final report

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Reducing Emissions from Livestock Research Program

Effect of starch-based concentrates with different rumen degradation characteristics on methane emissions

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Executive summary

Agriculture produces 16% of national greenhouse gas emissions in Australia mainly as methane (11.6%) and nitrous oxide (2.8%). Based on literature reviews and findings from this and previous RELRP research projects at Department of Primary Industries (DPI), Ellinbank, dietary supplements have the potential to substantially reduce enteric methane emissions from ruminants by 20%. This report covers research activities between 1 January 2012 and 30 April 2012.

1 Objectives

The main objectives of this project were to:

1.1 Quantify methane emissions and methane intensity in response to feeding dairy cows starch based supplements of different degradation characteristics.

1.2 Share biological samples with other Australian researchers working in the field of methane mitigation from ruminants.

2 Deliverables

The deliverable from this project is:

2.1 Draft research paper on starch supplements and methane emissions provided to funding body by 30/05/2012.

3 Summary of research activities

The main research activities associated with this project were:

3.1 Write experimental preschedule and submit for internal DPI review by 30/09/2011. A copy of the experimental preschedule was included in the milestone 2 report provided to the funding body in January 2012.

3.2 Write Animal Ethics Application and submit it to the DPI Animal Ethics Committee by 30/11/2011. A copy of the Animal Ethics Application was included in Milestone 2 report provided to the funding body in January 2012.

3.3 Conduct experiment (15/01/2012 – 1/04/2012). The feeding experiment including measurement of methane emissions was conducted at DPI Ellinbank between 9/01/2012 – 30/3/2012.

3.4 Submit first draft of a research paper to funding body by 30/05/2012. A draft manuscript containing experimental results is attached to this report.

3.5 Complete a research paper and submit it to DPI pubtracking by 30/12/2012.

This report describes findings from one experiment conducted at DPI Ellinbank concerning methane and production responses to feeding dairy cows on diets containing starch based supplements of different rumen degradation characteristics.

The three aims of this experiment were:

1. To determine if slowly rumen fermented starch (cracked corn grain) is a suitable dietary supplement to feed to dairy cows in order to reduce total enteric CH_4 emissions and intensity of CH_4 emissions (g CH_4/L milk), without adversely affecting milk production.
2. To determine if the SF_6 technique can be used in rumen cannulated cows to accurately measure CH_4 emissions.
3. To elucidate nutritional and biological mechanisms influencing enteric CH_4 emissions and the influence of CH_4 mitigating diets on quality attributes of milk by providing biological samples to collaborating scientists.

Summary of achievements

Key findings of this project were:

- Emissions from cows offered a diet containing 10 kg dry matter (DM) of crushed wheat averaged 219 g CH_4/cow per day or 11.1 g CH_4/kg dry matter intake (DMI), whereas when cows were offered a diet containing 10 kg DM of cracked corn, emissions averaged 424 g CH_4/cow per day or 19.5 g CH_4/kg dry matter intake. The unexpectedly very low CH_4 emissions from the wheat based diet are approximately 50% of what would be expected based on the current Australian inventory for CH_4 emissions from dairy cows.
- Milk volumes of cows on the wheat (27.8 l/cow per day) and corn (27.9 l/cow per day) based diets were similar; yields of milk protein were also similar (0.94 kg/cow per day for wheat and 0.91 kg/cow per day for the corn based diet, but milk protein% was slightly, but significantly higher on the wheat based diet (3.38 vs 3.25). Yields of milk fat were substantially decreased on the wheat diet compared to the corn based diet (0.77 vs. 1.18 kg/cow per day) because milk fat% was substantially decreased on the wheat based diet (2.75 vs. 4.23% milk fat).
- Methane emissions measured in the respiration chambers from rumen fistulated and non-fistulated cows were similar, even though the rumen cannulae allowed the ingress of air into the rumen headspace. This is an important experimental finding because it shows for the first time that rumen fistulated cows are valid subjects for research into enteric CH_4 abatement.
- When the SF_6 technique was used to measure CH_4 emissions from rumen cannulated dairy cows, emission estimates were not different to measurements made one week later on the same cows in respiration chambers. This novel finding counters recent speculation in the scientific literature which proposed that the SF_6 technique cannot be used to accurately measure CH_4 emissions from cannulated ruminants.
- Extensive additional measurements were made to elucidate the mechanisms responsible for methane inhibition. Feeding cows 10 kg DM of wheat resulted in a sudden increase in rumen acidity with rumen pH declining below pH 6.0. However, when the corn diet was fed, the decline in rumen pH was not as precipitous or severe. Samples of rumen fluid have been collected and sent to Dr Ben Hayes (DPI Vic) for genomic studies on rumen micro-organisms.

These findings have important implications for the Australian dairy and beef industries. High levels of wheat are already being fed to about 15% of Australian dairy herds. Similarly, high concentrate/forage rations are being fed to many cattle on beef feed-lots. Thus it is likely that the Australian Greenhouse Gas Inventory currently over estimates CH_4 emissions from the Australian dairy and beef industries.

Recommendations

The unexpected finding of low CH_4 emissions from cows fed the wheat based diet poses the following questions: If dairy cows were fed less wheat, would CH_4 suppression occur without such a serious decrease in milk fat yield as observed in this experiment? Are there carry-over effects on CH_4 emissions when cows are fed high levels of wheat? Accordingly, it is recommended that further research be carried out to investigate the CH_4 and production responses and CH_4 carry-over effects to various rates of wheat feeding.

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1 Background

1.1 Project Identification

Project Title	Effect of starch-based concentrates with different degradation characteristics on methane emissions
Project Number	B.CCH.1065
Project Leader	Dr Peter Moate (DPI, Vic)
Contact Details for P. Moate	Future farming Systems Research, Department of Primary Industries, Victoria, 1301 Hazledean Road, Ellinbank, 3820, Victoria, Australia
Project staff	Peter Moate, Richard Williams, Matthew Deighton, Di Mapleson, Brigid Ribaux, Tony Hookey, Serge Ziero, Jae Greenwood
RELRP collaborators	Athol Klieve, UQ. Mark Morrison, CSIRO. Carlos Ramirez, CSIRO. Ian Bland, University of Melbourne Dr Richard Eckard (UMelb)
Other collaborators	Ben Hayes, DPI Vic. Ben Cocks, DPI Vic. Roderick Williams, CSIRO. Todor Vasiljevic, Victoria University. Roderic Williams, CSIRO.
Commencement date	1/01/2012
Completion date	30/04/2012

1.2 Project Background

Enteric CH_4 contributes about 11% of national greenhouse gas emissions, the majority of which comes from beef, sheep and dairy cattle. Methane is predominantly produced in the rumen or first stomach of ruminants during the fermentation of ingested feed. In ruminants, between 4 and 10% of ingested energy is eructated as CH_4. Thus, CH_4 contributes not only to global climate challenges, but also presents an opportunity for efficiency gains in the beef, sheep and dairy industries. In south eastern Australia, these three industries mainly occupy relatively high rainfall, improved pasture zones or feedlots, where dietary and management interventions for reducing CH_4 are more feasible than in extensive rangeland zones.

