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Technologies for Reducing Greenhouse Gas Emissions and Providing Offset Options for the Beef and Dairy Industries

Principal Investigator: Karen Beauchemin[1]

Collaborators: Richard Eckard[2], Joe Jacobs[3], Peter Moate[3]

ERA Project Advisor: Xiaomei Li

[1]Lethbridge Research Centre
Agriculture and Agri-Food Canada
5403 1st Avenue South
Lethbridge, Alberta
T1J 4B1
Tel : 403-317-2235
karen.beauchemin@agr.gc.ca

[2]Primary Industries Climate Challenges Centre
University of Melbourne
Parkville, Victoria 3052
Australia

[3]Agriculture Victoria, Department of Economic
Development, Jobs, Transport and Resources
Ellinbank, Victoria 3821
Australia

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List of Abbreviations

AAFC	Agriculture and Agri-Food Canada
CO_2e	carbon dioxide equivalent
CPF	corn plus fat diet
CRN	corn diet of single rolled corn
DEDJTR	Department of Economic Development, Jobs, Transport and Resources, Victoria, Australia
D-GAF	Greenhouse Accounting Framework
DM	dry matter
DRB	double-rolled barley diet
EN	encapsulated nitrate
EO	microencapsulated essential oil blend
GHG	greenhouse gas
MON	monensin
NOP	3-nitrooxypropanol inhibitor
SRB	single-rolled barley diet
UEN	unencapsulated nitrate
U of A	University of Alberta
U of M	University of Melbourne
WHT	single-rolled wheat diet
WPF	wheat plus grain diet

Executive Summary

This collaborative effort among researchers in Canada and Australia explored innovative dietary approaches to reduce enteric methane emissions from milk and beef cattle, while further increasing production efficiency. The studies undertaken were aimed at near-market ready technologies to provide Alberta and Australian cattle producers with the opportunity to expand their participation in Carbon Offset Markets, in a cost effective manner.

A number of methane reduction technologies were investigated for dairy cows and beef cattle. The technologies investigated were: diet composition (feeding wheat and lipids), and use of feed additives (3-nitrooxypropanol inhibitor and nitrate), with some strategies examined in combination.

In growing and finishing feedlot cattle, a new experimental methane inhibitor 3-nitrooxypropanol reduced methane yield of feedlot cattle by 40%, without negative effects on animal productivity or carcass characteristics. The research also showed that a sustained reduction in methane using this inhibitor improved feed conversion efficiency of growing beef cattle by 3 to 5%. Optimum dose was shown to be lower in finishing diets than in backgrounding diets. Based on these results, a large scale pilot study to evaluate the inhibitor was initiated in Alberta. On the basis of the present research we conclude that feeding 3-nitrooxypropanol has great potential as a Carbon Offset Protocol. The results of the study will support registration of the product in Canada, giving Canadian beef producers access to this methane mitigation technology. As the cost of the inhibitor is not known at this time, profitability cannot be estimated.

Feeding wheat to dairy cows reduced methane production by 35 to 39% in short-term (5 weeks) studies, but it was not a reliable strategy for long-term mitigation of emissions. Feeding lipids derived from plants reduced methane emissions from dairy (5 to 12%) cows fed mixed diets and beef cattle (25%) fed forage diets. However, in forage based diets fiber digestibility was undesirably decreased, which may adversely affect animal productivity. Encapsulating calcium nitrate slowed its availability in the rumen (stomach) of cattle and reduced the risk of toxicity. Methane was reduced by up to 12% in one study, with no reduction in another study, indicating inconsistent effects.

The research showed that conducting multiple, long-term studies using conditions similar to those of commercial production systems is necessary to identify reliable methane mitigation strategies. Short-term studies were useful for initial screening, but

long-term studies were needed to show whether the methane reduction was maintained over time.

Whole farm modeling was helpful for assessing the net impact of a change in diet to reduce methane emissions on the total greenhouse gas emissions from producing meat and milk. Including all contributing greenhouse gas emissions to the farm-gate in the calculations helped identify best management practices for methane reduction that didn’t inadvertently increase emissions elsewhere in the system.

We conclude that international collaboration among scientists in Canada and Australia was a very effective approach for identifying enteric methane mitigation strategies for dairy and beef cattle production systems. We recommend 3-nitrooxypropanol for further development as a methane mitigation strategy.

Project Description

Introduction and Background

Ruminants, which include beef cattle, dairy cattle, sheep and goats, raised for meat and milk are important sources of protein in human diets worldwide. Their unique digestive system allows them to derive energy and protein from forages and byproduct feeds thereby avoiding direct competition for grains that can be used as human food. The forage and grasslands used to nourish ruminants also sequester vast amounts of carbon and provide grassland ecosystem serves that serve for other useful purposes such as providing areas for water catchment and habitat for wildlife. However, ruminants produce a large amount of enteric methane, which is a potent greenhouse gas (**GHG**). Reducing methane emissions from cattle in Canada would allow ruminant systems to have an expanded role in meeting growing Canadian and global demands for livestock products.

The livestock industry is challenged with reducing GHG emissions to limit the negative impacts of climate change. Using a life cycle approach, it has been estimated that livestock contribute about 14.5% of global anthropogenic GHG emissions (Gerber et al., 2013), with about 1/3 of these emissions attributed to enteric methane generated during feed digestion. As the demand for milk and meat products continues to rise in Canada and globally, methane emissions from livestock production will increase further unless mitigation strategies are adopted. In Canada, the nearly 1 million dairy cows and 12 million beef cattle contribute 16.6 million tonnes of CO_2 equivalent (**CO_2e**) annually from enteric methane to the national GHG inventory (Environment and Climate Change Canada, 2014). Methane is also a loss of potential energy from the animal representing up to 12% of the gross energy consumed (Johnson and Johnson, 1995). Thus, methane produced by ruminants is both an environmental concern and a potential loss in animal efficiency. Reducing the loss of methane from animals may enable more efficient use of energy for production of meat and milk.

There are opportunities to reduce methane emissions from cattle, but such strategies require further refinement and research is required to confirm their feasibility for Canadian dairy and beef production systems. This project represents a collaborative effort between researchers in Canada and Australia who have been exploring innovative dietary approaches to reduce the GHG emissions from milk and beef production, while further increasing production efficiency. The studies undertaken were aimed at further development of near-market ready technologies to provide Alberta cattle producers with the opportunity to expand their participation in the Carbon Offset Market, in a cost effective manner.

Technology Description

A number of methane reduction technologies were investigated for dairy cows and beef cattle. The technologies can be broadly classified as: 1) diet composition (feeding wheat rather than other grain sources, adding fat to the diet), and 2) use of new feed additives (3-nitrooxypropanol [**NOP**], nitrate). To achieve substantial reduction in enteric methane production it is important to consider various approaches, providing producers options that can be selected based on market conditions, farm management practices, and types of cattle. No single mitigation strategy is practical and cost effective for all farms.

Feeding wheat

An initial study conducted in Australia showed that feeding wheat to dairy cows (up to 45% of the diet) as a source of starch that is rapidly fermented in the rumen, reduced methane emissions by up to 50% compared with feeding more slowly fermentable starch sources (e.g., corn, slightly processed barley) or high forage diets (Moate et al., 2012). However, these results needed to be validated over the long term to establish if this effect is maintained, if wheat is to be recommended as a methane mitigation practice.

Feeding lipids (fats)

It is well documented that supplementation of cattle diets with vegetable fat is one of the most effective ways of increasing energy content of the diet while also lowering enteric methane emissions. Methane emissions typically decrease by 3 to 5% with each 1% added fat to the diet, up to a maximum of about 6% added fat. While the feeding of fat supplements to reduce methane production has been explored and offset methodologies developed, the use of fat supplements in conjunction with various starch forms or feed additives had not been studied. The use of fats is seen as uneconomical by many producers when used as a sole supplement. Research to explore the potential synergistic effects between fat and other feeding strategies is warranted to provide more economically viable alternatives to mitigate methane production.

3-Nitrooxypropanol

Previously, a number of methane inhibitors were identified, such as methane analogues (chloroform and bromochloromethane) and methyl-coenzyme M reductase analogues (bromoethanesulfonate). However, none of those inhibitors are licensed for feeding to cattle because of negative side effects on safety of animals, people, and the environment. The newly identified methane inhibitor, NOP, is an exception – it has been shown to have very low environmental and safety risk. NOP is a synthetic compound manufactured by DSM Nutritional Products (Kaiseraugst, Switzerland). In the rumen, NOP binds to the active site of the enzyme involved in methane synthesis (methyl-coenzyme M reductase) thereby blocking methane formation (Duin et al., 2016). In studies with cattle fed backgrounding diets, NOP added to the ration lowered enteric methane emissions by 33% to 59%, depending upon dose (Romero-Perez et al., 2014, 2015). These initial results are very promising, but further research was required to fully establish efficacy of NOP when used in longer-term feedlot studies.

Nitrate

Feeding nitrate (maximum of 3% of the dietary dry matter [**DM**]) was shown in earlier studies to reduce methane emissions, while providing a source of non-protein nitrogen for the animal (Lee and Beauchemin, 2014). Nitrate is reduced in the rumen to ammonia via nitrite as an intermediate. The reduction of nitrate competes for electrons with methane formation, and thus nitrate reduction acts as a competitive hydrogen sink in the rumen. The challenge with nitrate feeding is that it can be toxic if high concentrations are fed (> 4% of dietary DM) or if animals are not adapted beforehand. If nitrate and nitrite accumulate in the rumen, they are absorbed into blood through the rumen wall. Nitrite in blood binds to red blood cells and changes the ferrous form of hemoglobin to the ferric form (methemoglobin), which is incapable of carrying oxygen. High levels of methemoglobin in blood (> 50%) will cause the animal to asphyxiate and die. Thus, before starting this project more work was needed to identify the practical strategies needed to prevent toxicity. In a preliminary metabolism study with diets containing 55:45 forage:concentrate, we observed an 18% reduction in methane yield (defined as grams of methane per kilogram of DM consumed) with cattle fed 2.5% nitrate in an encapusulated form (**EN**) (Lee et al., 2015). Based on these findings, nitrate may offer potential as a methane mitigation strategy if the effects are maintained over the long-term and if the practicalities of feeding nitrate without risk of toxicity can be identified.

Project Goals

To provide beef and dairy producers in Alberta with a series of dietary solutions that could be used in a cost effective manner to lower enteric methane emissions.

Specific Objectives

- Determine effects of novel feed additives and diet manipulations on animal performance (feed efficiency, nitrogen use efficiency, weight gain or milk production).
- Determine management considerations for using novel feed additives and diet manipulations in commercial feedlots or dairies.
- Determine the enteric methane mitigation potential of novel feed additives, diet manipulations, and their effects when used in combination.
- Determine whether reductions in methane are sustained over the longer term.
- Determine safety and registration potential for using the novel feed additives for cattle.
- Provide the data necessary for development of Carbon Offset Protocols for the Alberta beef and dairy industries.

Work Scope Overview

International Collaboration: This project offered a unique opportunity for scientists in Canada and Australia to work together to develop methane mitigation technologies with relevance to Canadian dairy and beef producers. Prior to this collaboration, the researchers in these two locations had extensive research experience related to methane mitigation for dairy and beef cows. Working together enabled the Canadian team to focus on beef cattle and the Australian team to focus on dairy cows. Additionally, the organizations and industry partners in both locations committed in-kind and cash contributions to the project that were leveraged through Emissions Reduction Alberta to allow for a large-scale project that encompassed a number of methane mitigation strategies.

Beef Cattle Studies

The beef studies were conducted by Agriculture and Agri-Food Canada (**AAFC**) at the Lethbridge Research and Development Centre in co-operation with the University of

Alberta (**U of A**). The beef studies explored the potential use of two feed additives (NOP and EN) in rations by determining: 1) whether reductions in methane occurred, 2) whether the methane reductions were sustained over the long term, 3) practicalities of using these additives in commercial feedlots; 4) effects on animal performance (weight gain, feed efficiency), 5) safety, and 6) cost:benefit analysis. This information was needed to facilitate the registration of the compounds in Canada making them accessible to Canadian beef producers.

Dairy Cow Studies

The dairy studies were conducted by the Department of Economic Development, Jobs, Transport and Resources, Victoria, Australia (**DEDJTR**, formerly DEPI), and the University of Melbourne (**U of M**). Dairy cow feeding studies were conducted to determine: 1) the mechanisms responsible for enteric methane mitigation in response to feeding diets containing cereal grains that differ in their rates of starch fermentation, 2) whether reductions in methane due to high concentrations of starch are sustainable in the long term, 3) effects on animal performance (milk production, milk composition and cow health), and 4) the potential for synergy in terms of methane mitigation when two or more mitigants are included in the diets of ruminants.

Modeling and Integration

The modeling studies were conducted by AAFC and U of M. Modeling was used to integrate the various methane mitigation technologies investigated. The aim was to determine the broader potential for GHG reductions using low methane technologies. The modeling examined the impact of these technologies on GHG emissions from beef and dairy farms in Canada and Australia.

Outcomes and Learnings - Literature review

Methane Production by Ruminants

Increasing atmospheric concentration of GHG is a major worldwide concern. It is estimated that, globally, direct emissions from animal agriculture account for 7-10% of human derived GHG emissions (O'Mara, 2011), or 14.5% if indirect sources, such as feed production and deforestation for pasture or crop expansion are considered (Gerber et al., 2013). Ruminant production contributes directly to GHG emissions due to methane generated during feed digestion (i.e., enteric methane), methane and nitrous oxide from manure, as well as indirectly through nitrous oxide and CO_2 emissions from crop production and fuel use. Carbon can also be lost or gained in soil due to changes in land use. However, the largest single GHG source from ruminant production is enteric methane.

Methane is a loss of potential energy for the animal; 2 to 12% of the total gross energy consumed by ruminants is converted to methane and released via the breath (Johnson and Johnson, 1995). Enteric methane is a natural byproduct of microbial fermentation of feed in the rumen. Methane-producing Archaea (methanogens) in the animal's rumen reduce CO_2 to methane thereby preventing hydrogen from accumulating and having negative effects on feed digestion.

Methane Mitigation Strategies

There has been a lot of interest in developing mitigation options to reduce enteric methane emissions from ruminants, and many comprehensive reviews have been published (e.g., Beauchemin et al., 2009; Hristov et al., 2013; Knapp et al., 2014). Many dietary strategies target the process of methanogenesis in the rumen. One strategy is direct inhibition of methanogens using inhibitors that block methane formation, invoking the need to redirect hydrogen into alternative products to decrease the build-up of hydrogen in the rumen. Another strategy is to increase the concentration of starch in the diet by feeding higher-grain diets as a way of reducing the production of hydrogen in the rumen. A further strategy is to provide alternative sinks for hydrogen disposal in the rumen that can compete with methane formation (e.g., nitrate). However, ruminal microorganisms can potentially adapt to some mitigation strategies and return methane emissions to pre-treatment levels highlighting the need to evaluate methane reduction over the long term. In addition to dietary approaches to mitigating methane, a number of non-dietary means of targeting ruminal methanogens have been explored, including breeding animals for low methane production, immunization against methanogens, and administration of bacteriophages and bacteriocins. Most of the non-dietary approaches are experimental and warrant further investigation, but are not expected to be commercially viable in the near future. For this reason our project focused on promising dietary strategies.

Feeding Wheat

Wheat is a high quality source of starch that is rapidly fermented in the cow's rumen. The starch in wheat is more quickly degraded than the starch in barley (Herrera-Saldana et al., 1990), whereas the starch in barley is more quickly degraded than the starch in corn (Granzin, 2004). A short-term study conducted in Australia (Moate et al., 2012) showed that feeding wheat to dairy cows reduced methane emissions by up to 50% compared with feeding slowly fermentable starch (corn or lightly processed barley) or higher forage diets. Other than that study, no studies have compared the relative potencies of wheat, corn, and barley for their effects on enteric methane production in dairy cows.

Beauchemin and McGinn (2005) fed finishing beef cattle diets containing 81.4% of either dry-rolled corn or steam-rolled barley and reported different methane yields of 9.2 and 13.1 g of methane/kg of DM intake for the respective diets. Thus, the type of grain fed has the potential to substantially influence methane emissions. Based solely on assumed differences in the relative rates of starch degradability, one might expect that the barley diet would be associated with a lower methane yield than the corn diet. However, in the experiment of Beauchemin and McGinn (2005), the barley had a higher fiber concentration than the corn, and the ruminal fluid pH was greater in the barley-fed animals.

Feeding wheat to lower methane could be of interest to Alberta dairy and beef producers because wheat is commonly used in diets when cost effective. However, feeding practices based on starch supplementation that might decrease methane production have not been fully investigated. High levels of grain feeding are routine practice on most Alberta dairy farms (up to 50% of total DM intake) and feedlots (up to 90% of total DM intake). Processed wheat supplies rapidly fermentable starch, whereas corn contains slowly fermentable starch. The fermentability of barley, the traditional grain used on most cattle farms in Alberta, would be intermediate to wheat and corn. Both Canada and Australia produce substantial quantities of wheat and barley. Unfortunately, there is a paucity of published scientific literature comparing the enteric methane mitigation properties of grains that contain starch that degrades in the rumen at different rates.

Feeding Fat

Supplementation of diets with fats (excluding those that are protected from ruminal digestion) is a well-documented strategy for lowering enteric methane emissions from ruminants (Grainger and Beauchemin, 2011). The challenge, however, is to lower methane emissions without impairing animal production, given that adding unprotected fats to the diet can have negative effects on feed intake, carbohydrate digestion in the rumen, protein and fat concentration of milk, and organoleptic quality of milk (Beauchemin et al., 2009). Furthermore, fats can be costly in comparison with grains. Thus, higher metabolizable energy content of the diet and improved animal performance is necessary to offset higher feeding costs associated with using fat supplementation of diets for methane mitigation.

