheat (*Triticum aestivum*), 250 g/kg of cold-pressed canola, 46 g/kg of granulated dried molasses, and 21 g/ kg of mineral mix] and ad libitum alfalfa (*Medicago sativa*)hay. The algal meal supplement was added to the concentrate allowance and was fed during the morning and afternoon milking, whereas the alfalfa hay was fed individually in pens. Cows were gradually introduced to their diets over 7 d and then fed their treatment diets for a further 16 d. Dry matter intake and milk yield were measured daily, and milk composition was measured on a sample representative of the daily milk yield on Thursday of each week. For the last 2 d of the experiment, cows were individually housed in respiration chambers to allow measurement of CH4 emissions. Dry matter intake, milk yield and milk composition were also measured while cows were in the respiration chambers. Cows ate all their offered concentrates, but measured intake of alfalfa decreased with increasing dose of DHA by 16.2, 16.4, 15.1, and 14.3 kg of

DM/respectively. Milk yield (22.6, 23.5, 22.6, and 22.6 kg/cow per d) was not affected by DHA dose, but milk fat concentrations (49.7, 37.8, 37.0, and 38.3 g/kg) and, consequently, milk fat yields (1.08, 0.90, 0.83, and 0.85kg/d) decreased with addition of DHA. The feeding of algal meal high in DHA was associated with substantial increases in the concentrations of DHA (0.04, 0.36, 0.60, and 0.91 g/100 g of milk fatty acids) and conjugated linoleic acid C18:2 *cis*-9, *trans*-11 (0.36, 1.09, 1.79, and 1.87 g/100 g of milk fatty acids). Addition ofDHA did not affect total emissions of CH4 (543, 563,553, and 520 g/cow per d), nor emissions interms of milk production (24.9, 22.1, 24.3, and 23.4 g of CH4/kgof milk), but emissions were increased with respect to total intake (22.6, 23.5, 24.5, and 24.4 g of CH4/kg ofDM). These findings indicate that CH4 emissions werenot reduced when dairy cows were fed a forage-based diet supplemented with DHA from algal meal.

Moate PJ, Williams SRO, Torok VA, Hannah MC, **Ribaux, B. E.,** Tavendale M, Eckard RJ, **Jacobs JL,** Auldist MJ., Wales WJ (2013) Grape marc reduces methane emissions when fed to dairy cows. Journal of Dairy Science 97, 5073-5087.

Abstract
Grape marc (the skins, seeds, stalk, and stems remaining after grapes have been pressed to make wine)is currently a by-product used as a feed supplement by the dairy and beef industries. Grape marc contains condensed tannins and has high concentrations of crude fat; both these substances can reduce enteric methane (CH4) production when fed to ruminants. This experiment examined the effects of dietary supplementation with either dried, pelleted grape marc or ensiled grape marc on yield and composition of milk, enteric CH4 emissions, and ruminal microbiota in dairy cows. Thirty-two Holstein dairy cows in late lactation were offered 1 of 3 diets: a control (CON) diet; a diet containing dried, pelleted grape marc (DGM); and a diet containing ensiled grape marc (EGM). The diet offered to cows in the CON group contained 14.0 kg of alfalfa hay dry matter (DM)/d and 4.3 kg of concentrate mix DM/d. Diets offered to cows in the DGM and EGM groups contained 9.0 kg of alfalfa hay DM/d, 4.3 kg of concentrate mix DM/d, and 5.0 kg of dried or ensiled grape marc DM/d, respectively. These diets were offered individually to cows for 18 d. Individual cow feed intake and milk yield were measured daily and milkcomposition measured on 4 d/wk. Individual cow CH4emissions were measured by the $_{SF6}$ tracer technique on 2 d at the end of the experiment. Ruminal bacterial, archaeal, fungal, and protozoan communities were quantified on the last day of the experiment. Cows offered the CON, DGM, and EGM diets, ate 95, 98, and96%, respectively, of the DM offered. The mean milk yield of cows fed the EGM diet was 12.8 kg/cow per day and was less than that of cows fed either the CON diet(14.6 kg/cow per day) or the DGM diet (15.4 kg/cowper day). Feeding DGM and EGM diets was associated with decreased milk fat yields, lower concentrations of saturated fatty acids, and enhanced concentrations ofmono- and polyunsaturated fatty acids, in particular*cis*-9, *trans*-11 linoleic acid. The mean CH4 emissionswere 470, 375, and 389 g of CH4/cow per day for cows fed the CON, DGM, and EGM diets, respectively.
Methane yields were 26.1, 20.2, and 21.5 g of CH4/kg of DMI for cows fed the CON, DGM, and EGMdiets, respectively. The ruminal bacterial and archaeal communities were altered by dietary supplementation with grape marc, but ruminal fungal and protozoan communities were not. Decreases of approximately 20%in CH4 emissions and CH4 yield indicate that feedingDGM and EGM could play a role in CH4 abatement.