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

Prior to this project, CH_4 research at DPI Ellinbank focussed on the development of the capacity and skills to accurately measure CH_4 emissions from dairy cows. Two respiration chambers were built at Ellinbank and initial studies focussed on quantifying CH_4 emissions in response to various feed supplements including, whole cottonseed, brewers grains, hominy meal, cold-pressed canola, algae meal high in docosahexanoic acid and grape marc. In addition, the SF_6 tracer technique for measuring CH_4 emissions from grazing cattle was established at Ellinbank and research at Ellinbank showed the accuracy of the technique could be substantially increased by using more appropriate background correction for SF_6 and CH_4 in ambient air, and by recognizing that the release rate of SF_6 from permeation tubes is not constant but follows Michaelis-Menten kinetics.

This report covers research conducted at DPI Ellinbank between 1 January 2012 and 30 April 2012. During this period, research compared CH_4 emissions from cows offered daily supplements of 10 kg DM of either crushed wheat or cracked corn. This research has been co-funded by the Department of Agriculture, Fisheries and Forestry, Department of Primary Industries Victoria, and Dairy Australia.

1.3 Key Stakeholders

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

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

The next users of this research will be policy makers, and personnel within Dairy Australia and DAFF who are concerned with development of methodologies related to the carbon farming initiative.

2 Project objectives

To quantify methane emissions and methane intensity in response to feeding dairy cows starch based supplements of different degradation characteristics.

To share biological samples with other Australian researchers working in the field of methane mitigation from ruminants.

3 Methodology

Experimental design and procedures have been previously reported in detail in milestone 2 report. The following is a brief description of experimental details. The feeding experiment was conducted for 75 days (16/01/2012 – 30/04/2012) using 14 cows to investigate the effect of starch on CH_4 emissions from lactating dairy cows. The experiment was conducted at the Department of Primary Industries - Victoria, Ellinbank Research Centre (DPI Ellinbank, 38°14’S, 145°56’E), in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004).

Initially, eight non-fistulated and eight rumen fistulated, lactating, multiparous Holstein-Friesian cows were fed on each of 2 diets in sequence:

1) A treatment designated WHEAT in which cows received a diet comprising approximately 10.0 kg DM of lucerne hay, 2.0 kg DM of cold pressed canola meal, 0.12 kg DM of mineral mix, 0.12 kg DM of dried powdered molasses and 10.2 kg DM of crushed wheat grain.
2) A treatment designated CORN in which cows received a diet comprising approximately 10.0 kg DM of lucerne hay, 2.0 kg DM of cold pressed canola meal, 0.12 kg DM of mineral mix, 0.12 kg DM of dry powdered molasses and 10.2 kg DM of cracked corn grain.

Two of the non-fistulated cows had to be removed from the experiment (one cow because of mastitis and one cow because of behavioural problems). The canola meal was included in these diets to ensure that the diets would meet the cows dietary requirements for protein. All cows were offered both diets in a replicated, incomplete, randomized Latin-square design. Cows were assigned to each block in order of calving date to minimise the range in days in milk (DIM) when cows began their treatments.

All cows were transitioned to their diet for the first 7 days of each cycle.

Degradation rate of feeds was assessed in rumen-fistulated cows using the nylon bag technique, on days 16 to 21 and 44 to 49 of the experimental period. Incubation periods were 0, 1, 2, 4, 6, 8, 12, 18, 24, 48, 72, and 96 hours.

A SF_6 tracer technique was used to estimate CH_4 emissions on days 23 to 25 and 51 to 53. All cows were fitted with 2 gas collection canisters to simultaneously sample over 1 period of 24 hours and 2 periods of 12 hours. Rumen-fistulated animals were fitted with 2 additional canisters, 1 to sample gas leakage from the fistula and 1 to sample ambient air near the cows right flank.

Samples of rumen fluid were collected for measurement of pH, ammonia concentrations, VFA concentrations and numbers of protozoa. Samples of saliva and hair were collected on day 26 for DNA studies.

Two open circuit calorimeters were used to measure the flux of CH_4, CO_2 and O_2 on days 29 to 30 and 57 to 58 of the experimental period.

Feed consumption and milk yield were measured throughout the experiment. Representative samples of feed offered were analysed for nutritive characteristics. Samples of milk representative of a milking were collected for 6 consecutive milkings each week and for all 4 milkings while cows were in the calorimeters. Milk samples were analysed for fat, protein, lactose and somatic cell count

All data were analysed by analysis of variance using a replicated cross-over designs with diet (corn vs. wheat) and cow type (non-fistulated vs. rumen-fistulated) as factors.

4 Results

The experiment concluded 4 weeks ago on 30/04/2012. Laboratory analyses are continuing and statistical analyses have not yet been completed on all data. Accordingly, this report focuses on CH_4 response to feeding corn and wheat based supplements and effects on milk production. More details including results relating to the chemical composition of dietary feeds, influence of diet and rumen fistulation on rumen fluid pH, concentrations of volatile fatty acids, ammonia, and d-lactate in rumen fluid, counts of ciliated protozoa in rumen fluid, rumen headspace gas composition and the dry matter degradation characteristics of wheat and corn are reported in a draft manuscript in Appendix 6.4. Estimates of CH_4 emissions as estimated by the SF_6 technique in both the non-fistulated and rumen fistulated cows were similar to those made one week later in respiration chambers. This experiment has provided an extensive amount of experimental data sufficient for at least 4 scientific manuscripts.

4.1 Methane

Results relating to CH_4 emissions are shown in Table 1.

Table 1. Influence of diet and fistulation on methane emissions of cows while in chambers.