Feeding fat to ruminants reduces methane because it replaces dietary carbohydrates that are fermented in the rumen. In addition, lipids decrease numbers of protozoa and associated methanogens, and free fatty acids and medium-chain fatty acids are toxic to rumen methanogens (Grainger and Beauchemin, 2011). Hydrogenation of polyunsaturated fatty acids functions as a hydrogen sink, and in some situations, fat supplementation of diets depresses fiber digestion and DM intake, further decreasing the amount of feed fermented in the rumen, with the potential consequence that animal performance can be lowered. It is usually recommended that the amount of fat added to the diet should be limited to 3–4%, such that total fat concentration does not exceed 6% of dietary DM. Methane yield (g/kg DM intake) is reduced by about 3 to 5% for each percentage unit of fat added to the dietary DM (Beauchemin et al., 2009; Moate et al., 2011). It appears that feeding fats can be an effective methane mitigation practice in some situations, but that responses are highly variable. Factors such as level of supplementation, fat source and associated fatty acid profile, the form in which the fat is administered (i.e. as refined oil or as full-fat oilseeds), and the type of diet all impact the net effect of fat on methane production. Few studies have examined the combined effects of feeding fat and other methane mitigation strategies.

3-Nitroxypropanol Inhibitor

Research on the use of inhibitors to decrease enteric methane production has regained popularity with the development of NOP, a synthetic compound that inhibits the last step of methanogenesis (Duval and Kindermann, 2012). This compound is unique, because unlike other inhibitors (e.g., bromochloromethane, bromoethanesulfonate, chloroform and cyclodextrin) that have been shown to be toxic, NOP appears to be safe, with no unintended side-effects, although further study is needed.

3-Nitrooxypropanol has been shown to reduce methane production from sheep in Spain (Martinez-Fernandez et al., 2013), lactating dairy cows in Alberta, UK and USA (Haisan et al., 2013, 2014; Reynolds et al., 2014; Hristov et al., 2015), and growing beef cattle in Alberta (Romero Perez et al., 2014) with no signs of animal illness or intoxication. Although NOP reduced methane production in all studies, the magnitude of the reduction in methane varied from 4 to 60%, depending upon animal type, diet, and in particular, method of dosing the product. Either no effect, or very minor effects, on feed intake and digestibility were reported. From those studies, it was surmised that to maximize methane reduction, NOP needs to be incorporated into the animal’s ration rather than provided once daily. Another possibility is that future research may result in a method that provides for a slow and constant release of NOP into the rumen.

As those studies were short term (< 1 month feeding periods), we subsequently examined the effect of feeding NOP over a 4-month period in beef cattle with NOP mixed into the ration (Romero Perez et al., 2015). Methane production was consistently reduced by 60% with no signs of adaptation. Studies to examine safety of using NOP have shown that the product is not carcinogenic or mutagenic, and that the product is fully degraded in ruminal fluid to reaction products of 1,3 propandiol, nitrate and nitrate that naturally occur in the rumen (Duin et al., 2016). Thus, the risk of residues in meat or milk is extremely low, although further testing is needed to ensure consumer confidence.

Nitrate

Nitrate acts as an alternative hydrogen sink in the rumen. In other words, nitrate provides an alternative biochemical pathway for rumen microorganisms to dispose of hydrogen (reducing equivalents that are normally disposed of by forming methane). Fermentation of feed in the rumen by the microorganisms converts carbohydrates (from grains and forages) into energy precursors for the animal (volatile fatty acids) and produces metabolic hydrogen. The main hydrogen sink in the rumen is methane formation; the methane forming organisms reduce carbon dioxide ($\mathbf{CO_2}$) to methane, which utilizes hydrogen (Janssen, 2012). However, there are also other pathways in the rumen that ensure that hydrogen does not accumulate (for example, nitrates are reduced to ammonia thereby providing a sink for hydrogen). When nitrate is present in the rumen, nitrite and ammonia formation are favoured over methane production (Ungerfeld and Kohn, 2006). Ammonia is the major source of nitrogen for the ruminal microorganisms. Providing nitrate as an alternative hydrogen sink in the rumen is a novel approach to reducing methane from cattle because nitrate can be a means of reducing methane production plus it has the added benefit of contributing a source of nitrogen (non-protein nitrogen) to the animal. There is now overwhelming evidence to support the concept that feeding nitrate reduces methane emissions (Nolan et al., 2010; van Zijderveld et al., 2010, 2011; Hulshof et al., 2012; Li et al., 2012; El-Zaiat et al., 2014).

The major concern of cattlemen regarding feeding nitrate to reduce methane production is the potential for toxicity. Toxicity occurs when the nitrate level in the diet exceeds the capacity of the microbes in the rumen to convert it to nitrite and then ammonia. In that case, nitrate and/or nitrite are absorbed into the bloodstream and the nitrite causes toxicity by combining with hemoglobin to form methemoglobin, rendering the hemoglobin molecule incapable of transporting oxygen to the tissues. If high levels of methemoglobin are formed (>50%), the animal begins to suffer from oxygen starvation, and must be treated immediately. The key is to adapt the animals to nitrate slowly so the population of nitrite-reducing bacteria increases in size and capacity to reduce nitrite. One way of reducing the risk of toxicity may be to use an encapsulated form of nitrate that slows the release rate of nitrate in the rumen. Another important way to reduce the risk of toxicity is to adapt the animals slowly to the nitrate compound, by providing increasing levels of nitrate over time (i.e., ramping up the level in the diet). With further evaluation and development of protocols to reduce toxicity risk, nitrate feeding could become a viable strategy to lower methane emissions.

Experimental Procedures/Methodology

Experiment 1.1 Dose Response to Methane Inhibitor (AAFC)

Manuscript: Vyas, D., S. M. McGinn, S. M. Duval, M. K. Kindermann and K. A. Beauchemin. 2016. Optimal dose of 3-nitrooxypropanol for decreasing enteric methane emissions from beef cattle fed high-forage and high-grain diets. Anim. Prod. Sci. published online 26 May 2016 http://dx.doi.org/10.1071/AN15705

The experiment was approved by the Lethbridge Research and Development Center Animal Care and Use Committee under the guidelines of the Canadian Council on Animal Care (2009). Fifteen steers were used in this experiment, which was conducted in two sequential parts: high-forage backgrounding phase and high-grain finishing phase. Each phase was designed as an incomplete block with six treatments, five replications per treatment and two periods. Dietary treatments corresponded to the following six doses of NOP: 0 (Control), 50, 75, 100, 150 and 200 mg/kg DM. During both backgrounding and finishing phases, each treatment was replicated five times, such that each animal was assigned to two of six treatments. A washout phase (7 days) was included between the 28-day periods and no period effect was assumed in the study. Animals were gradually adapted to NOP over the first 10 days of each period.

During the first phase, steers were fed a high-forage backgrounding diet for 63 days (two periods and a washout; Table 1.1.1), followed by a 28-day transition where animals were offered diets with sequential increase in proportion of barley grain in the ration. Post-transition, the high-grain finishing diet (Table 1.1.1) was fed for 63 days (two periods and a washout).

Steers were weighed at the start and end of each period during backgrounding and finishing phases on two consecutive days. Experimental animals were penned individually. During each period, enteric methane was measured on days 27 and 28 from individual steers for 2 days using open-circuit calorimetry chambers as described by Beauchemin and McGinn (2006). Feed samples were collected and analyzed using standard procedures. Within each phase (i.e. backgrounding and finishing), the data were analysed using the mixed procedure of SAS (SAS Institute Inc., Cary, NC, USA), with animal as the experimental unit.

Table 1.1.1 Ingredient and chemical composition of the total mixed ration

	Backgrounding	Finishing
	Ingredient (%)	
Barley silage	65.0	8.0
Barley grain, dry rolled	19.1	76.1
Distillers dried grains	10.0	10.0
Supplement	5.9	5.9
	Chemical composition	
Dry matter, %	45.5 ± 1.67	79.6 ± 1.82
Crude protein, % DM	14.5	13.3
Neutral detergent fiber, % DM	41.7	27.0
Acid detergent fiber, % DM	19.1	7.2

Experiment 1.2 Methane Inhibitor and Feedlot Cattle Performance (AAFC)

Manuscript: Vyas, D., A. W. Alemu, S. M. McGinn, S. M. Duval, M. Kindermann and K. A. Beauchemin. 2018. The combined effects of supplementing monensin and 3-nitrooxypropanol on methane emissions, growth rate, and feed conversion efficiency in beef cattle fed high forage and high grain diets. J. Anim. Sci. (submitted)

The experiment was approved by Lethbridge Research and Development Center Animal Care and Use Committee under the guidelines of the Canadian Council of Animal Care (2009). The study was conducted with approval from the Veterinary Drugs Directorate of Health Canada (Experimental Studies Certificate; DSTS No. 186831).

Two-hundred and forty crossbred yearling steers were adapted to facilities and processed according to standard management procedures. The steers were stratified by weight (heavy, light) and blocked into 12 heavy and 12 light pens at the main feedlot (8 cattle/pen) and 4 pens each for heavy and light weight groups at a secondary feedlot (6 cattle/pen). Treatments were randomly assigned within each block at the main (6 pens/treatment) and secondary (2 pens/treatment) feedlots. Pens were equipped with fence-line feed bunks and automatic waterers.

Steers were used in a 238-d feeding trial and were fed high-forage diets based on barley silage for the first 105 d (backgrounding phase; Table 1.2.1). The animals were transitioned to the finishing diets for 28 d. The high-grain diets (i.e., finishing phase; Table 1.2.1) based on barley grain were fed for the last 105 d. The experiment was conducted as a randomized block design with 4 treatments arranged in a 2 × 2 factorial layout; 2 levels of NOP (with, without) were combined with 2 levels of monensin (**MON**, with, without). Treatments were as follows: 1) Control (no additive); 2) MON (33 mg/kg DM); 3) NOP (200 mg/kg DM for backgrounding or 125 mg/kg DM for finishing phase); 4) **MONOP** (33 mg/kg DM MON supplemented with 200 mg/kg DM in the backgrounding phase or 125 mg/kg DM NOP in the finishing phase). The MON was incorporated into the supplement at the time of manufacturing, while the NOP was homogenously mixed into the total mixed ration daily. Dietary NOP supplementation was discontinued after the end of the finishing phase. Steers were fed the control finishing diet for a minimum of 4 weeks prior to slaughter in accordance with the requirement of the Experimental Studies Certificate. All steers were slaughtered at a federally inspected facility the end of the finishing phase.

Twenty steers were used to measure methane and hydrogen in open-circuit calorimetry chambers. During the backgrounding phase, measurements were from 48 d to 79 d of the feeding period, while during the finishing phase these were taken from 63 d to 94 d coinciding with the mid-point of the feeding periods.

The data were analyzed as 2 × 2 factorial design using a MIXED procedure of SAS. Pen was the experimental unit for all variables, except gas emissions (animal). Statistical significance was declared at $P \leq 0.05$ and a tendency to significance was declared at $0.05 < P \leq 0.10$.

Table 1.2.1 Ingredient and chemical composition of the basal diets

Item	High-forage		High-grain	
	Control	Monensin	Control	Monensin
Ingredients				
Barley silage	65.0	65.0	8.0	8.0
Barley, dry rolled	25.0	25.0	87.0	87.0
Supplement	10.0	10.0	5.0	5.0
Canola meal	3.966	3.967	1.521	1.521
Barley, ground	5.520	5.504	2.170	2.154
Canola oil	0.051	0.051	0.052	0.052
Limestone	0.135	0.135	0.922	0.922
Salt	0.047	0.047	0.048	0.048
Urea	0.180	0.180	0.184	0.184
Molasses	0.056	0.056	0.057	0.057
Feedlot premix	0.045	0.045	0.046	0.046
Rumensin	-	0.016	-	0.016
Chemical composition				
Dry matter, %	50.7 ± 0.31	50.7 ± 0.31	86.8 ± 1.11	86.8 ± 1.10
Crude protein, %	14.2 ± 0.52	14.2 ± 0.61	13.9 ± 0.86	13.9 ± 0.88
Neutral detergent fiber, %	40.5 ± 1.52	40.4 ± 1.48	18.4 ± 0.63	18.4 ± 0.67

Acid detergent fiber, %	21.5 ± 0.50	21.5 ± 0.49	7.03 ± 0.7f2	7.05 ± 0.74
Starch, %	31.7 ± 1.81	31.8 ± 1.68	54.9 ± 2.86	54.8 ± 2.88

Experiment 2.1 Alternative Hydrogen Sink (Nitrate) (AAFC)

Manuscript: Lee, C., R. C. Araujo, K. M. Koenig, and K. A. Beauchemin. 2017. Effects of encapsulated nitrate on growth performance, nitrate toxicity, and enteric methane emissions in beef steers: backgrounding phase. J. Anim. Sci. 95:3700-3711 doi:10.2527/jas.2017.1460

Manuscript: Lee, C., R. C. Araujo, K. M. Koenig, and K. A. Beauchemin. 2017. Effects of encapsulated nitrate on growth performance, carcass characteristics, nitrate residues in tissues, and enteric methane emissions in beef steers: finishing phase. J. Anim. Sci. 95:3712-3726. doi:10.2527/jas2017.1461

The experiment was approved by Lethbridge Research and Development Center Animal Care and Use Committee under the guidelines of the Canadian Council of Animal Care (2009) as well as the Veterinary Drugs Directorate of Health Canada (Experimental Studies Certificate: OF15-21-G1443-400\4-103\175393; DSTS No. 175393). A total of 138 crossbred steers (250 kg) were purchased and used in a completely randomized experimental design. The cattle were processed using standard procedures and 24 head were selected for methane measurement. The other 108 steers were blocked by weight (6 blocks; 18 steers per block), and animals in each block were randomly assigned to 3 treatment pens (6 animals per pen); therefore, 18 pens (6 pens per treatment) were used. The pens (experimental unit) received 3 dietary treatments: Control, a typical backgrounding diet supplemented with urea (0.94% in dietary DM); 1.25% EN, the control diet supplemented with 1.25% encapsulated calcium ammonium nitrate in dietary DM, which partially replaced urea, or 2.5% EN, the control diet supplemented with 2.5% EN (DM basis), which fully replaced urea.

The 24 steers used for methane measurements were randomly assigned to 4 pens (6 animals per pen) receiving 4 dietary treatments. A subset of the animals in those pens (5 out of 6 animals in each pen) were used for individual measurement of methane production in respiratory chambers throughout the experiment. Dietary treatments were the same as described above with an additional fourth treatment diet: control diet supplemented with 2.3% unencapsulated nitrate (**UEN**).

The backgrounding study was conducted over 91 d in a randomized complete block design. Animals were adapted to diets for the first 3 weeks, during which nitrate concentration in the diets was gradually increased. Individual pens were bedded with straw and managed using standard feedlot management procedures, except that MON was not added to the diet and cattle were not implanted with steroids.

Following a 28-d transition period, the 132 cattle received the experimental finishing diets, maintaining the treatment and pen assignments designated at the start of the backgrounding period. Animals were subjected to methane measurement once during the backgrounding phase and twice during the finishing phase. The cattle were shipped to market after a 1-week withdrawal period (as required by the Experimental Studies Certificate). During the slaughter, muscle, fat, liver, and kidney of 2 randomly selected animals per pen from 6 pens per treatment for Control, 1.25% EN, and 2.5% EN. Other measurements and analyses were similar to those described for Experiment 1.2. Pen was the experimental unit except for methane.

Experiment 2.2 Alternative Hydrogen Sink (Nitrate) (AAFC)

Manuscript: Alemu, A.W., Romero-Pérez, A., Araujo, R.C. and Beauchemin, K. Effect of slow release nitrate and essential oil (Activo® Premium) on animal performance and methane emissions from feedlot cattle fed high forage diet. J. Anim. Sci. (in preparation)

Manuscript: Romero-Pérez, A., Alemu, A.W. Araujo, R.C. and Beauchemin, K. Effect of slow release nitrate and essential oil (Activo® Premium) on animal performance and methane emissions from feedlot cattle fed high grain diet. J. Anim. Sci. (in preparation)

The experiment was approved by Lethbridge Research and Development Center Animal Care and Use Committee under the guidelines of the Canadian Council of Animal Care (2009) as well as the Veterinary Drugs Directorate of Health Canada (DSTS No. 197834). A uniform group of 88 crossbred steers (250-270 kg) were used. The experiment was conducted as a completely randomized design with a 2 × 2 factorial arrangement of treatments with 22 animals per treatment. Each treatment was housed in one large pen. The treatments were: 1) control; 2) 2.5% encapsulated nitrate (EN) providing 1.875% nitrate in the dietary DM (2.5% EN); 3) 150 mg/kg DM of microencapsulated essential oil (**EO**) blend (Activo Premium, Grasp, Brazil); and 4) 2.5% EN + 150 mg/kg DM EO. EN and EO were mixed into the total mixed rations daily. Diets were isonitrogenous and were offered twice daily.

The experiment consisted of 3 phases: backgrounding (95 to 115 days), transition (28 d), and finishing (120 to 150 days). The control backgrounding diet contained (on a DM basis) 82% corn silage, 10% supplement, and 8% dry rolled barley grain. The finishing diet contained approximately 82% dry rolled barley grain, 10% supplement, 8% barley silage (DM basis). The cattle were re-randomized to new treatment assignments after the backgrounding phase. Ionophores and antibiotics for liver abscess control were not added to any of the diets. However, cattle were implanted with steroids.

During the first period (28-day period) of the backgrounding phase, the cattle that received the diets containing EN were acclimatized gradually using a step-up protocol, 0.625, 1.25, 1.875 and 2.5% EN in the diet DM. Each pen was equipped with 4 GrowSafe feed bunks to allow for determination of feed intake amount and pattern over the day. Pens were bedded with straw, and standard feedlot management procedures were used. Immediately upon completion of the study (no withdrawal), all animals were slaughtered commercially.