O'Neil B F, **Deighton MH,** O'Loughlin BM, Galvin N, O'Donovan M, Lewis E (2012) The effects of supplementing grazing dairy cows with partial mixed ration on enteric methane emissions and milk production during mid to late lactation. Journal of Dairy Science 95, 6582-6590.

Abstract
This study compared the enteric CH4 emissions and milk production of cows offered various grass-baseddiets during mid to late lactation. Forty-eight spring calving Holstein-Friesian dairy cows were randomly assigned to 1 of 3 nutritional treatments for 8 wk: (1) lowgrass allowance (LGA) + partial mixed ration (PMR), (2) high grass allowance (HGA), or (3) LGA. ThePMR group received an allocation of 13.9 kg of grass dry matter (DM)/cow per day and in addition were offered 4.1 kg of PMR DM/cow per day. The HGAgroup received an allocation of 19.3 kg of grass DM/cow per day and the LGA group received an allocation of 14.4 kg of grass DM/cow per day. The PMR offered was composed of 450 g of maize silage/kg of DM, 450g of concentrate blend/kg of DM, and 100 g of barley straw/kg of DM. Daily CH4 emissions were determined using a calibrated tracer technique,

using sulfur hexafluoride, for 5 consecutive days during 2 periods. Simultaneously, grassDM intake (DMI) was estimated using the n-alkane technique and the PMR DMI was also recorded. Cows offered PMR had higher DMI than either the HGA orLGA cows (16.5 vs. 14.9 and 13.9 kg of DM/d). The higher DMI of PMR cows increased milk production relative to HGA and LGA cows: milk yield (17.0 vs.14.6 and 13.1 kg) and fat and protein yield (1.29 vs.1.14 and 1.04 kg). Daily CH4 emissions were higher for the PMR group than for the HGA and LGA groups (406 vs. 384 and 349 g/cow per day). The enteric CH4emissions intensity per unit of DMI, milk yield, solidscorrectedmilk yield, and fat and protein yield did not differ between treatments. Effects observed in the PMRtreatment were due to an increase in DMI rather than to any nutritional characteristic of the PMR.

Reis LG, Chaves AV, **Williams SRO, Moate PJ** (2014) Comparison of enantiomers of organic acids for their effects on methane production *in vitro*. Animal Production Science 54, 1345-1349.

Abstract
This study aimed to evaluate the effect of organic acids on *in vitro*fermentation characteristics. Four organic acids (tartaric, malic, fumaric and citric) and their enantiomers (L-tartaric, D-tartaric, DL-tartaric, L-malic and DL-malic)were analysed using *in vitro*batch culture incubations, at four concentrations (0, 5, 10 and 15 mM). Cumulative total gas and methane (CH4) production (mL/gDM)were measured at 6, 12 and 24 h; ammonia, pH, volatile fatty acids (VFA) and *in vitro*dry matter digestibility (IVDMD) were determined after 24 h of fermentation. Overall, addition of acids at 5 to 15 mMincreased (P<0.0001) cumulative gas andCH4 production.Noeffect (P>0.10) of enantiomers, individual acid or interaction acid concentration was detected at 12 and 24 h for cumulative gas or CH4 production. Addition of DL-malic, L-malic andfumaric acids increased (P < 0.0001) the percentage of propionic acid in the ruminal fluid total VFA compared with all concentrations of the other organic acids or their enantiomers. Ammonia concentration was not affected (P _ 0.28) by the addition of organic acids, concentrations or interactions. These findings are evidence that ruminal microorganisms can metabolise both D- and L-enantiomers of organic acids. None of the organic acids and their enantiomers at four different concentrations demonstrated potential as CH4 mitigation agents.