Cow type	Non-fistulated		Fistulated			Contrast *P* value	
Diet	Corn	Wheat	Corn	Wheat	SED	Corn vs Wheat	Non-fistulated vs Fistulated
Number of cows	6	6	8	8		-	-
Alfalfa DMI (kg/cow/day)	10.1	9.7	10.1	9.8	0.16	0.006	0.808
Conc[1] DMI (kg/cow/day)	11.7	10.4	12.0	10.5	1.09	0.013	0.876
Total DMI (kg/cow/day)	21.8	20.2	22.1	20.3	1.15	0.008	0.856
Milk (kg/cow/day)	27.2	30.4	28.0	28.5	1.65	0.173	0.665
CH_4 (g/cow/day)	441 [b]	224 [a]	407 [b]	213 [a]	25.8	<0.001	0.269
CH_4 (g/ kg DMI)	20.5 [b]	11.5 [a]	18.5[b]	10.7 [a]	1.66	<0.001	0.315
CH_4 (g/kg Milk)	16.7 [b]	7.3 [a]	14.6 [b]	7.9 [a]	1.02	<0.001	0.396
CH_4 (g/kg ECM[2])	15.9 [b]	8.9 [a]	14.5 [b]	9.1 [a]	1.13	<0.001	0.571
CH_4 (g/kg FP[3])	217 [b]	125 [a]	198 [b]	126 [a]	15.7	<0.001	0.521

[1] The concentrate was composed on a dry matter basis, of 1.0% minerals, 1.0% molasses, 16.1% cold pressed canola and 81.9% of either crushed wheat or crushed corn.
[2] ECM = energy corrected milk yield
[3] FP = yield of milk fat plus milk protein
Means within a row followed by different letters differ significantly ($P < 0.05$)

4.2 Milk

Results relating to milk production are shown in Table 2.

Table 2. Influence of diet and fistulation on feed intake, milk production and milk composition in the two week period before cows entered the chambers.

Cow type	Non-fistulated		Fistulated			Contrast *P* value	
Diet	Corn	Wheat	Corn	Wheat	SEM	Corn vs Wheat	Non-fistulated vs Fistulated
Number of cows	6	6	8	8		-	-
Feed intake (kg DM/cow/d)							
Alfalfa hay	10.2 ^{b}	9.2 ^{ab}	10.0 ^{b}	8.9 ^{a}	0.33	0.008	0.303
Concentrate[1]	12.1 ^{ab}	11.8 ^{ab}	12.4 ^{b}	11.2 ^{a}	0.17	<0.001	0.722
Total DMI	22.3 ^{b}	21.0 ^{a}	22.4 ^{b}	20.2 ^{a}	0.35	<0.001	0.450
Milk (kg/cow/d)	27.5 ^{a}	28.6 ^{a}	28.3 ^{a}	26.9 ^{a}	0.72	0.587	0.600
ECM[2] (kg)	28.4 ^{b}	24.8 ^{a}	28.8 ^{b}	22.8 ^{b}	0.80	<0.001	0.371
Fat%	4.34 ^{b}	2.84 ^{a}	4.12 ^{b}	2.67 ^{a}	0.087	<0.001	0.222
Protein%	3.19 ^{a}	3.34 ^{bc}	3.31 ^{ab}	3.42 ^{c}	0.027	<0.001	0.201
Lactose%	5.15	5.14	5.00	5.05	0.021	0.242	0.176
log10 SCC	1.85	1.84	2.06	2.09	0.055	0.827	0.213
Fat (kg/cow/d)	1.19 ^{b}	0.82 ^{a}	1.17 ^{b}	0.72 ^{a}	0.043	<0.0001	0.208
Protein (kg/cow/d)	0.88 ^{a}	0.96 ^{b}	0.93 ^{ab}	0.92 ^{ab}	0.021	0.287	0.805
Lactose (kg/cow/d)	1.42	1.47	1.42	1.36	0.036	0.722	0.241
FP[3] (kg/cow/d)	2.07 ^{b}	1.77 ^{a}	2.10 ^{b}	1.63 ^{a}	0.059	<0.001	0.444

[1] The concentrate was composed on a dry matter basis, of 1.0% minerals, 1.0% molasses, 16.1% cold pressed canola and 81.9% of either crushed wheat or crushed corn.
[2] ECM = energy corrected milk yield
[3] FP = yield of milk fat plus milk protein
Means within a row followed by different letters differ significantly (*P* < 0.05)

5 Discussion / Conclusion

5.1 Research findings

This research has shown that feeding relatively high amounts of crushed wheat instead of crushed corn to dairy cows can result in approximately 50% decrease in CH_4 emissions (g CH_4/cow/day), 50 % decrease in CH_4 yield (g CH_4/kg DMI) and a 50% decrease in CH_4 intensity (g CH_4/kg milk). The findings of this experiment constitute one of the largest reductions in enteric CH_4 emissions that has been obtained by dietary manipulation. The mechanism for the reduction in CH_4 is not clear. We speculate the reduction may be a result of sub-acute rumen acidosis (SARA). Mean rumen pH was not different in cows fed corn (6.22) or wheat (6.13), but there was a longer duration below pH 6 in cows fed wheat than in cows fed corn (633 vs 334 min/day P=0.019). Cows fed wheat also showed some other signs of SARA including low milk fat concentration (2.84% vs 4.34% P<0.001), a relatively low acetate:propionate ratio (1.58 vs 3.65 P<0.001) and elevated concentration of d-lactic acid 9.6 vs 30.0 mM (P<0.001). However, there were no clinical signs of acidosis in the wheat fed cows.

A similar decrease in CH_4 occurred in both non-fistulated and fistulated dairy cows despite the fact that the rumen cannulae of fistulated cows allowed ingress of substantial quantities of air into the rumen headspace. Thus this research has shown that rumen fistulated animals can be validly used in research concerned with the quantification (by means of respiration chambers) of CH_4 emissions and identification of the CH_4 abatement potential of various dietary intervention strategies. Moreover, because similar results with respect to CH_4 emissions were obtained by the chamber technique and the SF_6 technique, we conclude that the SF_6 technique can be used to measure CH_4 emissions from rumen-fistulated cows.

5.2 Achievement of project objectives

Objective 1. To quantify methane emissions and methane intensity in response to feeding dairy cows starch based supplements of different degradation characteristics.This objective has been achieved because we have quantified, using respiration chambers, the CH_4 emissions and milk production in responses to feeding supplements of crushed wheat and crushed corn (see section 4 above). Furthermore, we have determined that CH_4 emissions from both rumen fistulated and non-fistulated cows as measured by the SF_6 technique are similar to emissions as measured using respiration chambers. Thus, we conclude that rumen-fistulated cows can be used in experiments employing either respiration chambers or the SF_6 technique to quantify CH_4 emissions.