Measurements were similar to those described for Experiment 1.2, with the exception of methane. Measurements of gases were carried out using a commercial head-chamber system (GreenFeed, C-lock, Rapid City, South Dakota, USA). The system was placed in each pen for 7 days on a rotating basis over the course of the study. The system measures methane emissions from individual cattle, and uses head position sensors to validate the measures obtained. Only data from days on which the animal accessed the feeder at least 3 times was used to estimate daily methane production over the measurement week for an individual animal.

Carcasses were evaluated at slaughter according to the Canadian grading system. Livers were scored for abscesses. For all traits, the animal was the experimental unit, with the analysis done separately for backgrounding and finishing periods.

Data were analyzed as a 2 × 2 factorial design using MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) for the backgrounding and finishing periods considering animal as experimental unit. Period was used as a REPEATED measure in the model. Statistical significance was declared at $P \leq 0.05$.

Experiment 3.1 Alternative Starch Source (DEDJTR and U of M)

Manuscript: Moate, P. J., S. R. O. Williams, J. L. Jacobs, M. C. Hannah, K. A. Beauchemin, R. J. Eckard, and W. J. Wales. 2017. Wheat is more potent than corn or barley for dietary mitigation of enteric methane emissions from dairy cows. J. Dairy Sci. 100:1-15. https://doi.org/10.3168/jds.2016-12482

This 35 day experiment examined the effect of the rate of dietary starch degradation on methane emissions, milk production, milk composition and ruminal digestion. The study used 32 lactating multiparous Holstein-Friesian cows (including 12 rumen-cannulated cows) producing an average of 32.3 kg/d of milk (71 days in milk) with an average body weight of 537 kg. Cows were allocated to 4 balanced groups. Each group of 8 cows (including 3 rumen-cannulated cows) was then randomly allocated to 1 of 4 dietary treatments (DM basis): (1) a corn diet (**CRN**) of 10.0 kg/d of single-rolled corn, 1.8 kg/d of canola meal, 0.2 kg/d of minerals, and 11.0 kg/d of chopped alfalfa hay; (2) a wheat diet (**WHT**) similar to the CRN diet but with the corn replaced by single-rolled wheat; (3) a barley diet (**SRB**) similar to the CRN diet but with the corn replaced by single-rolled barley; or (4) a double-rolled barley diet (**DRB**) similar to the CRN diet but with the corn replaced by double-rolled barley. The corn, wheat, and single-rolled barley had been passed once through a roller mill, but the double-rolled barley had been passed twice through the same roller mill to further reduce particle size. The amount of DM offered to each cow in all treatments was the same.

Methane emissions were measured using the sulphur hexafluoride tracer gas technique over 5 days (day 30 to 35). Cow body weights were measured at the start and end of the experiment. Rumen fluid was collected by stomach tube on day 35 and measured for pH, ammonia, volatile fatty acid concentrations and protozoa. The fistulated cows were used to measure the DM and starch degradation rates from the wheat and the barley using the *in situ* technique. These animals were also used to monitor ruminal pH profiles over the course of the experiment to determine the potential dietary effects on extent of sub-clinical ruminal acidosis.

Experiment 3.2. Rate of Dietary Starch Degradation and Methane/Milk Production, Long-term Effects (DEDJTR and U of M)

Manuscript: Moate, P. J., J. L. Jacobs, M. C. Hannah, G. L. Morris, K. A. Beauchemin, P.S. Alvarez Hess, R. E. Eckard, Z. Liu, S. Rochfort, W. J. Wales, and S. R. O. Williams. 2018. Adaptation responses in milk fat yield and methane emissions of dairy cows when wheat was included in their diet for 16 weeks. J. Dairy Sci. (submitted)

Cows were cared for according to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013). Animal use was approved by the Animal Ethics Committee of the Department of Economic Development Jobs Transport and Resources – Victoria. The objective was to study the long-term effects (over 112 days) of feeding diets containing either corn or wheat on milk production and methane emissions of dairy cows. Twenty-four multiparous, lactating Holstein-Friesian cows (including 12 ruminally fistulated) producing an average of 36.0 kg/d milk were used in a 2 × 2 factorial (fistulate×diet) treatment structure with 6 cows per treatment. Each of the 4 treatments was replicated 6 times. The diets were (DM basis) 1) CRN (10.0 kg/d of single rolled corn grain, 1.8 kg/d of canola meal, 0.2 kg/d of minerals and 11.0 kg/d of chopped alfalfa hay); and 2) WHT (10.0 kg/d of single rolled wheat grain, 1.8 kg/d of canola meal, 0.2 kg/d of minerals and 11.0 kg/d of chopped alfalfa hay). Throughout this experiment, the concentrate portion of the diet was offered to the cows separately from the hay portion of the diet, with both offered twice each day in two equal portions.

Covariate measurements of milk yield and composition, and body weight were made in the week prior to the experiment. Cows were transitioned to their treatment diets on days 1 to 8, and offered their full treatment diet for the remainder of the experiment. Water was offered ad libitum at all times.

Quantities of the ingredients of the concentrate offered to individual cows were weighed into individual feed bins. Refusals of concentrates were collected and weighed after each feed, with proportions of each grain being assumed to be the same as that offered on a wet basis. Dry matter concentration was determined for representative samples of grains, canola meal, hay and minerals collected each morning by drying the samples in a forced draft oven at 105°C for 24 h. Representative samples of feeds were obtained, dried, and analyzed using standard procedures.

Cows were milked twice daily and yield was measured for each cow at each milking, and milk components and milk fatty acids were measured. Energy-corrected milk, standardized to 4.0% fat and 3.3% protein, was calculated. Methane emissions from individual cows were measured using 6 open-circuit respiration chambers on days 24 and 25, 66 and 67, and 108 and 109. The availability of only 6 respiration chambers meant that the start of the experiment had to be staggered so methane production was measured on the same days of feeding for each cow.

Ruminal fluid samples were collected from cows after the morning feeding on days 26, 68 and 110 between 1100 to 1145 h. Ruminal fluid samples were collected from rumen fistulated and non fistulated cows using a sampling tube and a vacuum pump. When cows were in chambers, the ruminal fluid in fistulated cows was continuously monitored for pH by intra-ruminal boli.

Data were summarized for each cow within each 2-d chamber period (i.e. during week 4, 10 and 16) by taking the simple mean of each variable. The resulting data were analyzed by ANOVA specifying an additive treatment structure of fistulation status plus dietary treatment, crossed with week, and a factorial blocking structure of calving-cohort by chamber, split for week.

Experiment 3.3. Starch Sources and Oilseeds Effects on Methane/Milk Production (DEDJTR and U of M)

Manuscript: Alvarez Hess, P.S., S. R. O. Williams, J. L. Jacobs, M. C. Hannah, K. A. Beauchemin, R. E. Eckard, W. J. Wales, G. L. Morris and P. J. Moate. Effect of dietary fat supplementation on methane emissions from dairy cows fed wheat or corn. J. Dairy Sci (in preparation)

Cows were cared for according to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NHMRC, 2013). Animal use was approved by the Animal Ethics Committee of the Department of Economic Development Jobs Transport and Resources – Victoria. Thirty-two lactating, multiparous, rumen-cannulated Holstein-Friesian cows producing an average of 24.9 kg/d milk at 207 days of lactation were used. Eight blocks of 4 cows were defined by milk yield and four treatments were assigned to cows within each block. However, one cow from the CRN treatment group was removed from the experiment due to health reasons unrelated to the treatment. Thus, there were 7 cows in the CRN treatment group and 8 cows in each of the other treatment groups.

All four dietary treatments consisted of a base of (DM basis) 11.5 kg/d of chopped alfalfa hay, 1.8 kg/d of solvent extracted canola meal, 0.2 kg/d of minerals, and 42 mL/d of bloat drench. In addition to this, the CRN diet included 8.0 kg/d of corn grain, the WHT diet included 8.0 kg/d of wheat grain, the corn plus fat diet (**CPF**) included 8.0 kg/d of corn grain and 0.80 kg/d of canola oil, and the wheat plus fat diet (**WPF**) included 8.0 kg/d of wheat grain and 0.80 kg/d of canola oil. All cows were offered a common diet in the lead up to the experiment (i.e., covariate week). On days 8 to 14 of the experiment all cows were transitioned to their experimental diet. Days 15 to 28 served as an adaptation period. Methane measurements were undertaken on days 29 to 35.

Diets were offered to cows in two equal portions each day. Cows were fed in individual feed stalls within a well-ventilated animal facility. They were offered their concentrate first and then alfalfa. Water was offered to all animals at least once during each feeding period. Feeds were sampled and analyzed using routine procedures. Milk yield was measured for each cow at each milking, and milk samples were collected and analyzed.

The sulphur hexafluoride tracer technique was used to estimate methane emissions from individual cows from d 30 to 35. Ruminal fluid samples (~400 mL) were collected from each cow 4 h after feeding on day 35. The pH of ruminal fluid was continuously monitored by intra-ruminal boluses on d 28 to 35.

Average milk production and feed intake were summarized for each animal and analyzed with covariate adjustment, while no covariate was used for the other data.

Experiment 4.1 *In vitro* Assessments of Additivity and Synergy of Mitigation Strategies (AAFC, DEDJTR, and U of M)

Ruminal degradation of starch sources

Manuscript: Alvarez Hess PS, SRO Williams, JL Jacobs, PJ Moate, and RJ Eckard. The comparative ruminal in sacco degradation and in vitro gas and methane production of barley, corn and wheat (manuscript in preparation)

An *in situ* and an *in vitro* study was conducted by DEDJTR and U of M, respectively. For the *in situ* study, rolled corn, wheat, barley and double-rolled barley were incubated in the rumens of three dairy cows in an incomplete block design. The *in vitro* experiment incubated alfalfa hay (control), wheat, corn and barley with different degrees of processing, over 48 hours in a completely randomized design.

In vitro method development studies

Manuscript: Alvarez Hess, P.S., P.A. Giraldo, S.R.O. Williams, J.L. Jacobs, M.C. Hannah, P.J. Moate, and R.J. Eckard. The use of total gas collection for measuring methane production in vented in vitro systems. Animal Sci. J. (submitted)

Manuscript: Alvarez Hess PS, JL Jacobs, MC Hannah, PJ Moate, and RJ Eckard. Comparison of five methods for the estimation of methane production from vented in vitro systems. J. Sci. Food Agric. (submitted)

Novel methods to collect vented gas and ways to estimate gas and methane production from *in vitro* vented systems were developed. The ANKOM gas production system (ANKOM-RF Technology, New York, USA) is a commercially available *in vitro* digestion method that accurately measures total gas production over an incubation period. Inherent in this system is the release of excess gas to prevent gas from diffusing into the incubation medium. For this reason, a gas sample taken from the module's headspace at the conclusion of the incubation period may not be representative of the gas produced during the entire fermentation period. Collecting the vented gas in a gas bag would allow subsequent sampling of all the gas produced. However, since the vented gases would have to be collected through a gas line into a gas bag, it is possible that this could affect the venting process. This research involved two *in vitro* studies. In study 1, yeast and sugar were incubated in 310 ml serum bottles equipped with an ANKOM module. Incubations were made by three different methods: vented gas not collected, vented gas collected in gas bags through a 304 cm long gas sample line and vented gas collected in gas bags through a 22 cm long extension tube.

In study 2, alfalfa hay and wheat were incubated with rumen fluid in 310 ml serum bottles equipped with an ANKOM module, and incubations were made by systems described above. It was concluded that use of a 22 cm long extension tube was ideal for collecting vented gas without interfering with the venting process or with measurements.

In the previous studies using the ANKOM system, gas samples were taken from the module's headspace at the conclusion of the incubation period and methane concentration in the sample multiplied by the volume of gas produced, was used to estimate methane production. However, not all of the gas that occupied a headspace at the start of an experiment had been replaced by fermentation gas by the end of the experiment and unknown mixtures of original and fermentation gases are lost at each venting. Thus, a gas sample taken from the module's headspace after the incubation period may not be representative of gas produced during the entire fermentation period. There are several methods for estimating methane production from feedstuffs in vented *in vitro* systems. One method (A; “gold standard”) measures methane proportions in the incubation bottle’s head space and in the vented gas collected in gas bags (Cattani et al., 2014). Other methods measure methane proportion in a single gas sample from the incubation vessel’s head space, with different assumptions. Lopez et al. (2007) assumes the same methane proportion in the vented gas as in the head space. Hannah et al. (2016) assumes constant methane to CO_2 ratio. Cattani et al. (2016) uses an empirical conversion formula data, and Hannah et al. (2016) assumes constant individual venting volumes. This study compared the estimates of methane from these various methods to that of the gold standard method under different incubation scenarios.

Dose of 3-nitrooxypropanol

Manuscript: Vyas, D. and K. A. Beauchemin. In vitro dose response of adding 3-nitrooxypropanol to cattle diets (manuscript in preparation)

The aim of this *in vitro* study was to evaluate the effects of supplementing incremental doses of NOP on methane production, volatile fatty acid profile and nutrient digestibility using a high-forage diet. A 24-h batch culture *in vitro* study, with rumen fluid as the inoculum, was performed. Rumen fluid was collected from two cannulated cows receiving a barley silage based total mixed ration just before the morning feeding. The diet was dried and ground through a 1-mm sieve. Approximately 1.0 g DM of substrate was weighed and added directly into 120 mL glass vials. Anaerobic buffer medium was prepared and 60 mL was added to the vials inoculated along with 20 mL of ruminal fluid. The treatments included Control (backgrounding TMR diet) and NOP applied at 0, 0.0065, 0.0125, 0.0185, 0.025, 0.0375, 0.05, 0.075, 0.10, 0.20, 0.30, 0.40, and 0.50 mM dose levels. The doses were selected based on the range of dose levels supplemented during *in vivo* trials. Instead of supplementing NOP based on DM content we estimated molar doses by assuming 40 L rumen fluid volume and 10 kg DM intake. Gas and methane production over the incubation period of 3, 6, 9, 12, 18 and 24 h were measured.

NOP by diet interactions

Manuscript: Alvarez, P.S., P. J. Moate, J. L. Jacobs, K. A. Beauchemin and R. J. Eckard. 2017. Effects of basal substrate on in vitro methane inhibition by 3-nitrooxypropanol (in preparation)

The aim of this *in vitro* study was to compare the inhibitory effect of NOP on *in vitro* methane production from three different substrates: alfalfa hay, corn grain and wheat grain. Fermentations were conducted using an Ankom gas production *in vitro* system that consists of incubation bottles, pressure transducers with actuators to vent fermentation gas at a pre-set threshold, and gas bags to collect vented gas. This study consisted of six treatments: 1) alfalfa hay, 2) corn grain, 3) wheat grain, 4) alfalfa hay plus NOP (0.8 mg/g DM), 5) corn grain plus NOP and 6) wheat grain plus NOP. All substrates (1.0 g DM/bottle) were incubated separately in two different sources of pooled ruminal fluid. Each combination of substrate and ruminal fluid was incubated in three fermentation bottles. The experiment was replicated in two in vitro runs. After 24 hours of fermentation, methane production, total gas production, DM degradation, ammonia concentration, volatile fatty acid concentration and ruminal fluid pH were measured. Data were analysed by ANOVA with a factorial structure of basal diet and NOP and a blocking structure of run and ruminal fluid.

Release rate of encapsulated nitrate

Manuscript: Lee, C., R. C. Araujo, K. M. Koenig, and K. A. Beauchemin. 2017a. In situ and in vitro evaluations of a slow release form of nitrate for ruminants: rumen nitrate metabolism and the production of methane, hydrogen, and nitrous oxide. Anim. Feed Sci. Technol. 231:97-106. doi:10.1016/j.anifeedsci.2017.07.005

The study used *in situ* and *in vitro* techniques to determine nitrate release rate from EN in the rumen compared with UEN. The *in situ* technique was used to measure the rumen DM and nitrogen disappearance of EN. Three ruminally cannulated beef heifers were fed ad libitum a typical backgrounding diet based on barley silage. The feeds incubated were: soybean meal as a reference, EN and UEN. Samples (10 g) of each feed were weighed into small polyester bags in triplicate per incubation time and heifer. The bags were sealed and placed in large mesh sacs that permitted free percolation of rumen fluid. The mesh sacs were placed in the rumen of each heifer and incubated for 1, 2, 4, 6, 12, 18, 24, 48, and 72 h. For 0-h incubation, bags containing the feeds were immersed into warm water and then immediately washed according to the washing procedure described below. Upon removal at each time point from the rumen, bags were immediately rinsed with cold tap water for 10 s to remove large particles. Then, all bags were washed twice with cold water for 5 min using a washing machine. After washing, bags were dried and analyzed for total nitrogen concentration. A nonlinear regression model was fitted to the DM and nitrogen disappearance data by animal and ruminal effective degradation was calculated using an assumed fractional passage rate of 0.05/h.

An *in vitro* batch culture study was conducted using ruminal fluid from the same three ruminally-cannulated heifers that were used previously. Ruminal fluid was collected 2 h after feeding. Two types of inoculum were prepared: mixture of ruminal fluid and buffer in the ratio of 1:4 and buffer only. Substrates used with buffered-ruminal fluid were: 1) blank (no substrate), 2) substrate of urea, corn starch, and xylan, 3) substrate of UEN, corn starch, and xylan), and 4) substrate of EN, corn starch, and xylan). For the incubation with only buffer, there were only 2 treatments: 1) UEN and 2) EN; both treatments provided the same amount of nitrate. The incubation bottles were prepared in triplicate and incubated for 20 and 40 min and 1, 2, 4, 6, 12, and 24 h. Gas pressure and methane production were measured and bottles were opened and samples were taken for analysis.