Ross EM, **Moate PJ,** Bath CR, Davidson S, Sawbridge TI, Guthridge KM, Cocks BG, Hayes BJ (2012) High throughput whole rumen metagenome profiling using untargeted massively parallel sequencing. BMC Genetics 13, 53-66.

Abstract
Background: Variation of microorganism communities in the rumen of cattle (Bos taurus) is of great interest because of possible links to economically or environmentally important traits, such as feed conversion efficiency or methane emission levels. The resolution of studies investigating this variation may be improved by utilizing untargeted massively parallel sequencing (MPS), that is, sequencing without targeted amplification of genes. The objective of this study was to develop a method which used MPS to generate “rumen metagenome profiles”, and to investigate if these profiles were repeatable among samples taken from the same cow. Given faecal samples are much easier to obtain than rumen fluid samples; we also investigated whether rumen metagenome profiles were predictive of faecal metagenome profiles.
Results: Rather than focusing on individual organisms within the rumen, our method used MPS data to generate quantitative rumen micro-biome profiles, regardless of taxonomic classifications. The method requires a previously assembled reference metagenome. A number of such reference metagenomes were considered, including two rumen derived metagenomes, a human faecal microflora metagenome and a reference metagenome made up of publically available prokaryote sequences. Sequence reads from each test sample were aligned to these references. The “rumen metagenome profile” was generated from the number of the reads that aligned to each contig in the database. We used this method to test the hypothesis that rumen fluid microbial community profiles vary more between cows than within multiple samples from the same cow. Rumen fluid samples were taken from three cows, at three locations within the rumen. DNA from the samples was sequenced on the Illumina GAIIx. When the reads were aligned to a rumen metagenome reference, the rumen metagenome profiles were repeatable (P<0.00001) by cow regardless of location of sampling rumen fluid. The repeatability was estimated at 9%, albeit with a highstandard error, reflecting the small number of animals in the study. Finally, we compared rumen microbial profiles to faecal microbial

profiles. Our hypothesis, that there would be a stronger correlation between faeces and rumen fluid from the same cow than between faeces and rumen fluid from different cows, was not supported by our data (with much greater significance of rumen versus faeces effect than animal effect in mixed linear model).
Conclusions: We have presented a simple and high throughput method of metagenome profiling to assess the similarity of whole metagenomes, and illustrated its use on two novel datasets. This method utilises widely used freeware. The method should be useful in the exploration and comparison of metagenomes.

Ross EM, **Moate PJ,** Marrett L, Cocks BG, Hayes BJ (2013) Investigating the effect of two methane mitigating diets on the rumen microbiome using massively parallel sequencing. Journal of Dairy Science 96, 6030-6046.

Abstract
Variation in the composition of microorganisms in the rumen (the rumen microbiome) of dairy cattle (*Bostaurus*) is of great interest because of possible links to methane emission levels. Feed additives are one method being investigated to reduce enteric methane production by dairy cattle. Here we report the effect of 2 methanemitigatingfeed additives (grapemarc and a combinationof lipids and tannin) on rumen microbiome profilesof Holstein dairy cattle. We used untargeted (shotgun)massively parallel sequencing of microbes present in rumenfluid to generate quantitative rumen microbiomeprofiles. We observed large effects of the feed additiveson the rumen microbiome profiles using multiple approaches,including linear mixed modelling, hierarchical clustering, and metagenomic predictions. The effect onthe faecal microbiome profiles was not detectable usinghierarchical clustering, but was significant in the linear mixed model and when metagenomic predictions were used, suggesting a more subtle effect of the diets on the lower gastrointestinal microbiome. A differential representation analysis (analogous to differential expression in RNA sequencing) showed significant overlap in thecontigs (which are genome fragments representing different microorganism species) that were differentially represented between experiments. These similarities suggest that, despite the different additives used, the2 diets assessed in this investigation altered the microbiomes of the samples in similar ways. Contigs that were differentially represented in both experiments were tested for associations with methane production in an independent set of animals. These animals were not treated with a methane-mitigating diet, but did show substantial natural variation in methane emission levels. The contigs that were significantly differentially represented in response to both dietary additives showed a significant enrichment for associations with methaneproduction. This suggests that these methane-mitigating diets have altered the rumen microbiome towardnaturally low methane-emitting microbial profiles. The contig sequences are predominantly new and include *Faecalibacterium* spp. The contigs we have identified here are potential biomarkers for low-methane-emitting cattle.