Objective 2. To share biological samples with other Australian researchers working in the field of methane mitigation from ruminants. We have achieved this objective. We have provided samples of rumen fluid to Dr. Ben Hayes (DPI Vic) for genomic studies on rumen methanogens. Samples of rumen fluid are being stored at DPI Ellinbank to be provided on request to either Dr Mark Morrison (CSIRO) or Dr Athol Klieve (UQL) for genomic studies on rumen organisms. We have provided samples of whole blood to Dr Carlos Ramirez (CSIRO) for determination of CH_4 concentrations. Samples of blood and hair have also been provided to Dr Ben Hayes (DPI Vic) to extend a cow genomic database. Samples of milk have been provided to Drs Todor Vasiljevic (Victoria University) and Dr Roderick Williams (CSIRO) for measurement of quality attributes of milk.

5.3 Project Impact on Policy:

The research findings and future scientific publications that we envisage will result from this project will provide important information that may be used to adjust the Australian Greenhouse Gas Inventory and may also be useful for the development of a methodology for methane abatement under the Carbon Farming Initiative of The Australian Government's Department of Climate Change and Energy Efficiency.

6 Appendices

6.1 Manuscripts submitted to peer reviewed scientific journals

Ross, E. M., Moate, P. J., Bath, C. R., Davidson, S. E., Sawbridge, T. I., Guthridge, K. M., Cocks B. G., & Hayes B. J. (2011). High throughput whole rumen metagenome 1 profiling 2 using untargeted massively parallel sequencing. Submitted in March 2012 to Molecular Genetics and Computational Biology.

Williams, S. R., Clarke, T., Hannah, M. C., Marett, L. C., Wales, W. J., Moate, P. J., and Auldist, M. J. (2011). Energy partitioning in herbage-fed dairy cows offered supplementary grain during an extended lactation. Submitted 3 April 2012 to Journal of Dairy Science.

Williams S. R., Fisher PD, Berrisford T, Moate P. J., Reynard K (2012). Reducing methane on-farm may not always reduce net global emissions. Submitted 12 April 2012 to The International Journal of Life Cycle Assessment.

6.2 Television interviews

Peter Moate was filmed and interviewed on 2nd February 2012 by Al Jazeera for a seven minute story (on methane mitigation by feeding cows grape marc) which was broadcast world wide in late April to an estimated 500 million viewing audience in North America, South America, Europe, Asia, Middle East and Australia. The web link to this video is: http://www.youtube.com/watch?v=v4ELCYGc22Y

6.3 Extension activities

Project personnel have presented at a range of forums including conferences, workshops, seminars, field days, farmer meetings, and given talks to policy advisors, service providers and tertiary students. These presentations promote awareness of this project and other methane abatement research being conducted nationally. These events included:

14th February 2012 Peter Moate spoke about the methane project to an Education sub-committee group of Victorian parliamentarians visting Ellinbank.

15th and 16th of February 2012 Peter Moate gave talks about methane mitigation to farmer groups at the Sungold Field days held at Allansford in the Western district of Victoria.

24th February 2012 Peter Moate gave talks to groups of farmers on methane mitigation research at Ellinbank as part of the 2012 Australian Dairy Confernce.

28th February 2012 Peter Moate gave a talk on methane mitigation to a group of "Dairy Farmers of Tomorrow" at Dairy Australia headquarters in Melbourne.

16th March 2012 Peter Moate gave a talk on methane mitigation research to a group of DPI personnel at an Innovation in Agriculture workshop held at Attwood, Victoria.

27th March 2012 Peter Moate gave a presentation on this research project to an MLA workshop held in Canberra.

16th April 2012 Peter Moate gave a presentation on methane research to 49 Bioscience students from Monash University.

20th April 2012 Peter Moate gave a presentation on methane research to 50 Bioscience students from Monash University.

23rd April 2012 Peter Moate attended a meeting at Dairy Australia (Melbourne) concerned with the initiation and development of the CFI methodology (feed supplements) and to ensure that the key design elements are agreed

Richard Eckard, Richard Williams and Peter Moate have been involved in the production of a CCRP DVD on Feeding Fats and Oils.
(contact: erin.chillingworth@seftonpr.com.au)

6.4 Draft manuscript

A copy of the manuscript as at 1/05/2012 is attached.

Comparison of corn and wheat based diets for their effects on milk production and methane emissions.

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Article to be submitted to Journal of Dairy Science

INTRODUCTION

Methane (CH_4) is an important greenhouse gas contributing to global warming (Forster et al., 2007). Animal agriculture is responsible for approximately 11% of global greenhouse gas emissions with enteric CH_4 emissions constituting approximately 80% of these emissions (O'Mara, 2011). There are clear imperatives for developing practical strategies that will lead to a reduction in these emissions. The most successful mitigation strategies will achieve a substantial decrease in emissions and either lead to a profitable increase in milk or meat production or at least maintain profitability while reducing emissions.

Recent research (M. J. Auldist, Department of Primary Industries, Ellinbank, Victoria, Australia, personal communication) has shown that cows fed a partial mixed ration containing cracked corn produced more energy corrected milk than cows fed an iso-energetic diet containing crushed wheat. The reasons for this increase in production are thought to be associated with the fact that cows fed corn based diets tended to have a higher rumen fluid pH and greater feed intake than cows fed the wheat based diet. The mechanism is believed to relate to the fact that low pH in rumen fluid has been associated with lower rates of fiber digestion, hence lower rumen passage rates of digesta and therefore lower voluntary feed intake. Methane emissions (g CH_4/cow per day) from livestock are generally directly related to feed dry matter intake with research (P. J. Moate, unpublished) showing typical methane yields of 23 g CH_4/kg DMI. An important metric regarding CH_4 emissions is the intensity of emissions and in the dairy industry this equates to g CH_4/kg milk. In all production systems, animals first need to meet their energy requirements for maintenance before production responses from intake are possible. Given this necessity, there is general consensus amongst researchers that the lowest intensity of emissions can be obtained when cows are able to eat as much as possible and produce as much milk as possible. Thus, prima facie, diets containing corn grain would appear to enable elevated feed intake and hence lowered intensity of CH_4 emissions. However, there is also in vitro and in vivo evidence that low rumen fluid pH may be associated with low emissions of CH_4 (Lana et al. 1998; Russell 1998). Indeed, there appears to be a threshold rumen fluid pH somewhere between 5 and 6 at which methanogenesis is stalled.