Mode of action of NOP and nitrate

Manuscript: Guyader, J., E. M. Ungerfeld, and K. A. Beauchemin. 2017. Redirection of metabolic hydrogen by inhibiting methanogenesis in the rumen simulation technique (RUSITEC). Frontiers in Microbiol. 8:393. doi: 10.3389/fmicb.2017.00393

A decrease in methanogenesis is expected to improve ruminant performance by allocating ruminal metabolic hydrogen to more energy-rendering fermentation pathways for the animal. However, decreases in methane emissions of up to 30% are not always linked with greater performance. The aim of this study was to understand the fate of metabolic hydrogen when methane production in the rumen is inhibited by known methanogenesis inhibitors, including nitrate and NOP. The experiment was conducted using the Rumen Simulation Technique. Two apparatuses were used, each one consisting of a water bath maintained at 39ºC and 8 fermentation vessels of 900 mL and continuous mixing. The treatments were control diet alone (60% corn silage and 40% cereals and minerals on a DM basis) or supplemented with nitrate, NOP or anthraquinone (another methane inhibitor). The dose of each additive was selected with an aim of obtaining 75% methane decrease. Measurements started after 1 week adaptation.

Experiment 4.2 Animal Assessment of Additivity and Synergy of Mitigation Strategies (AAFC)

Thesis: Smith, M. L. 2017. Assessing the potential of a novel feed additive and an unsaturated fat alone and in combination to lower methane emissions from cattle and reduce their contribution to climate change. PhD dissertation, University of Delaware, Newark.

The objective of this study was to examine the combined effects of NOP and unsaturated fat (canola oil). All procedures were approved by the Lethbridge Research and Development Centre Animal Care Committee and conducted in accordance with the guidelines set forth by the Canadian Council on Animal Care (CCAC, 2009). Eight ruminally canulated beef heifers (Angus cross, 732 kg) were used in a double 4 × 4 Latin square design with four 28-d periods and four dietary treatments. The dietary treatments were: 1) control (no supplementation of NOP or oil), 2) canola oil alone (added at 5.0% of dietary DM), 3) NOP (200 mg/kg of dietary DM), and 4) NOP (200 mg/kg of dietary DM) and canola oil (5.0% of dietary DM) combined.

Animals were blocked according to body weight into two groups and randomly assigned to one of the four treatments using a double 4 x 4 Latin square design. Animals in this experiment were fed a high forage diet (90% forage and 10% concentrate) to create a ruminal environment that would favor hydrogen-yielding fermentation pathways (acetate) as opposed to hydrogen-consuming pathways (propionate and butyrate). Feed offered and refused was measured daily. Feeds and ingredients were sampled and analyzed using routine procedures. Body weight measurements were obtained during each period. Samples of ruminal content were obtained at 0, 3, 6, 9, and 12 h after feeding on days 14 and 17 of every period for analysis. Indwelling pH meters were inserted into each animal and used to record ruminal pH at one minute intervals for a total of 7 days. A 5 mL aliquot of ruminal fluid was obtained, preserved, and stored until protozoa were counted. Enteric gas production was measured from day 18 to 21 of every period using respiratory chambers. Total tract digestibility was measured by collecting total excretion of feces and urine. Data were analyzed using a MIXED model to account for animal, treatment, period, and random error. Repeated measure technique was used for variables measured over time.

Experiment 5.1 Conduct modeling studies to determine the broader potential for GHG reductions using low methane diet technologies, as applicable to Alberta and Victorian dairy farms (U of M and AAFC)

Manuscript: Alemu, A., S. Little, X. Hao, D. Thompson, A. Iwaasa, V. Baron, K. Beauchemin, H. Janzen, R. Kröbel. 2017. Assessment of grazing management on farm greenhouse gas intensity of beef production systems in the Canadian Prairies using life cycle assessment. Agric. Syst. 158:1-13. doi.org/10.1016/j.agsy.2017.08.003

Manuscript: Alvarez-Hess, P., S. Little, P. Moate, J. Jacobs, K. Beauchemin, R. Eckard. 2018. A partial life cycle assessment of the greenhouse gas mitigation potential of feeding 3-nitrooxypropanol and nitrate to cattle (in preparation)

Manuscript: Guyader, J., S. Little, R. Kröbel, C. Benchaar, and K. A. Beauchemin. 2017. Comparison of greenhouse gas emissions from corn- and barley-based dairy production systems in Eastern Canada. Agic. Syst. 152:38-46. doi.org/10.1016/j.agsy.2016.12.002

Manuscript: Little S. M., C. Benchaar, H. H. Janzen, R. Kröbel, E. J. McGeough, and K. A. Beauchemin. 2017. Demonstrating the effect of forage source on the carbon footprint of a Canadian dairy farm using whole-systems analysis and the Holos model: alfalfa silage vs. corn silage. Climate 5, 87 doi:10.3390/cli5040087

Modeling was conducted to determine the broader potential for GHG reductions using the methane reducing technologies investigated in the project. This information is critical prior to recommending these technologies be used on commercial beef and dairy farms. Baseline Australian and Canadian production scenarios for dairy production and beef production were first developed to determine baseline GHG emissions from the farming systems. Methane abatement strategies were then evaluated using the modelling framework and data from the *in vitro* and *in vivo* studies.

Canadian dairy farm case study

A case study representing a Canadian dairy farm with 60 milking cows was modelled. The GHG emissions were estimated using Holos 3.0.3, a whole-farm model developed by Agriculture and Agri-Food Canada. Holos considers all methane, nitrous oxide and CO_2 emissions from the farm and inputs used on the farm. The farm was assumed to be located in southern Alberta, Canada. The characteristics of the farm and the herd were based on the case studies designed by Mc Geough et al. (2012). The scenario was modeled over a period of 6 years to capture changes in herd dynamics. It included all animals, manure, production and purchase of feeds, and equipment use. The milking herd was housed in individual tie stalls during lactation and on a dry lot during dry months. The accumulated solid manure was stockpiled over the year and applied to the land used to grow feed crops. The average milk production for the herd was 30 kg/d with fat and protein concentrations of 3.8% and 3.2%, respectively. The lactation diet (DM basis) was composed of corn silage (27.5%), corn grain (22.5%), alfalfa hay (27.5%), soybean meal (20.5%) and a mineral supplement (2%).

Canadian beef farm case study

A case study Canadian beef farm was modelled with emissions estimated using Holos 3.0.3, a whole-farm model developed by Agriculture and Agri-Food Canada. All emissions on the farm and emissions associated with inputs on the farm were included. The farm was located in southern Alberta, Canada. The size and characteristics of the farm and its herd was based on the case study by Beauchemin et al. (2010). The simulated farm consisted of a beef production operation comprised of 120 cows, with the progeny retained on the farm and fattened in a feedlot. The farm also included cropland and native prairie pasture for grazing to supply the feed for the animals. The study was conducted over 8 years to fully account for the lifetime GHG emissions from the cows, bulls and progeny, as well as the beef marketed from cull cows, cull bulls, and progeny raised for market.

It was assumed that all feeds were produced on the farm. When the animals were grazing it was assumed that all of the waste was deposited directly onto the pasture. When the animals were confined, the manure system was deep bedding, and the accumulated manure was then applied to the field. Straw for bedding was obtained as a by-product from the barley grain produced on the farm for the feedlot diet.

Australian dairy case study

Two case study Australian dairy farms were modelled using the Greenhouse Accounting Framework (**D-GAF**). One farm represented a medium sized (340 cows), average producing dairy farm and the other represented a large (500 cows) high producing dairy farm. Both farms were located in Gippsland, Australia in a high rainfall area (≥700 mm/year). The size and characteristics of the farms and their herds were based on the dairy case studies designed by Browne et al. (2011) and Christie et al. (2012). The scenarios included all animals, manure, production and purchase of feeds, and equipment use. Both farms had improved pasture.

The average producing farm had a herd of 340 cows, 85 heifers older than 1 year, 96 heifers younger than 1 year and 3 bulls. The milking herd had a target daily DM intake of 20 kg/d for spring and autumn and 16 kg/d for summer and winter. In spring and autumn the diet was composed of 4 kg barley grain and 16 kg perennial ryegrass pasture (DM digestibility of 81.1-82.1%; crude protein of 19.0-20.6%). In summer and winter the diet DM was composed of 4 kg/d barley silage, 4 kg/d alfalfa hay and 8 kg/d perennial ryegrass pasture (DM digestibility of 70.7-71.2%; crude protein of 18.3-19.3%). Milk production per head/day for this farm was 27 kg/d in spring, 23 kg/d in summer, 16 kg/d in autumn and 4 kg/d in winter.

The high producing farm had a herd of 500 cows, 100 heifers older than 1 year, 112 heifers younger than 1 year and 3 bulls. The milking herd had a target daily DM intake of 22 kg/d during spring and autumn and 18 kg/d during summer and winter. In spring and autumn this was composed of 6 kg/d barley grain and 16 kg/d perennial ryegrass pasture (DM digestibility of 81.0-81.9 %; crude protein content of 19.7-18.2%). In summer and winter the diet DM was composed of 6 kg/d barley silage, 4 kg/d alfalfa hay and 8 kg/d perennial ryegrass pasture (DM digestibility of 69.9-70.1%; crude protein content of 17.8-18.7%). Average milk production for this farm was 29 kg/d in spring, 25 kg/d in summer, 18 kg/d in autumn and 5 kg/d in winter.

Ninety percent of the animal waste was considered to be deposited directly back onto the pasture as animals graze while the remaining 10% was stored in a lagoon and spread later.

Perennial ryegrass silage was produced on the farms with the excess pasture. The remaining feedstuffs were estimated to be produced on other farms, located at an average distance of 250 km from the case study farms. Transport of feedstuffs was also considered. Pre-farm emissions related to grain production were also included.

Australian beef farm case study

The Australian beef farm case study was adapted from Cullen et al. (2016). This was a 23,000 ha farm located in central-western Queensland, Australia in a low rainfall area (mean 435 mm/year). The farm included a cow-calf and grass-fed finishing operation and was modelled over a 10 year period in order to cover a breeding herd from birth to slaughter and six production cycles. The breeder herd began with the farm retaining 1,206 female and 21 male calves. With a mortality of 1.5% for females and 2% for males, 1,172 cows and 20 bulls reached adult age with a weight of 535 kg and 950 kg, respectively. First breeding was at 24 months of age and first calving was at 33 months of age, two months after calving cows were bred again. Weaning rate was 68% for heifers and 54% for adult cows (Cullen et al., 2016). Beef stock were kept on the farm and were sold for meat at an age of 18 months and a live weight of 370 kg, 19 female calves were kept to cover mortality and maintain the size of the breeding herd annually. Bulls were culled after they had sired calves in the sixth production cycle, while cows were culled after weaning the calves in the sixth production cycle, the final year was extended to complete the growth of the beef stock of the sixth production cycle. The first production cycle produced 389 steers and 368 heifers at an average weight of 370 kg, and production cycles 2 to 6 produced 308 steers and 289 each at an average weight of 370 kg. Diesel and electricity used was estimated as indicated by Cullen et al. (2016).

It was assumed that 100% of manure was deposited directly back onto the pasture while animals grazed. The GHG emissions (t CO_2e) were estimated based on the Australian National Greenhouse Gas Inventory method based on the animal numbers, live weight and growth using the Greenhouse Accounting Framework calculators for beef and dairy (Browne et al., 2011). The calculators incorporate the Australian NGGI methodology (Commonwealth of Australia, 2014) to estimate on-farm GHG emissions. The calculator considers methane, nitrous oxide and CO_2 emitted in the production of key farm inputs (Eckard and Taylor, 2016). Pre-farm emissions were calculated as those associated with the production and transport of purchased feeds and with the production of the enteric methane mitigant.

Results of Experiments and Model Simulations

Experiment 1.1 Dose Response to Methane Inhibitor (AAFC)

Results

For high forage diets, no treatment effects were observed on overall DM intake and DM intake inside chambers, averaging 8.61 and 6.37 kg/d, respectively (Table 1.1.2). Methane yield (g/kg DM intake) was decreased with increasing concentrations of NOP, with values significantly different from Control being observed when NOP was supplemented at 100, 150 and 200 mg/kg DM. For high-grain diets, overall DM intake and DM intake inside chambers averaged 9.98 and 8.13 kg/day, respectively, and neither was affected by NOP supplementation (Table 1.1.3). Methane yield decreased with NOP supplementation, with differences reaching significance at dose levels of 100, 150 and 200 mg/kg DM.

Discussion

Dietary composition used in the present study corresponds well with typical diets fed in western Canadian feedlots. It was found that the level of NOP supplemented affects the magnitude of methane mitigation. The six doses used in the study were between 0 and 200 mg/kg DM with the range selected based on previous studies. The present study confirmed the efficacy of NOP in mitigating enteric methane emissions from beef cattle. With both diets, increasing the dose of NOP caused a decrease in methane yield, but the lower doses (50 and 75 mg/kg DM) were not significantly different from the Control. In high-forage diets, methane yield decreased by 16%, 21% and 23% with 100, 150 and 200 mg NOP/kg DM, respectively, whereas for high-grain diets, the decreases were 26%, 33% and 45%, for the three doses respectively.

The greater efficacy of NOP in decreasing methane emissions (47.6 g/d for the high forage diet and 60.7 g/d on the high grain diet) when supplemented to high-grain diets than to high-forage diets in the present study had not been previously reported at the time of this study, as no other studies had been conducted with NOP added to high-grain beef-cattle diets.

In conclusion, the present study underscores the efficacy of NOP in lowering enteric methane emissions in beef cattle fed high-forage and high-grain diets. Among the various dose levels investigated in the present study, NOP supplemented at 100 to 200 mg/kg DM was effective in decreasing methane yield in steers fed high-forage and high-grain diets, without inducing any negative effects on DM intake.

Table 1.1.2 Enteric methane emissions from feedlot animals fed a high-forage diet supplemented with 0 (Control), 50, 75, 100, 150 and 200 mg/kg DM of 3-nitrooxypropanol

Variable	Dose							*P*-value	
	Control	50	75	100	150	200	SEM	NOP	Linear
DM intake, kg/d	8.10	8.30	8.96	8.32	8.35	8.65	0.34	0.54	0.95
DM intake in chambers, kg/d	6.23	6.54	6.47	6.73	6.73	5.53	0.67	0.81	0.70
Methane, g/d	143.8ab	154.0a	137.7ab	129.8abc	117.4bc	96.2c	12.5	0.05	0.10

Hydrogen, g/d	n.d.	n.d.	n.d.	0.16	0.64	1.03	0.24	0.11	0.15
Methane yield (g/kg DM intake)	23.6ab	25.4a	22.8abc	19.8cd	18.6cd	18.2d	1.65	<0.01	<0.01
Methane (% of gross energy intake	6.42ab	6.28a	5.93abc	5.18 cd	4.78 cd	4.71 cd	0.45	<0.01	<0.01

Values within a row with different letters are different (*P* < 0.05). SEM = standard error of the mean, n.d. = not detected.

Table 1.1.3 Enteric methane emissions from feedlot animals fed a high-grain diet supplemented with 0 (Control), 50, 75, 100, 150 and 200 mg/kg DM of 3-nitrooxypropanol

	Dose							*P*-value	
	Control	50	75	100	150	200	SEM	NOP	Linear
DM intake, kg/d	10.6	9.3	10.5	9.78	9.37	10.3	0.49	0.26	0.11
DM intake in chambers, kg/d	8.70	7.64	8.08	8.37	7.50	8.49	0.74	0.83	0.65
Methane, g/d	124.5ab	128.1a	110.6ab	89.2abc	67.0bc	63.8c	16.9	0.04	0.02
Hydrogen, g/d	n.d.	0.25	0.32	0.82	0.96	2.77	0.73	0.13	0.17
Methane yield (g/kg DM intake)	14.3ab	17.5a	15.2ab	10.6cd	9.64cd	7.83d	2.22	0.03	0.04
Methane (% of gross energy intake	4.11ab	4.72 a	4.42ab	2.95cd	2.71dc	2.13d	0.63	0.04	0.02

Values within a row with different letters are different (*P* < 0.05). SEM = standard error of the mean, n.d. = not detected.

Experiment 1.2 Methane Inhibitor and Feedlot Cattle Performance (AAFC)

Results

The study explored the individual and combined effects of MON and NOP using diets typical of western Canadian feedlots. There were no interactions between NOP and MON for most of the variables studied. Thus, the effects of NOP and MON were independent in both backgrounding and finishing diets.

For backgrounding diets, DM intake was reduced by 7% with NOP; but intake was not affected by MON (Table 1.2.2). Gain:feed ratio was increased by 4% with MON and by 5% with NOP. For finishing diets, both MON and NOP tended to reduce DM intake by 5% compared with control. Monensin tended to reduce average daily gain by 3%, while no effects on average daily gain were observed for NOP. Gain:feed ratio was improved by 3% with NOP while no effects were observed with MON.

For the backgrounding diets, there was an interaction between MON and NOP for total methane production (Table 1.2.3) because NOP reduced methane production to a greater extent when MON was not added to the diet. However, when methane production was corrected for intake and expressed as methane yield, the effect of NOP was consistent regardless of whether MON was supplied as evidenced by the lack of NOP × MON interaction. There was no longer an effect of MON on methane, but feeding NOP reduced methane yield by 42%. For cattle fed a high grain diet, total methane production and methane yield was lowered by 41 and 37% with NOP, respectively, whereas MON did not decrease methane production or yield.

Neither MON nor NOP affected carcass characteristics including hot carcass weight grade fat, rib eye area, marbling quality, marbling level, and saleable meat (Table 1.2.4). Similarly, liver score and dressing percentage was similar for all treatments.

Discussion

3-Nitrooxypropanol supplementation was shown to be a highly effective strategy for mitigating methane emissions in beef cattle fed high forage and high grain diets. The results from this study are consistent with previous studies that showed no negative effects on animal performance (Vyas et al., 2016). Ionophores such as MON are commonly used in beef cattle diets in North America to improve feed efficiency and nutrient utilization. A novel finding of the present research is that the effects of NOP were independent from those of MON in both high forage and high grain diets. Most variables examined showed a lack of significant interaction between NOP and MON.