Ross EM, **Moate PJ**, Marrett L, Cocks BG, Hayes BJ (2013) Metagenomic predictions: From microbiome to complex health and environmental phenotypes in humans and cattle. PLoS ONE 8(9): e73056.

Abstract
Mammals have a large cohort of endo- and ecto- symbiotic microorganisms (the microbiome) that potentially influence host phenotypes. There have been numerous exploratory studies of these symbiotic organisms in humans and other animals, often with the aim of relating the microbiome to a complex phenotype such as body mass index (BMI) or disease state. Here, we describe an efficient methodology for predicting complex traits from quantitative microbiome profiles. The method was demonstrated by predicting inflammatory bowel disease (IBD) status and BMI from human microbiome data, and enteric greenhouse gas production from dairy cattle rumen microbiome profiles. The method uses unassembledmassively parallel sequencing (MPS) data to form metagenomic relationship matrices (analogous to genomic relationship matrices used in genomic predictions) to predict IBD, BMI and methane production phenotypes with useful accuracies($r = 0.423$, 0.422 and 0.466 respectively). Our results show that microbiome profiles derived from MPS can be used to predict complex phenotypes of the host. Although the number of biological replicates used here limits the accuracy that can be achieved, preliminary results suggest this approach may surpass current prediction accuracies that are based on the host genome. This is especially likely for traits that are

largely influenced by the gut microbiota, for example digestive tract disorders or metabolic functions such as enteric methane production in cattle.

Ross EM, Petrovski S., **Moate PJ,** Hayes BJ (2013) Metagenomics of rumen bacteriophage from thirteen lactating dairy cows. BMC microbiology 13, 242-253.

Abstract
Background: The bovine rumen hosts a diverse and complex community of Eukarya, Bacteria, Archea and viruses (including bacteriophage). The rumen viral population (the rumen virome) has received little attention compared to the rumen microbial population (the rumen microbiome). We used massively parallel sequencing of virus like particles to investigate the diversity of the rumen virome in thirteen lactating Australian Holstein dairy cattle all housed in the same location, 12 of which were sampled on the same day.
Results: Fourteen putative viral sequence fragments over 30 Kbp in length were assembled and annotated. Many of the putative genes in the assembled contigs showed no homology to previously annotated genes, highlighting the large amount of work still required to fully annotate the functions encoded in viral genomes. The abundance of the contig sequences varied widely between animals, even though the cattle were of the same age, stage of lactation and fed the same diets. Additionally the twelve animals which were co-habited shared a number of their dominant viral contigs. We compared the functional characteristics of our bovine viromes with that of other viromes, as well as rumen microbiomes. At the functional level, we found strong similarities between all of the viral samples, which were highly distinct from the rumen microbiome samples.
Conclusions: Our findings suggest a large amount of between animal variation in the bovine rumen virome and that co-habiting animals may have more similar viromes than non co-habited animals. We report the deepest sequencing to date of the rumen virome. This work highlights the enormous amount of novelty and variation present in the rumen virome.

Torok VA, Percy NJ, **Moate PJ,** Ophel-Keller K (2014) Influence of dietary docosahexaenoic acid supplementation on the overall rumen microbiota of dairy cows and linkages with production parameters. Canadian Journal of Microbiology 60,267-275.