Diets high in rapidly fermented starch tend to result in rumen contents with lower pH than when the diet contains a high proportion of slowly fermented starch. Therefore, diets with a high proportion of quickly fermented starch might be expected to produce less CH_4 than diets containing a high proportion of slowly fermented starch. However, Benchaar et al. (2005) using the mathematical rumen model of Dijkstra et al. (1992) predicted that replacing rapidly degraded starch (barley) with slowly degraded starch (corn) can result in a 14% reduction in rumen methane emissions associated with a decrease in the acetate : propionate ratio in rumen fluid. Moreover, Carro and Ranilla (2003) carried out in vitro tests on 4 different cereal grain diets and found that a corn based diet was associated with the lowest CH_4 emissions. The situation is also complicated when CH_4 intensity (g CH_4/L milk) is considered. Partial mixed rations containing slowly fermented starch from corn have recently been found to result in higher yields of milk and milk solids than diets containing rapidly fermented starch. Thus, the future practicality of corn grain as a major supplement for dairy cows may depend upon a reconciliation of its effects on both production and CH_4 emissions.

We hypothesized that dairy cows fed starch that ferments slowly in the rumen (corn grain) to will have higher CH_4 emissions (g CH_4/cow/day), CH_4 yield (g CH_4/kg

DMI) and intensity of CH_4 emissions (g CH_4/L milk), in comparison to when they are fed a diet containing starch that ferments quickly in the rumen (wheat grain).

MATERIALS AND METHODS

The experiment was conducted at the Department of Primary Industries, Ellinbank Research Centre, Victoria, Australia (DPI Ellinbank, 38°14’ S, 145°56’ E) and was conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004). Animal use was approved by the Animal Ethics Committee of the Department of Primary Industries – Victoria.

Cows, Diets, Feeding and Management

Eight rumen-fistulated (cannula internal diameter 10 cm; Robyn’s Rumen Cannula, Lilydale, Victoria, Australia) and 6 non-fistulated, lactating, multiparous Holstein-Friesian cows were fed in the DPI Ellinbank animal house on each of 2 diets in a cross-over sequence. The initial design was a partial cross-over design with four treatments in 4 blocks of size 4, and two periods within each block. The four treatments consisted of the 2 by 2 factorial in diet (wheat, corn) by cow type (fistula, non-fistula). Each block consisted of 4 cows: one of each treatment combination. In the second period of each block, each cow changed diet, from wheat to corn, or from corn to wheat. Blocks were temporally staggered with a lag time of one week between consecutive blocks. Cows were allocated to blocks according to calving date.

1) A treatment designated WHEAT in which cows received a diet comprising approximately 10.0 kg DM of alfalfa hay, 2.0 kg DM of cold pressed canola meal, 0.12 kg DM of mineral mix, 0.12 kg DM of dry molasses powder and 10.2 kg DM of crushed wheat grain.

2) A treatment designated CORN in which cows received a diet comprising approximately 10.0 kg DM of alfalfa hay, 2.0 kg DM of cold pressed canola meal, 0.12 kg DM of a mineral pellet, 0.12 kg DM of dry molasses powder and 10.2 kg DM of cracked corn grain.

Thus, the concentrate portion of the diet was composed on a dry matter basis, of 1.0% minerals, 1.0% molasses, 16.1% cold pressed canola and 81.9% of either crushed wheat or crushed corn. The canola meal was included in these diets to ensure that the diets met the cows' dietary requirements for protein and fat (NRC 2001). The feeding sequence was 4 randomized Latin squares. Due to the staggered start imposed by having only 2 calorimeters, cows were assigned to each block in order of calving date to minimize the range in DIM when cows began their treatments.

For the first 7 days after a cow entered the experiment, she was introduced to her first experimental diet. On day 29 she entered the respiration chamber and measurements on CH_4 emissions took place on day 29 and day 30. On the morning of day 31 (at 0800 h) she was removed from the respiration chamber, but continued to be fed her first experimental diet until 1130 hours on day 31. At 1130 hours, her rumen contents were sampled via a stomach tube. At 1500 hours on day 31 she commenced her introduction to her second dietary treatment and the schedule of feeding, and measurement of CH_4 emissions repeated with the cow being in the respiration chamber for a second period on days 57 and 58.

Cows were milked twice daily, at ~0600 and ~1500 h. Each cow was offered 1 kg DM of her concentrate mix during milking as a contentment feed. Any remainder was transferred to the animal house and added to the remainder of that cows ration. Cows were offered the remainder of their grain in the animal house before being given access to their allocation of alfalfa hay.

Measurements

All fresh quantities of concentrates and alfalfa hay offered and of concentrates and hay refused were weighed for individual cows, every feed. Samples of hay offered each day were collected and DM concentration determined each day while samples of concentrates offered were collected once each week for DM determination. Representative samples of hay, crushed wheat, cracked corn grain and cold-pressed canola offered were collected over the duration of the experiment, bulked and frozen. These were subsequently freeze dried, ground to pass through a 0.5 mm sieve and then chemically analysed for neutral detergent fibre (NDF), crude protein (CP) and ether extract (EE). Particle size distribution of wheat and of corn was determined using sieves.

Fat content of all feeds was analysed by soxhlet extraction using petroleum spirit (AACC method 30-20, AOCS Aa 4-38). All feeds were analysed by near-infrared spectroscopy for CP. NDF was determined by Van Soest extraction (Van Soest et al., 1991) using the Foss® FiberCap technique (Foss Tecator, 2002). The estimated ME concentration of the feeds was based on analysis by near infrared spectroscopy by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY).

Rumen digestion kinetic studies were carried out using the 8 fistulated cows on days 23-26 and days 51-54 after entering the experiment. Samples of ground alfalfa hay, ground canola meal, crushed corn, ground corn, crushed wheat and ground wheat were incubated in nylon bags in the rumen of each fistulated cow. Nylon bags were placed into the rumen in 2 batches. In the first batch, inserted at 0700 h to coincide with the morning feeding, 1 nylon bag of each feed type was placed into a mesh laundry bag with a 190 g weight then 7 laundry bags were inserted into the rumen. One laundry bag was removed at each of 1, 2, 4, 6, 8, 12, 18 h after insertion. In batch

2, inserted at 1500 h to coincide with the afternoon feeding, up to 3 nylon bags of each feed type were placed into a mesh laundry bag with a 190 g weight. Four of these laundry bags were inserted into the rumen and one of each of these laundry bags was removed at 24, 48, 72 and 96 h after insertion. Upon removal, nylon bags were plunged into iced water, rinsed under cold running water, smoothed flat on a tray then frozen at -18°C. These bags and contents were then freeze dried and then reweighed. Parameters relating to degradability of dry matter in these bags were then calculated (Ørskov, E.R. and I. McDonald. 1979) by means of a non-linear regression using the Genstat 13 computer program.