During the backgrounding phase, NOP supplemented at 200 mg/kg DM intake (1.23 g/d) decreased total methane production and methane yield by 38 and 29%, respectively and the results are in agreement with previous studies providing either similar (Vyas et al., 2016) or greater amounts of NOP (2.7 g/d) in beef cattle fed high-forage diets (Romero-Perez et al., 2014). The dose of NOP used in the finishing phase of this study was considerably lower (125 mg/kg DM intake) than levels used during the backgrounding phase. Despite using a lower dose in the finishing diets, methane yield was decreased by 41 and 37% in the finishing phase, respectively. Vyas et al. (2016a) showed NOP was highly potent in high-grain diets where a dose of 200 mg/kg decreased total methane production and methane yield by 84 and 80%, respectively. The variability in the efficacy of NOP with changes in dietary composition is attributed to lower methane emissions from cattle fed high-grain diets.

Cattle producers may be more willing to adopt methane mitigation practices if associated with improvement in gain:feed ratio or average daily gain. The present study demonstrates that feed conversion efficiency, measured as gain:feed ratio, was improved in beef cattle fed high-forage diets by 5% and high-grain diets by 3% when supplemented with NOP.

In conclusion, the results demonstrate efficacy of NOP in reducing enteric methane emissions and subsequently improving feed conversion efficiency in cattle fed backgrounding and finishing diets. In both phases of the study, NOP lowered methane yield and the reduction in methane was not affected by whether MON was included in the diets. Both compounds improved feed conversion efficiency in the backgrounding phase, but only NOP improved feed conversion in the finishing phase. We conclude that NOP is a potent methane inhibitor that can be added to conventional feedlot diets containing MON without incurring negative effects on performance or carcass characteristics. Furthermore, the study suggests a possible link between sustained reduction in methane and improved feed conversion efficiency, which may encourage producers to adopt this methane mitigation approach.

Table 1.2.2 Performance of feedlot cattle fed high-forage diets supplemented with (+) and without (-) monensin (MON) and 3-nitrooxypropanol (NOP) (No significant interactions between MON and NOP)

	-MON		+MON			Effect, *P*-value	
	-NOP	+NOP	-NOP	+NOP	SEM	MON	NOP
High forage diets							
No. of steers (pens)	60 (8)	60 (8)	60 (8)	60(8)			
Initial body weight, kg	308.3	308.0	307.5	309.6	2.22	0.86	0.69
Final body weight, kg	461.8	459.3	463.9	463.9	3.32	0.31	0.71
DM intake, kg/d	8.41	7.64	8.08	7.64	0.10	0.12	<0.01
Gain:feed	0.172	0.184	0.183	0.189	0.002	<0.01	<0.01
Average daily gain, kg/d	1.45	1.43	1.47	1.46	0.02	0.21	0.41
High grain diets							
No. of steers (pens)	60 (8)	60 (8)	60 (8)	60(8)			
Initial body weight, kg	506.9	503.9	512.2	513.3	3.80	0.06	0.81
Final body weight, kg	697.5	692.2	693.6	696.5	5.24	0.97	0.82
DM intake, kg/d	12.1	11.4	11.4	11.0	0.27	0.06	0.06
Gain:feed	0.150	0.152	0.152	0.159	0.002	0.58	<0.01
Average daily gain, kg/d	1.80	1.79	1.73	1.74	0.04	0.08	0.98

Table 1.2.3 Enteric methane emissions from feedlot animals fed diets with (+) and without (-) monensin (MON) and 3-nitrooxypropanol (NOP) (No significant interactions between MON and NOP, except methane, g/animal per day for high forage diets, $P < 0.01$)

	-MON		+MON			Effect, *P*-value	
	-NOP	+NOP	-NOP	+NOP	SEM	MON	NOP
High forage diets							
DM intake, kg/d	6.79	6.23	5.19	6.11	0.34	0.09	0.71
Methane, g/d	190.3^{a}	87.4^{c}	138.8^{b}	102.6^{c}	10.4	<0.01	<0.01
Methane yield, g/kg of DM intake	28.2	15.7	28.1	17.1	1.48	0.65	<0.01
Hydrogen, g/d	0	2.26	0	1.95	0.44	0.77	<0.01
High grain diets							
DM intake, kg/d	10.2	9.90	8.12	8.57	0.69	0.04	0.91
Methane, g/d	160.1	73.9	155.7	112.4	20.5	0.45	0.01
Methane yield, g/kg of DM intake	15.9	8.32	19.1	13.8	2.16	0.09	0.01
H_2, g/animal per day	0.09	8.01	0.02	1.63	1.48	0.06	<0.01

Table 1.2.4 Carcass characteristics in feedlot animals fed high grain diet supplemented with diets with (+) and without (-) monensin (MON) and 3-nitrooxypropanol (NOP) (No significant interactions between MON and NOP)

	-MON		+MON			Effect, *P*-value	
	-NOP	+NOP	-NOP	+NOP	SEM	MON	NOP
Body weight, kg	730.0	734.2	732.4	735.7	11.2	0.85	0.73
Hot carcass weight, kg	425.5	432.0	434.3	432.4	6.91	0.49	0.73
Fat cover (1 mm)	22.2	21.5	22.6	23.7	0.90	0.14	0.82
Fat cover (2 mm)	22.2	21.0	21.9	22.8	0.87	0.36	0.90
Grade fat, mm	19.9	18.6	19.7	20.6	0.86	0.28	0.89
Rib eye area, cm	89.0	93.1	93.0	94.0	1.57	0.11	0.10
Marbling quality	2.90	2.93	2.90	2.87	0.07	0.60	0.99
Marbling score	29.4	27.5	23.9	28.0	3.58	0.45	0.73
Saleable meat, %	49.4	50.8	50.0	49.5	0.72	0.64	0.52
Liver score	1.70	1.58	1.57	1.74	0.14	0.95	0.89
Dressing percentage	58.3	58.8	59.3	58.8	0.44	0.28	0.95

Experiment 2.1 Alternative Hydrogen Sink (Nitrate) (AAFC)

Results

Feeding nitrate to cattle during the backgrounding phase had no effect on animal performance Table 2.1.1). There was a 6 to 10% reduction in methane yield for the encapsulated and non-encapsulated forms of nitrate, respectively, but this reduction was not statistically significant (Table 2.1.2). In the finishing phase, methane production was not affected by feeding nitrate, but there was a linear increase in gain:feed ratio with increasing level of EN.

Discussion

Supplemental nitrate has been shown to be an effective methane mitigation strategy in other studies, thus the lack of effect of nitrate on methane yield in the present study was unexpected. The lack of difference between EN and UEN indicated that the lack of decline in methane production was not due to encapsulation of the nitrate source. The study also showed that inclusion of nitrate in a backgrounding diet to up to 2% nitrate did not cause nitrate toxicity or any health problems. However, sorting against EN in the diets was observed, indicating EN altered the organoleptic properties of the diets and therefore potentially changed eating behavior without affecting DM intake and growth.

We conclude that the lack of effect of nitrate on methane mitigation in this study may have been due to the few number of animals used for the measurements (5/treatment). Because this was a relatively long term study, another possibility is that the microbiome of the rumen adapted to the nitrate over time. The improvement in gain:feed ratio in the finishing study was unexpected and difficult to explain. It may be that the nitrate caused the animals to consume much smaller meals (observed in a previous study), which may have attenuated the effects on rumen acidosis.

Table 2.1.1 Dry matter intake and growth performance of beef steers (experimental unit, pen; n = 7) fed a backgrounding diet supplemented with encapsulated nitrate (EN) in a feedlot

	Treatments				Treatment
	Control	1.25% EN	2.5% EN	SEM	P-value
Backgrounding phase					
DM intake, kg/d	8.12	8.10	8.10	0.203	0.98
Nitrate consumed, g/d	15.0^{c}	96.6^{b}	174.7^{a}	4.14	< 0.01
Initial body weight, kg	291	292	292	7.84	0.20
Final body weight, kg	400	402	403	10.41	0.77
Average daily gain, kg/d	1.17	1.21	1.19	0.036	0.57
Gain:feed ratio, kg/kg	0.144	0.148	0.146	0.0037	0.73
Finishing phase					
DM intake, kg/d	10.7^{a}	10.7^{a}	9.9^{b}	0.14	< 0.01
Nitrate consumed, g/d	3.0^{c}	118.8^{b}	183.4^{a}	2.43	<0.01
Initial body weight, kg	450.1	452.4	447.6	10.7	0.71
Final body weight, kg	656.0	667.4	657.0	9.00	0.25
Average daily gain, kg/d	1.36	1.43	1.39	0.033	<0.01
Gain:feed ratio, kg/kg	0.127^{c}	0.133^{b}	0.141^{a}	0.0029	<0.01

^{a,b,c}Within a row, means without a common superscript letter differ ($P < 0.05$).

Table 2.1.2 Dry matter intake and methane production of beef steers (5 per treatment) fed a backgrounding diet supplemented with encapsulated (EN) or unencapsulated (UEN) nitrate in environmental chambers

	Treatments				SEM	Treatment P-value
	Control	1.25% EN	2.5% EN	UEN		
Backgrounding phase						
Dry matter intake, kg/d	6.48	6.28	5.85	5.96	0.37	0.65
Methane,[1] g/d	187.7	173.8	156.3	152.3	10.39	0.099
Methane, g/kg DMI	29.0	27.6	27.2	25.9	1.51	0.56
Finishing phase						
Dry matter intake, kg/d	7.8	7.9	7.2	7.1	0.49	0.59
Methane,[1] g/d	141.9	173.1	135.3	142.8	20.36	0.57
Methane, g/kg DMI	18.3	21.6	19.3	20.5	2.08	0.70

[1] Control vs. 2.5% EN, *P* = 0.058; Control vs. UEN, *P* = 0.026.

Experiment 2.2 Alternative Hydrogen Sink (Nitrate) (AAFC)

Results

There were almost no significant interactions between EN and EO, meaning that feeding EO did not alter the animal's response to EN. In the backgrounding phase (Table 2.2.1), feeding EN rather than urea as the source of non-protein nitrogen reduced methane yield by 12%, but had no positive effects on animal performance. There was a reduction in DM intake with EN, but that did not result in an improvement in gain:feed ratio. The feeding behavior analysis indicated that feeding EN slowed down the feeding rate (g of DM/min), which resulted in animals with their heads in the feed bunk longer during meals. As a result, they ate more meals per day. During the finishing phase (Table 2.2.2), feeding EO reduced the DM intake and the final body weight of the cattle at slaughter, but average daily gain was not significantly affected. The decrease in DM intake resulted in an improvement in gain:feed ratio of 9.7%. Feeding EN rather than urea reduced methane yield by 10.2% and N altered feeding behavior; it slowed down the feeding rate (g of DM/min), increased meal frequency, and increased meal duration.. Due to the lighter slaughter weight of cattle fed EN, they also had lighter carcass weights and smaller rib eye area (Table 2.2.3).

Table 2.2.1 Dry matter intake, feeding behaviour and methane production of beef steers fed backgrounding diets supplemented with and without encapsulated nitrate (EN) and with and without essential oil (EO)

	Without EO		With EO		SEM	Effect[1], *P-value*	
	Without EN	With EN	Without EN	With EN		EN	EO
Initial body weight, kg	371	360	363	359	5.0	0.13	0.40
Final body weight, kg	467	446	458	443	7.8	0.02	0.42

ADG, kg/d	1.07	1.07	1.16	1.02	0.04	0.12	0.64
DM intake, kg/d	8.3	7.8	8.2	7.5	0.18	0.004	0.42
Gain : Feed	0.13	0.13	0.14	0.13	0.00	0.54	0.68
Meal duration, min/d	183.6	188.2	183.4	186.6	4.71	0.41	0.85
Head down duration, min/d	80.9	97.0	84.0	91.6	4.83	0.02	0.81
Meal frequency, events/d	9.2	10.4	9.6	10.3	0.29	0.002	0.49
Feed rate, g/min	46.2	42.7	46.1	41.1	1.29	0.001	0.51
Head duration per meal, min/meal	9.6	10.2	9.3	9.5	0.68	0.57	0.52
Methane, g/d	184	151	197	166	5.8	0.02	<.0001
Methane yield, g/kg of DM intake	22.4	19.4	24.9	22.3	0.8	0.001	0.001

[1] No significant interactions between EN and EO.

Table 2.2.2 Dry matter intake, feeding behaviour and methane production of beef steers fed finishing diets supplemented with and without encapsulated nitrate (EN) and with and without essential oil (EO)

	Without EO		With EO		SEM	Effect[1], *P-value*	
	Without EN	With EN	Without EN	With EN		EN	EO
Initial body weight, kg	535.5	513.8	527.6	510.5	9.0	0.03	0.53
Final body weight, kg	747.5	711.9	735.4	720.6	11.4	0.03	0.88
ADG, kg/d	1.93	1.87	1.91	1.91	0.04	0.57	0.76
DM intake, kg/d	12.5	11.3	12.1	11.4	0.2	<0.001	0.52
Gain : Feed	0.15	0.17	0.16	0.17	0.01	0.001	0.42
Meal duration, min/d	79.0	90.0	80.7	83.5	2.9	0.02	0.40
Head down duration, min/d	33.5	34.7	32.1	29.8	2.2	0.81	0.16
Meal frequency, events/d	6.5	7.6	6.9	7.6	0.2	<0.001	0.38
Feed rate, g/min	164.6	126.7	153.7	142.8	5.6	<0.001	0.64
Head duration per meal, min/meal	5.7	4.9	5.0	4.2	0.4	0.04	0.10
Methane, g/d	207	167	207	169	7.2	<0.001	0.90
Methane yield, g/kg of DM intake	16.6	15.2	17.1	15.0	0.7	0.008	0.83

[1] No significant interactions between EN and EO, except for feeding rate ($P = 0.02$).

Table 2.2.3 Carcass characteristics of beef steers fed finishing diets supplemented with and without encapsulated nitrate (EN) and with and without essential oil (EO)

	Without EO		With EO		SEM	Effect, *P-value*	
	Without EN	With EN	Without EN	With EN		EN	EO
Body weight, kg	747.5	711.9	735.4	720.6	11.4	**0.03**	0.88
Hot carcass weight, kg	435.8	411.2	431.2	415.6	6.5	**0.003**	0.98
Fat cover (1 mm)	21.2	22.0	22.6	21.1	1.1	0.75	0.78
Fat cover (2 mm)	20.0	20.7	22.0	19.1	1.2	0.38	0.88
Grade fat	18.7	19.0	19.7	17.3	1.1	0.35	0.74
Rib eye area, cm^2	93.2	82.4	90.1	89.1	2.3	**0.01**	0.42

Marbling quality	3.00	2.95	2.91	2.91	0.1	0.65	0.18
Marbling level	24.1	27.3	22.3	31.8	5.1	0.21	0.79
Saleable meat, %	51.0	49.0	49.5	51.3	1.0	0.95	0.74
Liver score	2.6	2.5	2.8	2.4	0.3	0.29	0.86
Dressing percentage	58.7	58.1	59.0	57.9	1.3	0.54	0.96

Discussion

Unlike in Experiment 2.1 where no significant reduction in methane occurred with feeding EN rather than urea, in the present study methane production decreased by about 10%, confirming the potential for nitrate as a methane mitigant. In the present study, EN was fed in combination with EO to determine whether potential negative taste or flavor issues with nitrate feeding could be overcome by feeding EO as a flavoring agent. The lack of interactions between EN and EO indicates the relative independence of these compounds. There was no added benefit from feeding them in combination because EO had no effects on feed intake. The study confirms the observation from Experiment 2.1 that feeding EN improves feed conversion efficiency of cattle fed high grain diets. This is a substantial positive benefit of feeding nitrate. In Experiment 2.1 we hypothesized that the improvement in gain:feed ratio was due to a change in feeding behavior, although behavior was not measured. By measuring feeding behavior in the present study, we were able to confirm that feeding nitrate slows down the rate of intake and may provide some protection against the risk of acidosis, which may have led to an improvement in feed efficiency. Whether this improvement occurs when cattle are fed ionophores is not known. Another important finding from the study is that there were no issues related to nitrate toxicity. We conclude that feeding a protected source of nitrate to feedlot cattle can decrease methane emissions by about 10 to 12%, while improving feed conversion efficiency by 10% in rations that do not include ionophores. However, final weight of cattle fed EN at slaughter may be slightly lower, therefore, slightly longer days on-feed may be required, offsetting some of the advantages of improved gain:feed ratio.

Experiment 3.1 Alternative Starch Source (DEDJTR and U of M)

Results

Feeding wheat deceased ($P < 0.001$) methane yield by about 35% compared with feeding corn, barley, or double rolled barley (Table 3.1.1). However, wheat also decreased ($P < 0.01$) DM intake, energy corrected milk production and the fat concentration of milk. The relationship between rumen pH and methane yield for the various grains is shown in Figure 3.1.1.

Discussion

The large (35%) decrease in methane yield due to feeding wheat indicates that it has the potential to play an important role in the abatement of methane from dairy production. The close positive association between methane yield and minimum rumen pH would indicate that the reduction in methane was due to low rumen pH. The methanogens in the rumen that produce methane are known to be susceptible to pH. A similar trend has been reported between daily mean of ruminal fluid pH and methane emission (Hünerberg et al., 2015). The reduction in energy corrected milk due to feeding wheat was partially due to lower feed intake, which may have been caused by increased incidence of rumen acidosis.