Abstract: The rumen microbiota contributes to greenhouse gas emissions and has an impact on feed efficiency and ruminant product fatty acid composition. Dietary fat supplements have shown promise in reducing enteric methane production and in altering the fatty acid profiles of ruminant-derived products, yet *in vivo* studies on how these impact the rumen microbiota are limited. In this study, we investigated the rumen bacterial, archaeal, fungal, and ciliate protozoan communities of dairy cows fed diets supplemented with 4 levels of docosahexaenoic acid (DHA) (0, 25, 50, and 75 g·cow−1·day−1) and established linkages between microbial communities and production parameters. Supplementation with DHA significantly ($P < 0.05$) altered rumen bacterial and archaeal, including methanogenic archaeal, communities but had no significant ($P > 0.05$) effects on rumen fungal or ciliate protozoan communities. Rumen bacterial communities of cows receiving no DHA were correlated with increased saturated fatty acids (C18:0 and C11:0) in their milk. Furthermore, rumen bacterial communities of cows receiving a dietsupplemented with 50 g DHA·cow−1·day−1 were correlated with increases in monounsaturated fatty acids (C20:1n-9) and polyunsaturated fatty acids (C22:5n-3; C22:6n-3; C18:2 *cis*-9, *trans*-11; C22:3n-6; and C18:2n-6 *trans*) in their milk. The significant diet associated changes in rumen archaeal communities observed did not result in altered enteric methane outputs in these cows.

Williams SRO,Moate PJ, Hannah MC, **Ribaux BE,** Wales WJ, Eckard RJ (2011) Background matters with the $_{SF6}$ tracer method for estimating enteric methane emissions from dairy cows. Animal Feed Science and Technology 170, 265-276.

Abstract
Since its inception, the sulfur hexafluoride (SF_6) tracer technique for estimating ruminal methane (CH4) 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 SF_6 (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 SF_6 with background concentrations measured during indoor experiments. A literature search found 52 scientific papers which reported use of the SF_6 tracer technique with 17 reporting use indoors, 31 outdoors and 4 were desktop reviews or an uncommon implementation ofSF_6 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◦14S, 145◦56E)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◦41S, 144◦41E). This difference was thought to be due to the CH4 emissions from cows in the Ellinbank area. During the same period, the SF_6 concentration in open air at DPI Ellinbank increased from 4.9 pmol/molin 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 on2 sides, the accumulation of gases during experiments varied in a quadratic manner along the line of feeding stalls with the CH4 concentration ranging from 4 to 10 _mol/mol and SF_6ranging from 4 to 26 pmol/mol. Vertically, background concentration of CH4 trended from4.6 _mol/mol at 225 mm above the floor to 12.3 _mol/mol at 1775 mm while SF_6trended 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 SF_6 measurements from individual animals.

Williams SRO, Clarke T, Hannah MC, Marrett LC, **Moate PJ,** Auldist MJ, Wales WJ (2013) Energy partitioning in herbage-fed dairy cows offered supplementary grain during an extended lactation. Journal of Dairy Science 96, 484-494.

Abstract
An experiment was conducted to quantify the changes in energy partitioning resulting from grain supplementation in herbage-fed dairy cows at 4 stages during a 670-d lactation. The experiment used 16 lactating Holstein-Friesian cows, with a control and a grain treatment being randomly allocated to 8 cows each. During 4 measurement periods (each of 4 d in a metabolism stall and 3 d in an indirect calorimeter) beginning at approximately 110, 270, 450, and 560 d in milk (DIM), the energy balance of each cow was measured. Cows in both groups were individually offered freshly cut ryegrass pasture (*Lolium hybridum* L.) in periods 1and 3 and ryegrass pasture silage and alfalfa (*Medicagosativa*L.) hay in periods 2 and 4. In all periods, cows in the grain group were offered an additional 4.4 to 5.0kg of dry matter of cereal grain/cow per day. Adding grain to the diet increased yields of fat and protein and tended to increase yields of milk and lactose, but did not affect milk composition. Gross energy intake (GEI) declined as lactation progressed. Adding grain to the diet decreased the percentage of GEI in faeces and urine,but the extent of these reductions did not change as lactation progressed. Adding grain to the diet similarly reduced the percentage of GEI lost to heat, but again the extent of the reduction remained similar as lactation progressed. The magnitude of the increase in milk energy resulting from grain supplementation did not change with advancing lactation, but tissue energy retention was greater in the first 300 DIM compared with after 300 DIM. For herbage-based diets, CH4 emissions ranged from 6.2 to 7.6% of GEI, which corresponds to 24.0 to 25.8 g of CH4/kg of dry matter intake. For diets supplemented with cereal grains, CH4 emissions ranged from 6.3 to 7.3% of GEI, which corresponds to21.6 to 25.2 g of CH4/kg of dry matter intake. It was concluded that, for cows producing <24 kg of milk/dand consuming herbage-based diets supplemented with grain, the efficiency of utilizing the additional energy in the grain, as measured by the loss of energy in heat, and its partitioning to milk, did not change as lactationprogressed from 110 to 560 DIM.