Milk yield from individual cows' was measured each milking each day. When not in the calorimeters milk yield measurements were made using a DeLaval ALPRO milk metering system (DeLaval International, Tumba, Sweden) and milk samples for composition analysis were collected from Tuesday afternoon milking to Friday morning milking (inclusively). While in the calorimeters (2 days for each cow) milk yield measurements were made by collecting and weighing the milk from individual cows and milk samples (1% of the yield from individual cows at each milking) were collected, preserved (with 1 M Bronopol @ 4 ml preservative/L of milk), refrigerated then bulked over the 2 days for each cow. From each bulked sample, 2 sub-samples were taken for composition analysis, and 1 sub-sample was taken for N analysis (by the Dumas method).

Fat, protein, and lactose concentrations in milk were measured by means of a near-infrared milk analyser (model 2000, Bentley Instruments, Chaska, MN, USA). Somatic cells were counted by a Fossomatic SC300 cell counter.

Methane Emissions

Methane emissions from each cow were measured using the Indirect Calorimeters at DPI Ellinbank. General characteristics of the calorimeters have been described by Grainger et al. (2007). In this experiment, air was exhausted from the chamber at ~1800 L/min. Both the exhaust air and the replacement air were analyzed with concentrations of O_2, CO_2, and CH_4 recorded every 10 seconds. Calibration of the chambers was performed as reported previously (Williams et al. 2012).

Ruminal Fermentation, and Protozoa

Gas from the rumen headspace from individual cows was sampled by means of rumenocentesis for non-fistulated cows and rumenocentesis through the rubber rumen cannula at 0700 h on days 25 and 53 of the experiment. These samples were sealed then stored until the end of the experiment. Samples were analyzed by gas chromatography to determine the gas composition (% by volume) (CO_2, CH_4, O_2 and H_2). The dinitrogen (N_2) percentage was estimated as 100 – ($C0_2$, CH_4, H_2 and O_2). The percentage of the sample made up of fermentation gases (FG) was calculated as the sum of CO_2, CH_4 and H_2.

Temperature and pH of rumen fluid in cannulated cows were continuously monitored by intra-rumenal boluses (KB1000, Kahne Limited, Auckland, New Zealand) while the cows were in the calorimeters and the week prior. These devices were calibrated according to the manufacturer’s instructions and their pH measurement compared with pH measured by a Mettler-Toledo FG2 pH meter (Schwerzenbach, Switzerland). Three boluses were used in each cannulated animal. One was weighted (~750 g) so it resided at the bottom of the rumen, one was placed into a mesh laundry bag then tied to the cannula so it resided at the top of the rumen raft, and one was unmodified so it floated within the rumen raft as designed. This was to check if the diurnal variation in pH recorded by the free-floating rumenal bolus represents the overall diurnal variation in rumen pH or simply a reflection of the movement of the free-floating bolus within the rumen over time.

One week before each cow entered the calorimeters, samples of rumen material were collected via a stomach tube. The pH of the rumen fluid was immediately measured using a Mettler-Toledo FG2 pH meter (Schwerzenbach, Switzerland).

Ciliate protozoa were counted from a 0.5 ml sub-sample of rumen fluid transferred to a 12 ml plastic vial. This was immediately diluted with 4.5 ml of a stain solution containing 10% formalin, 0.9% saline and 0.06% methyl green. These samples were stored at 4°C until counted on-site for ciliate protozoa.

Volatile fatty acids in rumen fluid were measured from a 5 ml sample of rumen fluid to which two drops of concentrated H_2SO_4 was immediately added, then stored at -18°C. Analysis was by capillary gas chromatography using Supelco Bulletin 749D. The concentration of d-lactate in rumen fluid was measured by a Boehringer Mannhein kit (Cat No. 11 112 821 035) after deproteinisation with perchloric acid and analaysed on an Olympus AU400 Autoanalyser. . The ammonia-N concentration in rumen fluid was determined from a 5 ml sample of acidified rumen fluid. The analysis involved a direct enzymatic method using Infinity ™ Ammonia Liquid Stable Reagent (Cat No.TR60101 supplied by Beckman Coulter Australia) on an Olympus AU400 Autoanalyser (AHL NTM-56). Analyses for VFA's, d-lactate and ammonia-N concentration in rumen fluid were performed at Animal Health Laboratories, DAFF, South Perth, Australia.

Statistical Analyses

Milk production and milk composition data were analysed according to a mixed model having fixed nested effects for block and period within block, and factorial effects for diet by cow-type. Random effects were nested: day within period within cow. The same fixed and random structures were used for production data prior to cows being in chambers and production and methane data whilst cows were in respiration chambers.

Rumen fluid pH, VFAs, protozoa, and headspace gas data were analysed using a similar mixed model but with the exclusion of the random effect for day, as there were no repeated measures within periods for these data.

Dry matter and starch digestion dynamics data, measured in fistulated cows only, were analysed according to a mixed model having fixed factorial effects for diet by feed-type and nested random effects for sample-bag within period within cow.

Distributional assumptions of normality and constant variance were checked visually using graphs of residuals against fitted values, and histograms and normal quantile plots of residuals.

RESULTS

The following are preliminary results that still require quality assurance checks.

Feed composition

Table 1. Composition of dietary ingredients (g/kg DM).

Parameter	Crushed wheat	Crushed corn	Alfalfa hay	Cold pressed canola	Molasses	Mineral pellet
DM (g/kg FW)	935	913	929	932	997	816
Crude protein	136	97	174	372	92	75
Soluble protein	127	92	147	334	92	72
ADF	66	44	389	216	42	66
NDF	138	116	501	293	132	143
Lignin	11	10	107	112	0	24
NFC	727	732	266	289	669	122
Starch	497	608	5	15	0	52
Ash	17.2	15.9	80.1	87.2	110	620.4
TDN	850	900	520	710	720	280
Crude fat	20	55	18	66	0	60
Sodium	0.1	0.1	1.4	0.8	9.4	0.3
Potassium	4.8	4.6	24.4	13.8	31.1	6.1
Calcium	0.8	0.1	8.5	6.4	21.4	111.2
Magnesium	1.6	1.7	2.5	5.7	4.2	101.7
Phosphorus	3.4	3.7	2.7	11.1	3.1	59.8
Sulfur	1.6	1.1	2.3	6.4	3.8	6.1
Chloride	1.1	1.1	9.8	1.0	7.5	1.5