Table 3.1.1 Influence of diet on methane emissions, and energy correct milk (ECM) and dry matter intake (DMI) during the measurement of methane

Parameter	Corn	Wheat	Barley	Double rolled barley	SED	*P*-value
Dry matter intake, kg/d	22.2^{b}	21.1^{a}	22.6^{b}	22.7^{b}	0.44	0.002
Milk, kg/d	32.1	32.3	31.3	30.6	0.80	0.16
Energy corrected milk, kg/d	31.2^{b}	27.6^{a}	30.7^{b}	30.7^{b}	1.10	0.010
Milk composition (%)						
Fat	3.82^{b}	2.83^{a}	3.95^{b}	4.14^{b}	0.238	<0.001
Protein	3.17	3.19	3.27	3.16	0.052	0.162
Methane, g/d	446^{b}	300^{a}	518^{b}	533^{b}	43.6	<0.001
Methane, g/ kg DM intake	20.3^{b}	14.3^{a}	22.9^{b}	23.4^{b}	1.80	<0.001

^{a,b,c} Means in the same row followed by different superscripts differ significantly ($P < 0.05$)

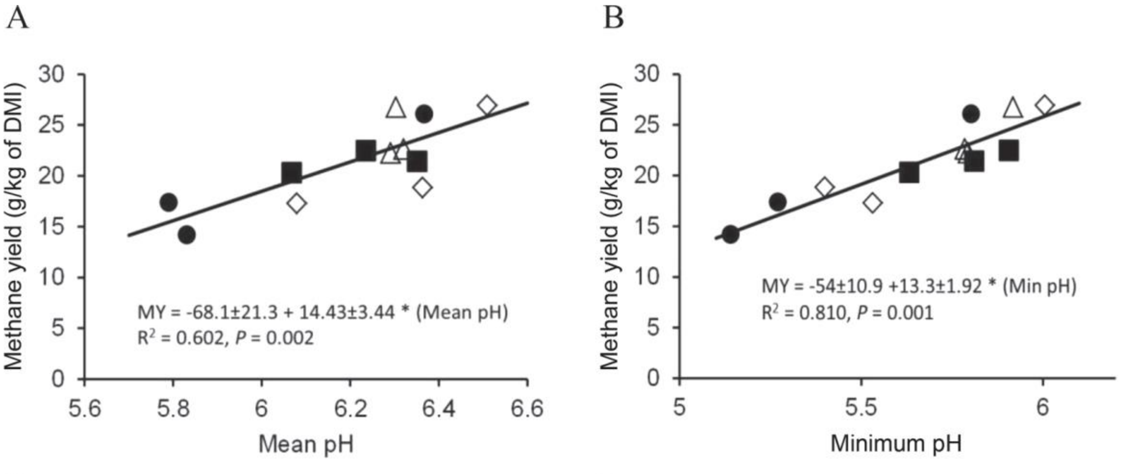


Figure 3.1.1 Relationship between methane yield and minimum rumen pH. wheat (●), corn (■), double-rolled barley (Δ), or single-rolled barley (◊) diets.

Experiment 3.2. Rate of Dietary Starch Degradation and Methane/Milk Production, Long-term Effects (DEDJTR and U of M)

Results

Over the 16 week experimental period, total DMI remained relatively constant and similar for the two dietary treatment groups (Table 3.2.1). Effects of the two diets and duration of feeding on milk production parameters are shown in Table 3.2.2. At week 4, in comparison to the CRN diet, the WHT diet significantly ($P < 0.05$) reduced milk fat yield and milk fat concentration, but these differences had disappeared by week 16. At week 4, methane emission, methane yield, milk fat yield and milk fat concentration were substantially less ($P < 0.05$) in cows fed the WHT diet compared to the same metrics in cows fed the CRN diet; but these differences were not apparent at weeks 10 and 16 (Table 3.2.2). The responses over time in these metrics were not similar in all cows (Figure 3.2.1). In 4 cows fed the WHT diet (cows A, B, C and D, designated "poor adapters), milk fat concentration and methane yield remained relatively constant from week 4 to week 16, whereas for 5 cows fed the WHT diet (cows E, F, G, H, and I, designated "good adapters"), their milk fat concentrations and methane yields approximately doubled between weeks 4 and 16 (Figure 3.2.1). In addition, this association or relationship between milk fat concentration and methane yield was apparent in cows fed the WHT diet and not in cows fed the CRN diet (Figure 3.2.2).

Table 3.2.1 Feed intake, milk yield, milk composition and bodyweight from cows on each treatment during weeks 4, 10 and 16 of the experiment

Parameter	Week 4		Week 10		Week 16		*P*-value		
	CRN[1]	WHT[1]	CRN	WHT	CRN	WHT	TRT	Week	TRT × Week
No. of cows	11	10	11	10	11	10			
Feed intake, kg DM/d[4]									
Alfalfa hay	10.0^{b}	8.9^{a}	10.3^{b}	9.8^{b}	10.4^{b}	10.1^{b}	0.049	0.001	0.021
Corn	9.9^{b}	0^{a}	10.0^{c}	0^{a}	9.9^{b}	0^{a}	0.001	0.138	0.138
Wheat	0^{a}	9.8^{c}	0^{a}	9.0^{b}	0^{a}	9.9^{c}	0.001	0.001	0.001
Canola meal	1.8	1.8	1.8	1.8	1.8	1.8	0.932	0.211	0.663
Minerals	0.2	0.2	0.2	0.2	0.2	0.2	0.939	0.467	0.542
Total	21.9^{b}	20.7^{a}	22.4^{b}	20.8^{a}	22.3^{b}	22.1^{b}	0.003	0.001	0.005
Crude protein	3.7	3.8	3.8	3.8	3.9	3.9	0.732	0.001	0.608
Neutral detergent fiber	5.6^{a}	5.6^{a}	5.8^{ab}	5.8^{ab}	6.0^{b}	5.8^{ab}	0.603	0.001	0.263
Starch	6.7^{b}	6.6^{ab}	6.6^{ab}	6.2^{a}	6.7^{b}	6.6^{b}	0.267	0.008	0.281
Fat	0.80	0.73	0.81	0.73	0.82	0.74	0.272	0.006	0.881
Grain, g/kg[5]	454^{b}	476^{c}	449^{ab}	432^{a}	445^{ab}	451^{b}	0.610	0.001	0.001
Milk yield, kg/cow/d	32.9^{bc}	35.2^{c}	30.8^{ab}	33.9^{bc}	29.9^{a}	34.3^{c}	0.047	0.001	0.090
ECM	31.7	27.6	29.5	29.5	28.2	30.3	0.722	0.777	0.001
Fat	1.24^{b}	0.80^{a}	1.15^{b}	1.02^{b}	1.09^{b}	1.07^{b}	0.066	0.171	0.001
Protein	1.03^{bc}	1.11^{c}	0.97^{ab}	1.07^{bc}	0.92^{a}	1.08^{c}	0.030	0.001	0.056

Lactose	1.71^{b}	1.83^{b}	1.61^{a}	1.79^{b}	1.58^{a}	1.81^{b}	0.033	0.017	0.136
Milk composition, g/kg									
Fat	37.9^{d}	22.9^{a}	37.6^{d}	30.2^{b}	36.7^{cd}	31.7^{bc}	0.002	0.004	0.001
Protein	31.3	31.6	31.4	31.6	30.7	31.5	0.562	0.210	0.421
Lactose	51.7	52.0	52.3	52.8	52.8	52.9	0.633	0.010	0.746
Body weight, kg	561^{a}	570^{ab}	576^{abc}	584^{abc}	588^{bc}	593^{c}	0.557	0.001	0.698

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

[1] CRN = corn diet, WHT = wheat diet; ECM = energy corrected milk.

Table 3.2.2 Influence of diet on methane emissions (CH_4), methane yield, methane intensity and methane emissions as percentage of gross energy intake (GEI) at weeks 4, 10 and 16 of the experiment

Parameter	Week 4		Week 10		Week 16		*P*-value		
	CRN[1]	WHT	CRN	WHT	CRN	WHT	TRT	Week	TRT × Week
CH_4, g/d	404^{b}	233^{a}	433^{b}	375^{b}	410^{b}	409^{b}	0.025	0.001	0.001
CH_4, g/kg DMI	18.4^{b}	11.2^{a}	19.3^{b}	17.9^{b}	18.3^{b}	18.3^{b}	0.040	0.001	0.001
CH_4, % gross energy intake	5.68^{b}	3.28^{a}	5.97^{b}	5.24^{b}	5.49^{b}	5.64^{b}	0.033	0.001	0.001

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

[1] CRN = corn diet, WHT = wheat diet

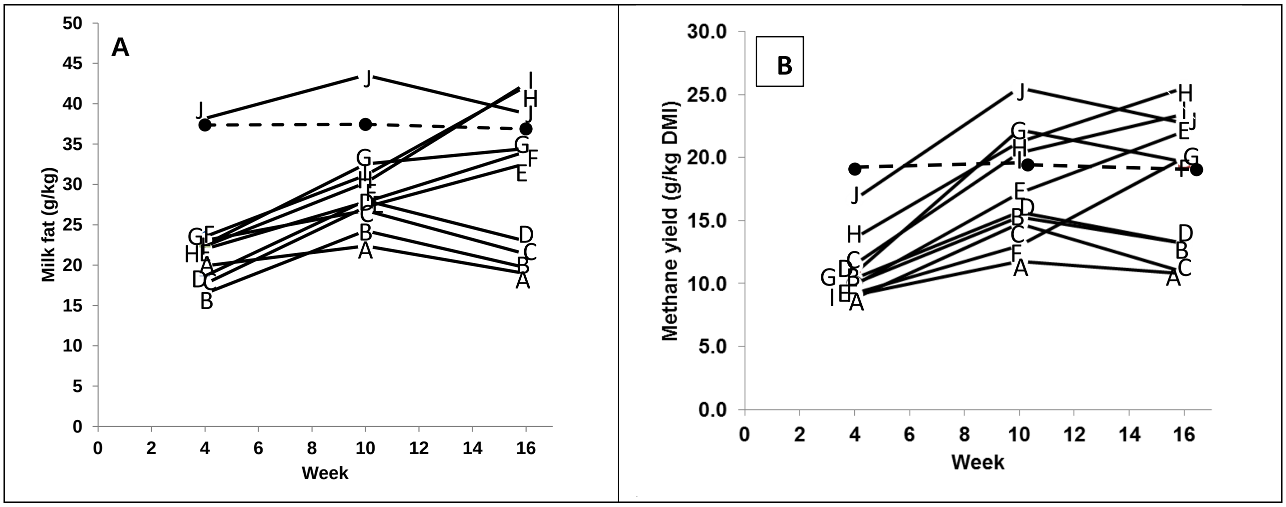


Figure 3.2.1 Responses in milk fat concentration (panel A) and methane yield (panel B), at week 4, week 10 and week 16. Data from individual cows fed the WHT dietary treatment are depicted by letters A to J. Cows A, B, C and D were considered “non-adaptive” while cows E, F, G, H and I were considered “adaptive”. The solid lines joining data are included to facilitate the depiction of the time course trajectories of individual cow responses over the experimental period. The (●) markers depict the means for cows fed the CRN treatment.

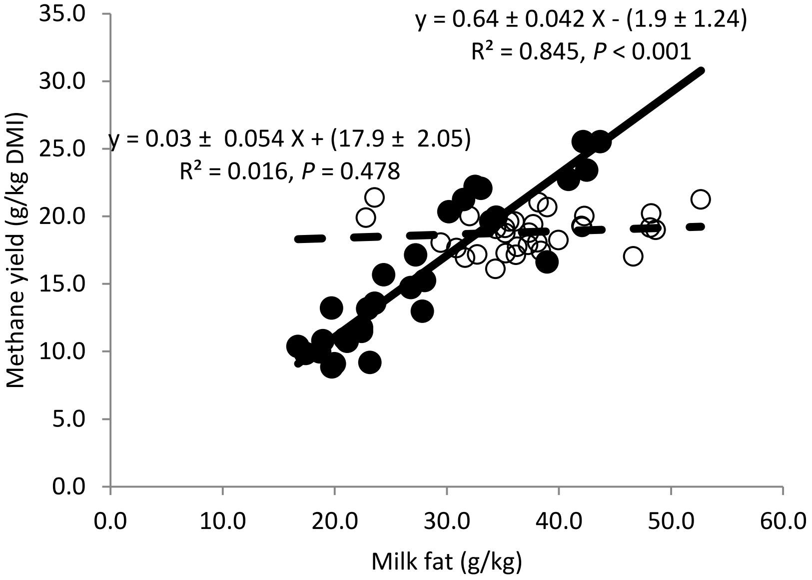


Figure 3.2.2 Relationships between milk fat concentration and methane yield for cows fed the CRN (○) and WHT (●) diets.

Discussion

This is the first report to compare over an extended period, the effects of feeding wheat based diets with corn based diets for effects on milk yield, milk fat yield, milk composition and methane emissions. In the short term (4 weeks), the inclusion of approximately 45% wheat instead of corn in the diet of cows resulted in a 39% reduction in methane yield, 35% reduction in milk fat concentration and 40% reduction in milk fat yield. However these inhibitory effects did not persist to week 10 or beyond. Our data suggest cows do not all respond in the same way with some “adaptive” cows showing a marked increase in methane yield, milk fat concentration and milk fat yield after week 4, while in other “non-adaptive” cows, these metrics were persistently inhibited to 16 weeks.

In the current experiment, the substantially lower milk fat concentration and milk fat yield at week 4 in cows fed the WHT diet compared to these metrics in cows fed the CRN diet are consistent with findings from Experiment 3.1 in which cows were fed similar diets. However, as far as we can ascertain, the adaptation response; i.e., the increase in milk fat concentration over time, and the recovery in milk fat yield, in cows fed a wheat based diet has not previously been reported in the scientific literature, and may partly explain why there has been variable findings in the scientific literature with respect to the effect of wheat feeding on milk fat concentration. At this juncture, we have no explanation why there is an association between milk fat concentration and methane yield in cows fed the WHT diet but not in cows fed the CRN diet, and this phenomenon requires further study. In addition, further research is required to elucidate why some cows fed a high wheat diet are adaptive and spontaneously recover from milk fat depression and low methane yield while other cows fed the same diet continue to exhibit milk fat depression and low methane yields for extended periods.

Experiment 3.3 Starch Sources and Oilseeds Effects on Methane/Milk Production (DEDJTR and U of M)

Results

Cows fed WHT consumed a diet that contained slightly more concentrate than cows fed the other diets (Table 3.3.1) However, cows fed CRN had lower DM intake than cows fed diets containing fat (CPF and WPF). Adding canola oil to the diet increased fat concentration by about 3 percentage points, for a total of approximately 6% dietary fat.

Milk yield and milk composition was not different between the CRN and WHT diets, but cows fed the CRN diet had less protein and lactose yield and greater milk fat concentration than cows fed the CPF diet, while there was no difference in these parameters between cows fed the WHT and WPF diets. Energy corrected milk and milk fat concentration were greater for cows fed WHT than for cows fed WPF.

Methane emission (g/d) and methane yield (g/kg of DM intake) or as a percentage of gross energy intake of cows fed the WHT diet were greater than for cows fed CRN. Feeding fat decreased methane emissions by 11% for cows fed the WPF diet, but there was no decrease in methane emissions associated with the CPF diet. Similarly, the fat in the WPF diet reduced methane yield by 12%, but the fat in the CPF diet reduced methane yield by just 5%.

Mean ruminal fluid pH was lower for cows fed WHT compared with those fed CRN, and feeding fat further lowered pH for both grain sources. Cows fed diets containing corn (CRN and CPF) had lower concentrations of total volatile fatty acids in ruminal fluid than cows fed diets containing wheat (WHT and WPF). Dietary supplementation with fat had no effect on volatile fatty acids. Cows fed diets containing corn (CRN and CPF) had greater ruminal fluid concentrations of acetate than cows fed wheat (WHT, WPF). Dietary fat supplementation decreased concentrations of acetate. Ruminal fluid from cows fed CRN had fewer total protozoa than that from cows fed WHT. There was no effect of fat supplementation on total protozoa.

Table 3.3.1 Key results from feeding wheat and corn with and without fat

Parameter	CRN	WHT	CPF	WPF	SED	*P*-value		
						Grain	Fat	Grain x Fat
DM intake, kg/d	20.7^{a}	21.3^{ab}	21.7^{b}	21.8^{b}	0.33	0.12	0.003	0.46
Grain, %	36.1^{ab}	37.4^{b}	35.5^{a}	36^{a}	0.59	0.07	0.02	0.37
Milk yield, kg/d	21.1^{a}	23.8^{ab}	26.1^{b}	24.9^{b}	1.25	0.57	0.002	0.05
Energy corrected milk, kg/d	23.2^{ab}	26.1^{b}	25.8^{b}	21.4^{a}	1.49	0.29	0.41	0.005
Milk composition, %								
Fat	4.91^{c}	4.80^{c}	3.99^{b}	3.04^{a}	0.175	<0.001	<0.001	0.005
Protein	3.41	3.40	3.29	3.18	0.118	0.41	0.05	0.56
Lactose	4.97^{b}	4.94^{ab}	5.04^{b}	4.78^{a}	0.087	0.02	0.49	0.10
Methane, g/d	524^{a}	637^{c}	523^{a}	569^{b}	19.2	<0.001	0.02	0.03
Methane, g/kg of DM intake	25.5^{a}	29.9^{b}	24.1^{a}	26.2^{a}	1.07	<0.001	0.004	0.17
Methane, % of gross energy intake	7.6^{a}	9.1^{b}	7.0^{a}	7.7^{a}	0.31	<0.001	<0.001	0.09
Mean pH	6.64^{c}	6.29^{b}	6.38^{bc}	6.01^{a}	0.12	<0.001	0.005	0.95
Total VFA, mM	97^{a}	128^{b}	104^{a}	129^{b}	8.4	<0.001	0.52	0.62
Acetate, %	68.8^{c}	66.5^{b}	68.3^{c}	64.1^{a}	0.87	<0.001	0.03	0.16
Propionate, %	16.9^{a}	17.5^{a}	16.5^{a}	20.4^{b}	1.33	0.02	0.20	0.12
Protozoa, 10^3 cells/mL	188^{a}	525^{b}	268^{ab}	363^{ab}	75.8	0.035	0.856	0.10

$^{a,b,c}P < 0.05$.