Williams SRO, Fisher P, Berrisford T, **Moate PJ,** Reynard K (2014) Reducing methane on-farm may not always reduce net global emissions. The International Journal of Lifecycle Assessment 19, 69-78.

Abstract
Purpose To consider whether feed supplements that reduce methane emissions from dairy cows result in a net reduction in greenhouse gas (GHG) intensity when productivity changes and emissions associated with extra manufacturing and management are included.

Methods A life cycle assessment was undertaken using a model farm based on dairy farms in Victoria, Australia. The system boundary included the creation of farm inputs and on-farm activities up to the farm gate where the functional unit was 1 L of fat and protein corrected milk (FPCM). Electricity and diesel (scaled per cow), and fertiliser inputs (scaled on farm size) to the model farm were based on average data from a survey of farms. Fertiliser applied to crops was calculated per area of crop. Animal characteristics were based on available data from farms and literature. Three methane-reducing diets (containing brewers grain, hominy or whole cotton seed) and a control diet (cereal grain) were modelled as being fed during summer, with the control diet being fed for the remainder of the year in all cases.
Results and discussion Greenhouse gas intensity (kg CO2- eq/L FPCM) was lower than the control diet when the hominy (97 % compared with control) and brewers grain (98 %) diets were used but increased when the whole cottonseed diet was used (104 %). On-farm GHG emissions (kg CO2-eq) were lower than the control diet when any of the methane-reducing diets were used (98 to 99.5%of emissions when control diet fed). Diesel use in production and transport of feed supplements accounted for a large portion (63 to 93 %) of their GHG intensity (kg CO2-eq/t dry matter). Adjusting fertiliser application, changing transport method, changing transport fuel, and using nitrification inhibitors all had little effect on GHG emissions or GHG intensity.
Conclusions Although feeding strategies that reduce methane emissions from dairy cows can lower the GHG emissions up to the farm gate, they may not result in lower GHG intensities (g CO2-eq/L FPCM) when pre-farm emissions are included. Both transport distance and the effect of the feed on milk production have important impacts on the outcomes.

Williams SRO, Moate PJ, Deighton MH, Wales WJ (2014) Methane emissions of dairy cows cannot be predicted by the concentrations of C8:0 and total C18 fatty acids in milk. Animal Production Science 54, 1757-1761

Abstract.

Methane (CH4) emissions from dairy cows are technically difficult and expensive to measure. Recently, some researchers have found correlations between the concentrations of specific fatty acids in milk fat and the CH4 emissions from cows that could obviate the need for direct measurement. In this research, data on individual cow CH4 emissions and concentration of caprylic acid (C8:0) and total C18 fatty acids in milk were collated from eight experiments involving 27 forage-based diets and 246 Holstein-Friesian dairy cows. Linear regressions between CH4 and both C8:0 and total C18 in milk were produced for published data and used to calculate 95% prediction regions for a new observation. The proportion of observed methane emissions from eight experiments that fell outside the 95% prediction region was 27.6% for the C8:0 model and 26.3% for the total C18 model. Neither model predicted CH4 emission well with Lin's coefficient of concordance of less than 0.4 and the Nash–Sutcliffe efficiency coefficient of approximately zero for both the C8:0 and total C18 models. In addition, general linear model analysis showed significant differences between experiments in their intercepts ($P < 0.001$) and slopes ($P < 0.001$). It is concluded that the relationships tested cannot be used to accurately predict CH4 emissions when cows are fed a wide range of diets.