Milk Production

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

Cow type	Non-fistulated		Fistulated			Contrast *P* value	
Diet	Corn	Wheat	Corn	Wheat	SEM	Corn vs Wheat	Non-fistulate vs fistulated
Number of cows	6	6	8	8		-	-
Feed intake (kg DM/cow/d)							
Alfalfa hay	10.2 [b]	9.2 [ab]	10.0 [b]	8.9 [a]	0.33	0.008	0.303
Concentrate[1]	12.1 [ab]	11.8 [ab]	12.4 [b]	11.2 [a]	0.17	<0.001	0.722
Total DMI	22.3 [b]	21.0 [a]	22.4 [b]	20.2 [a]	0.35	<0.001	0.450
Milk (L/cow/d)	27.5 [a]	28.6 [a]	28.3 [a]	26.9 a	0.72	0.587	0.600
ECM[2] (kg)	28.4 [b]	24.8 [a]	28.8 [b]	22.8 [b]	0.80	<0.001	0.371
Fat%	4.34 [b]	2.84 [a]	4.12 [b]	2.67 a	0.087	<0.001	0.222
Protein%	3.19 [a]	3.34 [bc]	3.31 [ab]	3.42 [c]	0.027	<0.001	0.201
Lactose%	5.15 [a]	5.14 [a]	5.00 [a]	5.05 [a]	0.021	0.242	0.176
log10 SCC	1.85 [a]	1.84 [a]	2.06 [a]	2.09 [a]	0.055	0.827	0.213
Fat (kg/cow/d)	1.19 [b]	0.82 [a]	1.17 [b]	0.72 [a]	0.043	<0.0001	0.208
Protein (kg/cow/d)	0.88 [a]	0.96 [b]	0.93 [ab]	0.92 [ab]	0.021	0.287	0.805
Lactose (kg/cow/d)	1.42	1.47	1.42	1.36	0.036	0.722	0.241
FP[3](kg/cow/d)	2.07 [b]	1.77 [a]	2.10 [b]	1.63 [a]	0.059	<0.001	0.444

[1] The concentrate was composed on a dry matter basis, of 1.0% minerals, 1.0% molasses, 16.1% cold pressed canola and 81.9% of either crushed wheat or crushed corn.
[2] ECM = energy corrected milk yield
[3] FP = yield of milk fat plus milk protein
Means in the same row followed by different superscripts differ significantly ($P < 0.05$)

Methane emissions

Table 3. Influence of diet and fistulation on CH_4 emissions of cows while in chambers.

Cow type	Non-fistulated		Fistulated			Contrast *P* value	
Diet	Corn	Wheat	Corn	Wheat	SED	Corn vs Wheat	Non-fistulate vs fistulated
Number of cows	6	6	8	8		-	-
Alfalfa DMI	10.1	9.7	10.1	9.8	0.16	0.006	0.808
Conc[1] DMI	11.7	10.4	12.0	10.5	1.09	0.013	0.876
Total DMI (kg/cow/day)	21.8	20.2	22.1	20.3	1.15	0.008	0.856
Milk (kg/cow/day)	27.2	30.4	28.0	28.5	1.65	0.173	0.665
CH_4 (g/cow/day)	441 ^{b}	224 ^{a}	407 ^{b}	213 ^{a}	25.8	<0.001	0.269
CH_4 (g/ kg DMI)	20.5 ^{b}	11.5 ^{a}	18.5 ^{b}	10.7 ^{a}	1.66	<0.001	0.315
CH_4 (g/kg Milk)	16.7 ^{b}	7.3 ^{a}	14.6 ^{b}	7.9 ^{a}	1.02	<0.001	0.396
CH_4 (g/ ECM[2])	15.9 ^{b}	8.9 ^{a}	14.5 ^{b}	9.1 ^{a}	1.13	<0.001	0.571
CH_4 (g/kg FP[3])	217 ^{b}	125 ^{a}	198 ^{b}	126 ^{a}	15.7	<0.001	0.521

[1] The concentrate was composed on a dry matter basis, of 1.0% minerals, 1.0% molasses, 16.1% cold pressed canola and 81.9% of either crushed wheat or crushed corn.
[2] ECM = energy corrected milk yield
[3] FP = yield of milk fat plus milk protein
Means in the same row followed by different superscripts differ significantly ($P < 0.05$)

Rumen fermentation

Table 4. Influence of diet and fistulation on rumen fluid pH.

Cow type	Non-fistulated		Fistulated			Contrast *P* value	
Diet	Corn	Wheat	Corn	Wheat	SED	Corn vs Wheat	Non-fistulate vs fistulated
Number of cows	6	6	8	8		-	-
Mean rumen fluid pH at 10.30AM[1]	6.48 [b]	5.64 [a]	6.11 [b]	5.66 [a]	0.173	<0.001	0.285
Mean within cow pH	N.A.	N.A.	6.22	6.13	0.054	0.160	N.A.
SD within cow pH	N.A.	N.A.	0.25 [a]	0.49 [b]	0.032	<0.001	N.A.
Maximum pH	N.A.	N.A.	6.78	7.04	0.084	0.028	N.A.
Nadir of pH	N.A.	N.A.	5.80 [b]	5.42 [a]	0.053	<0.001	N.A.
Total duration below pH 6 (min)	N.A.	N.A.	334 [a]	633 [b]	87	0.019	N.A.
pH area below 6 pH.min	N.A.	N.A.	39 [a]	237 [b]	31.0	0.001	N.A.

[1] Rumen fluid pH measurements at 10.30 AM were on samples collected by stomach tube. All other measurements were made by Kahne intra-ruminal electronic boli weighted so as to reside in the bottom of the rumen of the rumen fistulated cows.

N. A. = Not applicable.

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

Table 5. Influence of diet and fistulation on concentrations in rumen fluid of ammonia, total volatile fatty acids (VFA), individual VFAs, and rumen protozoa.

Cow type	Non-fistulated		Fistulated			Contrast P value	
Diet	Corn	Wheat	Corn	Wheat	SED	Corn vs Wheat	Non-fistulate vs fistulated
Number of cows	6	6	8	8		-	-
Rumen fluid NH_3 (mg/L)	222 [b]	98 [a]	259 [b]	111 [a]	24.4	<0.001	0.246
Rumen fluid Total VFAs (mM)	77 [a]	118 [b]	100 [a]	121 [b]	7.9	<0.001	0.052
Individual VFAs (mM%)							
Acetic	64.8 [b]	54.2 [a]	63.8 [b]	55.9 [a]	1.41	<0.001	0.753
Proprionic	17.7 [a]	34.8 [b]	18.8 [a]	31.6 [b]	2.09	<0.001	0.401
Iso-Butyric	0.99 [b]	0.30 [a]	0.93 [b]	0.32 [a]	0.055	<0.001	0.514
n-Butyric	12.4 [b]	7.5 [a]	13.0 [b]	8.7 [a]	0.961	<0.001	0.135
Iso-Valeric	1.54 [b]	0.34 [a]	1.46 [b]	0.51 [a]	0.107	<0.001	0.533
n-Valeric	2.41	1.98	1.74	1.93	0.416	0.907	0.050
Caproic	0.17 [a]	0.85 [b]	0.29 [a]	1.06 [b]	0.306	0.006	0.296
D-lactate	9.6 [a]	30.0 [b]	4.3 [a]	67.3 [b]	13.62	<0.001	0.214
A:P ratio	3.65 [b]	1.58 [a]	3.42 [b]	1.78 [a]	0.216	<0.001	0.819
BCFA	2.54 [b]	0.63 [a]	2.40 [b]	0.84 [a]	0.154	<0.001	0.731
Log10 (Protozoa)							
Entodinia	5.53 [b]	2.64 [a]	5.66 [b]	2.35 [a]	0.445	<0.001	0.836
Epidinia	4.09 [b]	2.14 [a]	4.64 [b]	2.22 [a]	0.366	<0.001	0.300
Other	2.91 [a]	2.49 [a]	3.83 [b]	3.17 [b]	0.852	0.253	0.047
Total	5.48 [b]	3.13 [a]	5.73 [b]	3.53 [a]	0.428	<0.001	0.508