CRN = corn, WHT = wheat, CPF = corn plus fat, WPF = wheat plus fat, SED = standard error of the difference.

Discussion

Feeding cows a diet containing a high proportion of wheat grain resulted in greater daily methane emissions and methane yield than a diet containing a high proportion of corn, with no effect on DM intake, milk yield or milk composition. The results for methane production were not expected and differ from observations in our previous study (Moate et al., 2017). In that study cows fed wheat grain at 10 kg DM/d produced 33% less methane and had a 30% lower methane yield than cows fed the equivalent amount of corn. Previously, we attributed the lower methane production of cows fed wheat to its faster rate of ruminal starch degradability (Moate et al., 2017). Starch in wheat grain is known to be more quickly fermented in the rumen than starch in corn grain, thus the pH in the rumen of cows fed wheat is usually lower than that of cows fed other grains.

There are several factors that may explain why methane production of cows fed wheat in the present study was not lower than that of cows fed corn. Firstly, cows fed the CRN diet had a lower neutral detergent fiber intake than cows fed the WHT diet. It has previously been reported that decreasing fiber concentration in the diet leads to decreased methane production. Secondly, the cows fed CRN had a greater fat intake than cows fed WHT, and fat intake is associated with lower methane production. Thus, lower methane production from cows fed CRN compared with those fed WHT may in part be due to lower dietary NDF concentration and greater fat concentration. Thus, when considering methane mitigation from diets containing a high proportion of grain, the fiber and fat concentrations in the diet should be considered.

In the study by Moate et al. (2017) it was reported that minimum pH of the ruminal fluid was strongly associated with methane yield (g/kg DM intake) and that milk fat concentration also decreased with less methane production. Figures 3.3.1 and 3.3.2 illustrate our data combined with the data of Moate et al. (2017) and show that for these two parameters our data is consistent with the study by Moate et al. (2017). However, the minimum pH of the cows fed WHT in the present study was not as low as in the previous study. Thus, these results suggest that a decline in ruminal pH is needed for wheat to be a methane mitigation strategy.

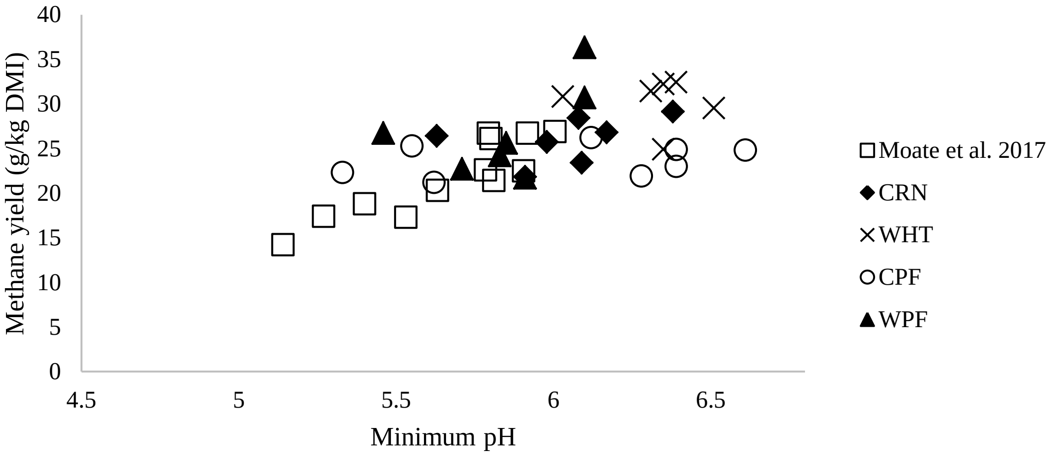


Figure 3.3.1 Relationship between minimum pH and methane yield from the cows fed the CRN (♦), WHT (x), CPF (○) and WPF (▲) diets and the experiment by Moate et al. 2017 (□)

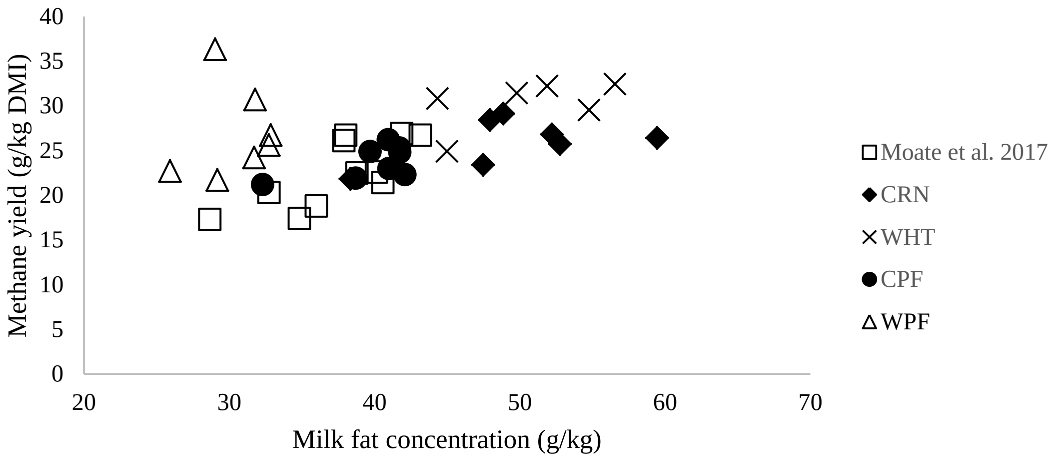


Figure 3.3.2 Relationship between milk fat concentration and methane yield from the cows fed the CRN (♦), WHT (x), CPF (○) and WPF (▲) diets and the experiment by Moate et al. 2017 (□)

Increasing the fat concentration of the diet reduced daily methane emissions and methane yield in cows fed wheat but not in cows fed corn. Thus, the effect of fat as a methane mitigation agent may depend on the composition of the diet. It has been reported that methane yield is reduced by 3 to 5% with each increase of 10 g/kg DM in dietary fat concentration (Beauchemin et al., 2008; Moate et al., 2011). For the WHT diet, an increase in dietary fat of 40 g/kg DM resulted in a 12.4% reduction in methane yield as expected (Moate et al., 2011). However, there was no effect on methane yield when dietary fat concentration was increased in the CRN diet. An important novel finding of this research is that the efficacy of fat supplementation for methane mitigation may be dependent on the type of grain in the basal diet. The differing effects of dietary fat on methane yield in the WHT versus CRN diets may be related to the effects of fat on ruminal fermentation and protozoal populations in the WHT versus CRN diet. This is supported by our results as ruminal fluid from cows fed the WHT diet contained a total protozoa count which was numerically, albeit not significantly (*P* > 0.05), greater than that of cows fed the WPF diet. Moreover, ruminal fluid from cows fed the WHT diet had a greater percentage of acetic acid and smaller percentage of propionic acid, and consequently greater A:P ratio than that of cows fed the WPF diet. However, for these parameters there were no differences between the CRN and CPF diets. We consider our results provide further evidence that the effectiveness of a fat supplement for mitigating methane emissions in lactating cows is dependent on the composition of the basal diet.

Our study was conducted on cows in late lactation. However, other authors have reported that in their studies methane emissions remained constant across the entire lactation (Cammel et al., 2000; Münger and Kreuzer et al., 2006) or increased until week 10 of lactation and then decreased (Garnsworthy et al., 2012). Thus, we propose that there is currently inadequate evidence to suggest that stage of lactation *per se* influenced our results. Perhaps the most likely explanation for why the WHT diet did not reduce methane emissions in comparison to the CRN diet relates to adaptation. Experiment 3.3, was conducted with cows fed similar diets to those in Experiment 3.2. Over the 16 weeks of Experiment 3.2, cows adapted to the WHT diet so that by the end of the experiment, there was no difference in methane yields of cows fed the CRN and WHT diets. In the current experiment, cows had been fed a high grain diet for 12 weeks prior to treatment allocation and a further 4 weeks before methane was measured. Thus, in the current experiment, adaptation of ruminal microorganisms to a high grain diet may have limited the inhibitory effect of wheat on ruminal fermentation parameters and methane response.

It is concluded that feeding fats to dairy cows reduces enteric methane emissions when cows are fed a diet containing wheat but not when cows are fed a diet containing corn. For wheat to reduce methane emissions, a substantial decline in rumen pH is required, however, this may be undesirable in terms of rumen health.

Experiment 4.1 *In vitro* Assessments of Additivity and Synergy of Mitigation Strategies (AAFC, DEDJTR, and U of M)

Results and Discussion: Ruminal degradation of starch sources

Wheat had faster rate of *in situ* degradation of crude protein, starch and organic matter compared with the other two grain sources. Ground wheat and barley produced less *in vitro* methane yield per unit of degradable DM than rolled wheat and barley. Ground wheat produced 25% less *in vitro* methane yield per unit of degradable DM with a higher pH than ground corn and was not significantly different from ground barley. It was concluded that the rate of starch degradability had a greater effect on methane production than starch concentration of the sample and that the results from *in situ* degradability were in agreement with the *in vitro* gas production rate and reduced methane production with increased grain processing.

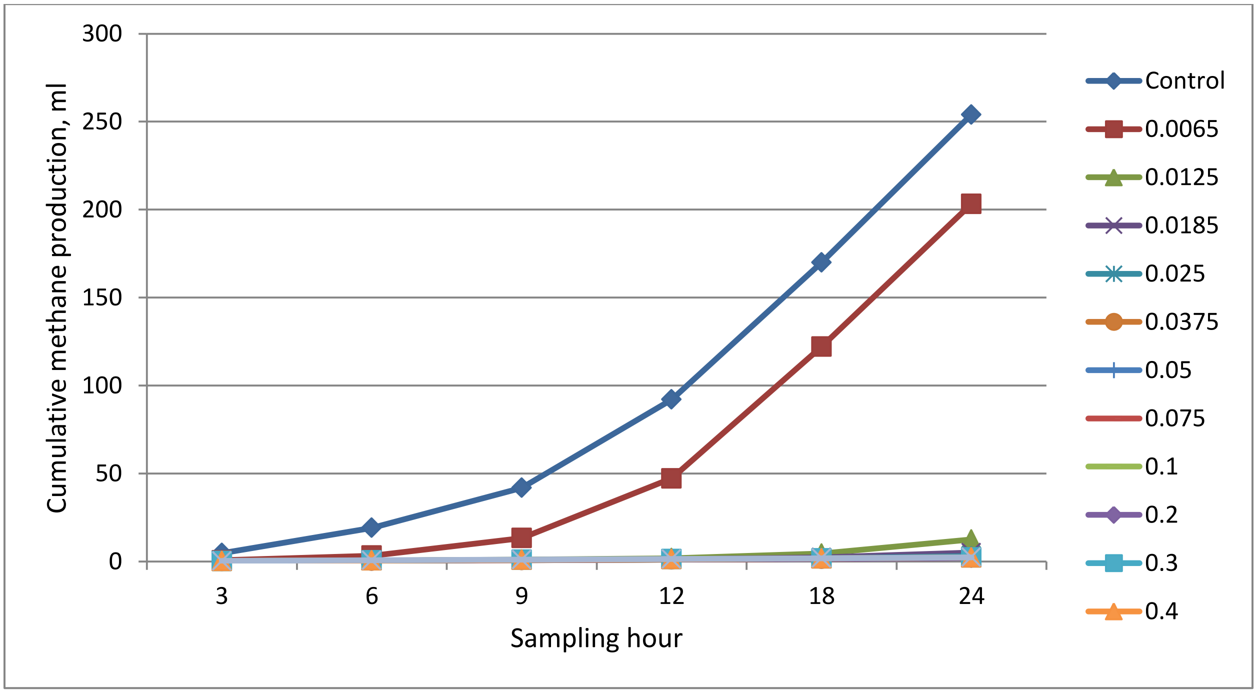
Results and Discussion: In vitro method development studies

A method involving collecting gas through a long (304 cm), narrow (1 mm internal diameter) gas line estimated greater gas production than a control method not involving collection of gas. A method involving collecting gas through a short (22 cm), wide (4 mm internal diameter) gas line did not affect head space methane percentage or estimates of gas production. It is concluded that *in vitro* methane production can be accurately estimated by using the Ankom system together with collection of vented gases into gas collection bags, but only if the Ankom system is connected to the gas collection bag via a short wide gas line.

A method that assumes constant methane to carbon dioxide ratio, a method developed from empirical data, and a method that assumes constant individual venting volumes had greater concordance (0.81 to 0.85), lower root mean square errors (0.72 to 0.85) and lower mean bias (-0.35 to 0.35,) compared with the gold standard method that collects and samples all of the gas produced. Based on precision, accuracy implementation, it is recommended that, when the gold standard methane cannot be used, other methods that can be used with good accuracy and precisions to estimate methane production from vented *in vitro* systems.

Results and Discussion: Dose of 3-nitrooxypropanol

All doses of NOP reduced methane production after 3 and 6 h of incubation, but only a dose ≥ 0.025 mM maintained substantial reduction in methane after 12 h of fermentation. The dose of 0.025 mM is equivalent to 120 mg/kg DM. A decrease in methane production was associated with an increase in hydrogen production.



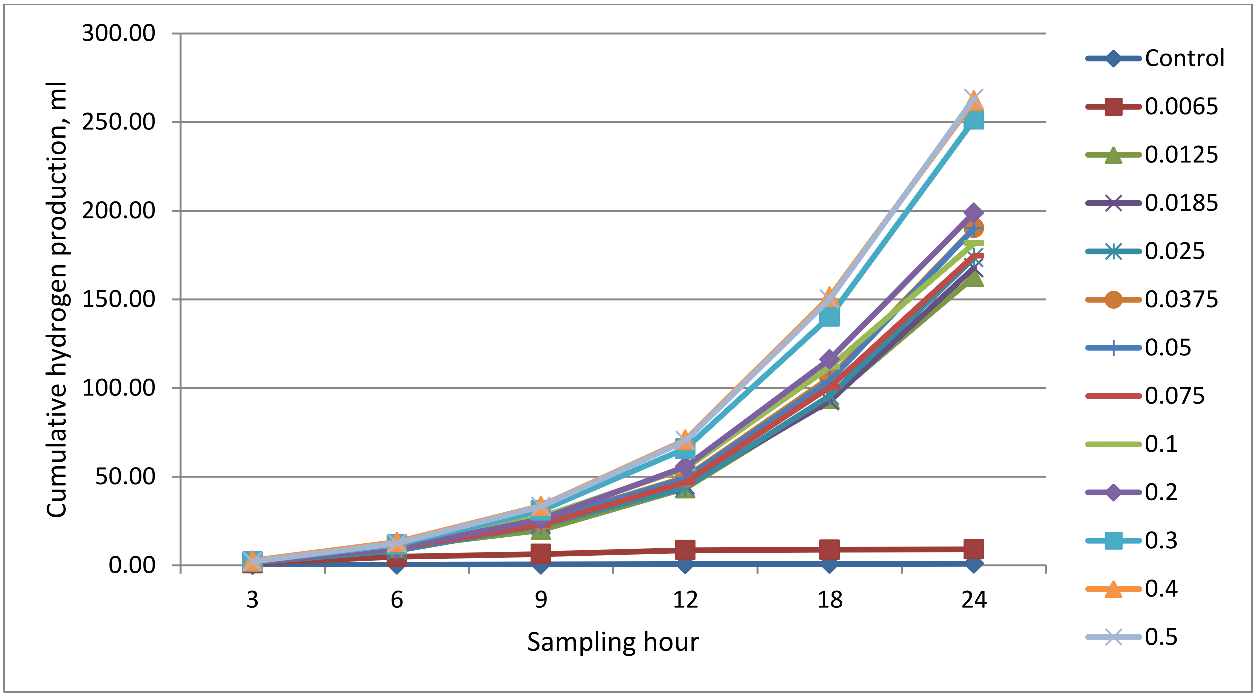


Figure 4.1.1 Effects of dose of NOP (mM) on *in vitro* methane (top) and hydrogen (bottom) production

Results and Discussion: NOP by diet interactions

3-Nitrooxypropanol reduced methane production by 52%, and there was no interaction between NOP and basal diet. NOP had no effect on ruminal fluid pH or DM degradation. The reduction in methane production due to NOP was within the range previously reported *in vivo* (Haisan et al., 2014; Hristov et al., 2015).

Results and Discussion: Release rate of encapsulated nitrate

Nitrate was released from encapsulated nitrate at a slower rate in rumen fluid compared with immediate solubilisation of un-encapsulated nitrate. Methane production was considerably lowered by un-encapsulated and encapsulated nitrate compared with urea, although the degree of methane mitigation was less for encapsulated nitrate. There were no negative effects of encapsulated nitrate on microbial substrate fermentation while un-encapsulated nitrate decreased total gas production, indicating that encapsulated nitrate was not toxic to microbes unlike un-encapsulated nitrate. Therefore, encapsulated nitrate was more efficiently metabolized without nitrate and nitrite accumulation and with less hydrogen and nitrous oxide production compared with un-encapsulated nitrate. These results indicate that encapsulation of nitrate lowers methane production, while decreasing the negative effects on digestion thereby reducing the potential for toxicity.

Results and Discussion: Mode of action of NOP and nitrate

Nitrate mostly decreased hydrogen availability by acting as an electron acceptor competing with methanogenesis. As a consequence, nitrate decreased methane production (−75%), dissolved dihydrogen concentration (−30%) and the percentages of reduced volatile fatty acids (butyrate, caproate and others) except propionate, but increased acetate molar percentage, ethanol concentration and the efficiency of microbial nitrogen synthesis (+14%) without affecting production of hydrogen gas. NOP decreased methanogenesis (−75%) while increasing both gaseous and dissolved hydrogen concentrations (+81% and +24%, respectively). NOP also decreased acetate and increased butyrate. Overall, NOP increased the amount of reduced volatile fatty acids, but part of hydrogen spared from methanogenesis was lost as hydrogen gas.