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

Table 6. Influence of diet and fistulation on rumen headspace gas composition[1].

Cow type	Non-fistulated		Fistulated			Contrast *P* value	
Diet	Corn	Wheat	Corn	Wheat	SED	Corn vs Wheat	Non-fistulate vs fistulated
Number of cows	6	6	8	8		-	-
CO_2	55.4 [b]	56.3 [b]	15.5 [a]	10.1 [a]	2.74	0.121	<0.001
CH_4	26.4 [b]	24.5 [b]	4.2 [a]	0.3 [a]	3.34	0.21	<0.001
H_2	0.15 [b]	0.43 [b]	0.04 [a]	0.02 [a]	0.078	0.058	<0.001
FG	87.0 [b]	80.6 [b]	20.1 [a]	10.9 [a]	4.3	0.066	<0.001
N_2	11.6 [a]	18.2 [a]	68.4 [b]	76.0 [b]	3.7	0.057	<0.001
O_2	1.33 [a]	1.29 [a]	11.5 [b]	13.1 [b]	0.78	0.255	<0.001

[1] Measurements were made at 0700 h, which was just after the morning milking, but before the morning feeding

[2] FG represents fermentation gases and is defined as the sum of CO_2, CH_4 and H_2.

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

Table 7. Influence of diet on dry matter and starch digestion kinetics of wheat and corn.

Table 5 Rumen degradation characteristics of dry matter (DM) from feeds.

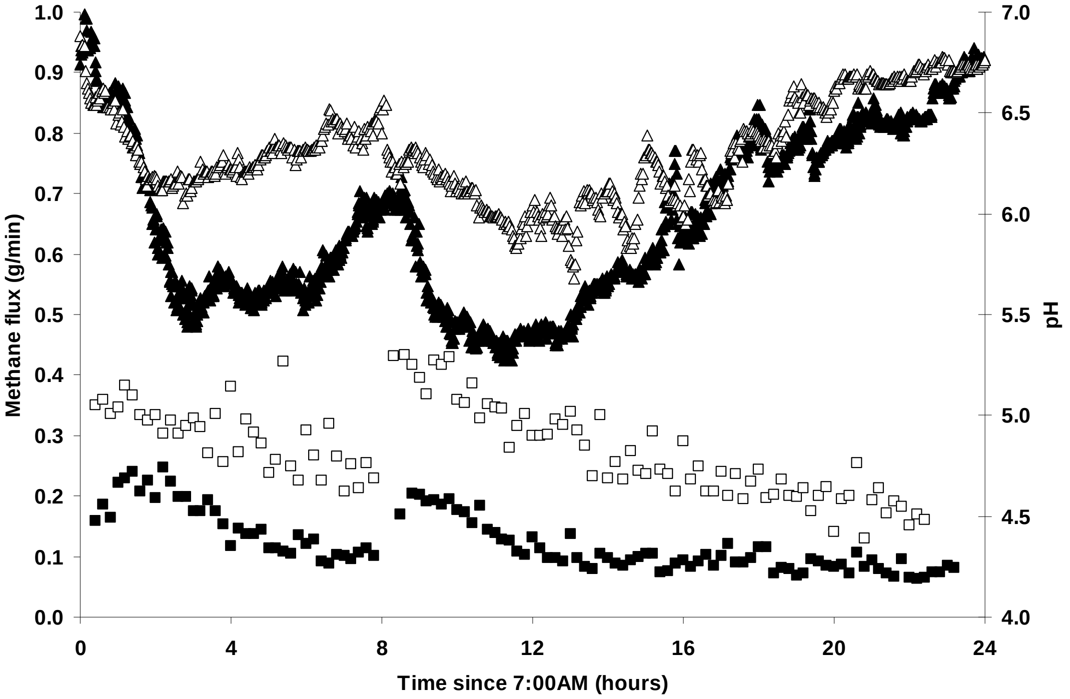
P (effective degradability %) = A + (B*R)/(C+R)

Where A is the zero time intercept (the percentage of the feed that is immediately solubilized), B is the percentage of the feed that is potentially degraded in the rumen, T is the total percentage of the feed that disappears from the bags by 96 hours, C is the rate of degradation (%/h) of the B portion of the feed, and R is the assumed solid outflow rate from the rumen (8.0 %/h).

Feed	A (%)	B (%)	T (%)	C (%/h)	P (%)
Ground wheat	55.6 ^{d}	38.3 ^{c}	94.1 ^{cd}	36.2 ^{c}	86.2 ^{f}
Crushed wheat	37.9 ^{c}	54.6 ^{d}	92.4 ^{c}	35.8 ^{c}	82.4 ^{e}
Ground corn	30.6 ^{b}	66.2 ^{e}	96.9 ^{e}	10.9 ^{ab}	68.3 ^{c}
Crushed corn	21.0 ^{a}	73.8 ^{f}	94.7 ^{d}	6.1 ^{a}	51.4 ^{a}
Ground alfalfa	39.3 ^{c}	32.5 ^{b}	71.8 ^{a}	12.1 ^{ab}	57.7 ^{b}
Ground canola	60.5 ^{e}	22.8 ^{a}	83.2 ^{b}	14.2 ^{b}	74.3 ^{d}

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

Figure 1. Diurnal patterns in rates of production of methane in a corn fed cow (empty squares) and the same cow when fed wheat (solid squares), and patterns in rumen fluid pH in the same cow when fed corn (empty triangles) and wheat (solid triangles). The rumen pH measurements were made by means of the Kahne intra-ruminal bolus weighted so as to reside in the bottom of the rumen.



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