Experiment 4.2. Additivity and Synergy of Mitigation Strategies (AAFC)

Results

Animals fed oil were heavier by the end of the experiment likely due to the greater energy density of the diet and the greater intake of the oil-containing treatments (Table 4.2.1). NOP had no effect on digestibility, but feeding oil decreased DM and fiber (neutral detergent fiber) digestibility. Mean rumen pH was greater for NOP and oil compared with control, but not when NOP and oil were combined. Total volatile fatty acids in rumen fluid were lower for NOP and oil treatments compared with the control. Both NOP and oil decreased acetate proportion and increased propionate concentration compared with the control. Methane emissions corrected for dry matter intake (methane yield) was decreased by 25% for oil alone, 32% for NOP alone, and by 52% for NOP+Oil. Hydrogen gas emissions increased compared with the control when NOP was fed either alone or with oil.

Discussion

Adding fat to the diet to reduce methane emissions from cattle is a proven mitigation strategy. In the present study added fat alone reduced methane emissions by 25%, which is consistent with the expected reduction of 5% per 1% added fat. However, adding fat to forage based diets can decrease digestibility. The decrease in DM and fiber digestibility observed in the present study where fat was added at 5% of DM clearly demonstrates the potential negative effects of added fat. A decrease in fiber digestibility corresponds to a decrease in the efficiency of nutrient use from high-fiber feeds such as forages and by-products used in cattle diets.

Adding NOP to the diet also decreased methane production. The observed 32% reduction is consistent with observations from previous beef studies where forage based diets were fed (Romero-Pérez et al., 2014, 2015; Vyas et al., 2016). The decrease in methane resulted in an increase in hydrogen emissions, possibly indicating a lack of hydrogen sinks in the rumen. The study also showed that NOP did not decrease diet digestibility, which is consistent with previous studies (Romero-Pérez et al., 2014). Adding NOP to the diet altered fermentation in the rumen towards greater proportion of propionate (alternative hydrogen sink), and lesser proportion of acetate, as expected.

The novel finding of the study is that when oil was fed with NOP, the decrease in methane emissions (52% reduction), was greater than when either component was fed alone. Methane emission expressed as a percentage of gross energy intake (i.e., Ym) for the combination was 2.75%, which is similar to the expected Ym value for a high concentrate corn-based diet. Thus, the combination resulted in a substantial reduction in methane. However, the reduction in diet digestibility due to the oil was also observed when oil was combined with NOP, which would be a limitation of oil feeding. Thus, in commercial feeding operations, a smaller amount of added oil would be recommended (ie., 2 to 3% maximum) to avoid depression of digestibility.

We conclude that NOP can be used alone or with added fat to reduce methane production from cattle.

Table 4.2.1 Key results for the effects of feeding NOP with and without added fat in the form of canola oil

	Treatment[1]				SEM	*P*-value
	CON	OIL	NOP	NOP+OIL		
Body weight[2], kg	716^{b}	732^{a}	714^{b}	734^{a}	14.1	<0.0001
DM intrake, kg/d	7.19^{b}	7.35^{a}	7.08^{c}	7.31^{a}	0.03	<0.0001
Digestibility, %						
Dry matter, %	66.64^{a}	60.83^{b}	66.95^{a}	60.62^{b}	0.99	<0.0001
Neutral detergent fiber, %	58.97^{a}	48.28^{b}	60.96^{a}	46.82^{b}	1.25	<0.0001
Rumen pH, minimum	5.76^{b}	5.66^{bc}	5.91^{a}	5.61^{c}	0.05	<0.0001
Rumen pH, mean	6.49^{c}	6.53^{b}	6.57^{a}	6.48^{c}	0.05	<0.0001
Total volatile fatty acids, m*M*	101.3^{a}	94.8^{b}	94.8^{b}	88.3^{c}	3.13	<0.0001
Acetate, mol/100 mol	64.3^{a}	60.4^{b}	56.9^{c}	55.9^{c}	0.79	<0.0001
Propionate, mol/100 mol	17.6^{b}	20.4^{a}	20.5^{a}	20.8^{a}	0.44	<0.0001
Methane, g/d	191.1^{a}	140.2^{b}	124.9^{c}	93.2^{d}	9.31	<0.0001
Methane yield, g/kg of DM intake	26.24^{a}	19.57^{b}	17.88^{b}	12.69^{c}	1.23	<0.0001

Methane, % of gross energy intake[4]	5.94^a	4.21^b	4.11^b	2.75^c	0.27	<0.0001
Hydrogen emissions, g/d	0.00^c	0.09^c	1.11^a	0.61^b	0.12	0.001

[a-c] Least squares means within a row with different superscripts differ ($P < 0.05$).

[1] Treatments: CON= control, OIL= canola oil, NOP= 3-nitrooxypropanol, NOP+OIL= 3-nitrooxypropanol and canola oil.

Experiment 5.1. Conduct modeling studies to determine the broader potential for greenhouse gas reductions using low methane diet technologies, as applicable to Alberta and Victorian dairy farms (U of M and AAFC)

Results and Discussion

The methane reductions from the studies conducted using NOP and nitrate were used to evaluate the net impact on whole farm GHG emissions, the GHG intensity of meat and milk production, and profitability for Australian and Canadian dairy and beef farms. The scenarios show the profitability of adopting these strategies for GHG abatement, and do not account for additional revenue that would occur due to an increase in animal performance. Two carbon prices were assumed: $11.82 (current price in Australia) and $20 per tonne of CO_2 abated. However, as neither NOP nor nitrate is commercially available in Canada as a feed supplement, hypothetical prices were used so that a net revenue for carbon abatement could be calculated. The price (per kilogram) of NOP was estimated at $30/kg, and nitrate was estimated at $0.10/kg (as the incremental cost of substituting urea nitrogen with calcium nitrate nitrogen). It is recognized that the prices of these compounds would need to be adjusted if a full economic assessment was conducted. However, the intent of this exercise was to demonstrate the potential additional revenue/loss that may be possible from carbon abatement for beef and dairy farmers.

Results and Discussion: Canadian dairy farm – 3-nitroxypropanol and nitrate

Table 5.1.1 shows the net GHG abatement per year estimated for a Canadian dairy farm (by feeding NOP or nitrate to the milking cows only or to the entire herd. Total reduction in the GHG intensity of milk leaving the farm gate by feeding NOP was 13% and 18% respectively for the two scenarios. For nitrate, the reduction in GHG intensity was 3% and 4%, respectively. At both carbon prices, neither product was profitable. Thus, additional value from feeding NOP or nitrate in terms of milk production or milk composition would be needed to breakeven.

Table 5.1.1 Effect of enteric methane mitigation using 3-nitrooxypropanol (NOP; 80 mg/kg DM intake) and nitrate (21 g/kg DM intake) on whole farm GHG emissions and intensity (kg CO_2/kg fat and protein corrected milk), and gross revenue from carbon abatement using a carbon price of \$11.82 and \$20.00 per tonne of CO_2e for a Canadian dairy farm. NOP or nitrate was fed to either the lactating cows or the entire herd. Hypothetical cost of NOP was \$30/kg and incremental cost of substituting urea with calcium nitrate was assumed to be \$0.10/kg of nitrate.

	Control	NOP		Nitrate	
		Milking cows only	Entire herd	Milking cows only	Entire herd
t CO_2e/year					
Enteric CH_4	431	326	293	378	362
Manure CH_4	124	124	124	124	124
Direct N_2O	76	76	76	76	76
Indirect N_2O	33	33	33	33	33
Energy CO_2	33	33	33	33	33
Pre-farm emissions	62	64	65	88	97
Total CO_2e	758	656	623	732	725
Net abatement	0	103	135	26	33
GHG intensity, kg CO_2e/kg milk	1.21	1.05	0.99	1.17	1.16
Gross revenue from GHG reduction (\$11.82/t)					
Total, \$/year	0	1214	1597	311	394
Per cow, \$/cow/d	0	0.034	0.018	0.009	0.004
Profit (\$30/kg NOP), \$/cow/d	0	-0.002	-0.002	-0.033	-0.019
Gross revenue from GHG reduction (\$20/t)					
Total, \$/year	0	2054	2702	526	667
Per cow, \$/cow/d	0	0.057	0.031	0.015	0.008
Profit (\$30/kg NOP), \$/cow/d	0	-0.035	-0.020	-0.041	-0.023

Results and Discussion: Australian Dairy Farm – 3-nitroxypropanol

Emissions produced by the Australian dairy farms are shown in Table 5.1.2. The GHG intensity of milk production (kg CO_2/kg fat and protein corrected milk) was 1.09 for the average herd (340 cows) and 0.97 for the high-producing herd (500 cows). Feeding NOP to either herd reduced overall farm GHG emissions by 13.0% if NOP was fed for the entire lactation of 300 days, or by 6% if fed only for the first 120 days. Thus, feeding NOP has potential to generate additional revenue from carbon abatement. Factoring in the cost of feeding NOP, the profit (per cow per day) was \$0.014 to \$0.020 for a high carbon price, depending on farm size and how long the product is fed. At a low carbon price (\$11.82/t CO_2e abated), NOP was only profitable in the high producing farm when fed to the dairy cows during 120 days of peak lactation.

Table 5.1.2 Effect of enteric methane mitigation using 3-nitrooxypropanol (NOP, 200 mg/kg DM intake) on whole farm GHG emissions per year and intensity (kg CO_2/kg fat and protein corrected milk), and potential profit with a carbon price of \$11.82 and \$20.00 per tonne of CO_2e abated for Australian dairy farms. Scenarios include an average producing farm (340 cows) and a high producing farm (500 cows), with NOP fed to lactating cows for 300 or 120 days/year. Hypothetical cost of NOP was \$30/kg.

	Average farm (340 cows)			High-producing farm (500 cows)		
	Control	NOP for 300 d	NOP for 120 d	Control	NOP for 300 d	NOP for 120 d
t CO_2e/year						
Enteric CH_4	927	725	840	1509	1168	1361
Manure CH_4	133	133	133	218	218	218
Direct N_2O	136	136	136	210	210	210
Indirect N_2O	66	66	66	123	123	123
Energy CO_2	175	175	175	293	293	293
Pre-farm emissions	71	76	73	202	210	205
Total GHG	1,508	1,310	1,423	2,556	2,222	2,410
Net abatement	0	198	85	0	334	145
Milk production, kg/year	1,384	1,384	1,384	2,625	2,625	2,625
GHG intensity, kg CO_2e/kg milk	1.09	0.95	1.03	0.97	0.85	0.92
Gross revenue from GHG reduction (\$11.82/t)						
Total, \$/year	0	2,336	999	0	3,949	1,719
Per cow, \$/cow/d	0	0.023	0.024	0	0.026	0.029
Profit (\$30/kg NOP), \$/cow/d	0	-0.002	-0.001	0	-0.002	0.001
Gross revenue from GHG reduction (\$20/t)						
Total, \$/year	0	3,952	1,690	0	6,681	2,908
Per cow, \$/cow/d	0	0.039	0.041	0	0.045	0.048
Profit (\$30/kg NOP), \$/cow/d	0	0.014	0.016	0	0.016	0.020

Results and Discussion: Canadian beef farm - 3-nitrooxypropanol

Emissions from a Canadian beef farm are shown in Table 5.1.3. Feeding NOP to the backgrounded cattle reduced the GHG intensity of beef production by 2.4%, while feeding NOP to both the backgrounders and finishers reduced it by 5.8%, and feeding NOP to the growing stock as well as to the cow herd for 6 months reduced GHG intensity by 19%. Feeding NOP to beef cattle for GHG abatement was profitable with a high carbon price but not with a low carbon price. At the higher carbon price, the profit was \$28/year if NOP was fed only to the backgrounding cattle, or \$554/year if fed to the entire herd. The observed 3% to 5% improvement in gain:feed ratio observed in our studies would further increase profitability. Gross cost of production savings is estimated at $15-20/animal finished, assuming NOP was fed throughout the backgrounding and finishing periods. Factoring in the cost of NOP, the net profit would be $3 to $8/animal marketed.

Table 5.1.3 Effect of enteric methane mitigation by feeding 3-nitrooxypropanol (NOP) (200 mg/kg DMI) and monensin (MON) (33 mg/kg DMI) on whole farm GHG emissions (t CO_2e), GHG intensity (kg CO_2/kg liveweight), and potential profit at a carbon price of $11.82 and $20.00 per tonne of CO_2e abated for a Canadian beef farm. NOP was fed to the backgrounding group (B), finishing group (F), backgrounding and finishing groups (BFC), and backgrounding and finishing groups plus adult breeding cows for six months of the year (BFC). Hypothetical cost of MON was $30/kg.

Item	Control	NOP			
		B	F	B+F	B+F+C
t CO_2e/year					
Enteric CH_4	529.4	510.2	502.0	482.8	375.1
Manure CH_4	39.7	39.7	39.7	39.7	39.7
Direct N_2O	157.2	157.0	156.7	156.5	155.0
Indirect N_2O	29.5	29.4	29.3	29.3	28.9
Energy CO_2	60.5	60.1	59.7	59.2	56.0
Pre-farm emissions	0.0	0.7	0.8	1.4	4.1
Total GHG	816.3	797.0	788.3	768.9	658.8
Net abatement	0.0	19.3	28.0	47.4	157.4
GHG intensity (kg CO_2/kg liveweight)	15.2	14.8	14.6	14.3	12.2
Gross revenue from GHG reduction/year					
Carbon, $11.82/t	0.0	228.0	330.7	559.9	1,861.1
Profit, $/year	0.0	-129.7	-192.3	-320.9	-733.8
Carbon, $20/t	0.0	385.8	559.6	947.3	3,149.0
Profit, $/year	0.0	28.1	36.6	66.6	554.1

Results and Discussion: Canadian beef farm – nitrate

The analysis of feeding nitrate to Canadian beef farms for methane abatement is shown in Table 5.1.4. Because the net abatement was small, the reduction in GHG intensity was also relatively small (maximum of 3.7%, when fed to the entire herd). Thus, nitrate feeding for methane abatement would only be profitable if the price of carbon continues to rise well above $20/t, or if the cost of feeding nitrate decreases. Thus, this strategy is not economically feasible solely for methane abatement under current market conditions, but may become feasible in the future. As nitrate has nutritional value because it is a source of non-protein nitrogen, and thus can be used to replace urea, or slow release urea in the case of encapsulated nitrate, a more comprehensive economic assessment is warranted. This would need to take into account the additional benefits to the animal of using encapsulated nitrate, such as improve feed conversion efficiency of finishing cattle.

Table 5.1.4 Effect of enteric methane mitigation through nitrate feeding on whole farm GHG emissions (t CO_2e) and intensity (kg CO_2/kg live weight), potential gross revenue from GHG abatement using carbon prices of \$11.82 and \$20.00 per tonne of CO_2e for a Canadian beef farm over an 8-year cycle. Nitrate was fed at a rate of 18.7 g/kg DM intake to the backgrounders (B), finishers (F), the backgrounding and finishing cattle and the backgrounders, finishers and adult breeding cows (C) for six months of the year. Incremental cost of substituting urea with calcium nitrate was assumed to be \$0.10/kg of nitrate.

GHG source		Nitrate Feeding			
	Control	B	F	B + F	B+F+C
t CO_2e/year					
Enteric CH_4	529	523	519	513	478
Manure CH_4	40	40	40	40	40
Direct N_2O	157	157	157	157	157
Indirect N_2O	29	29	29	29	29
Energy CO_2	60	60	60	60	60
Pre-farm	0	3	7	10	22
Total GHG	816	813	811	808	786
Net abatement	0	3	5	8	30
GHG intensity, t CO_2e/kg beef	15.15	15.10	15.06	15.01	14.59
Gross revenue from GHG reduction/year					
Carbon, \$11.82/t	0	33	59	92	355
Profit/loss, \$/year		-164	-348	-512	-951
Carbon, \$20/t	0	56	100	155	601
Profit/loss, \$/year		-141	-308	-449	-705

Results and Discussion: Australian beef farm – 3-nitrooxypropanol

The analysis of feeding NOP to the Australian beef farm for methane abatement is shown in Table 5.1.5. Feeding NOP to the young growing stock only reduced GHG intensity by 4.5%, feeding NOP to the older growing stock only reduced GHG intensity by 5.7%, feeding NOP to all growing stock reduced GHG intensity by 10.2% and feeding NOP to all growing stock plus adult cows reduced GHG intensity by 29.8%. In this farm, feeding NOP to beef cattle was only profitable at a high carbon prince ($20/t CO_2e abated) and when fed to the all growing stock plus cows.

Table 5.1.5 Effect of feeding 3-nitrooxypropanol (NOP, 200 mg/kg DM intake) on whole farm GHG emissions GHG intensity (kg CO_2/kg liveweight), and gross revenue at a carbon price of $11.82 and $20.00 per tonne of CO_2e abated for an Australian beef farm. NOP was fed to young growing animals (6-12 months), older growing animals (2-18 months), all growing animals (6-18 months) and all growing animals plus adult breeding cows. Hypothetical cost of NOP was $30/kg.

	Control	NOP			
		Young growing stock only	Older growing stock only	All growing stock	All growing stock plus cows
t CO_2e/year					
Enteric CH_4	1,581	1,479	1,455	1,352	929
Manure CH_4	2	2	2	2	2
Direct N_2O	511	511	511	511	511
Indirect N_2O	0.15	0.15	0.15	0.15	0.15
Energy CO_2	64	64	64	64	64
Pre-farm emissions	0.0	3.5	4.2	7.7	9.7
Total GHG	2,159	2,060	2,036	1,938	1,516
GHG intensity	15.37	14.67	14.50	13.80	10.80
Net abatement	0	99	123	221	643
Gross revenue from GHG reduction/year					
Carbon, $11.82/t	0	1,166	1,450	2,616	7,595
Profit,$/year	0	-1,041	-1,187	-2,227	-3,801
Carbon, $20/t	0	1,973	2,453	4,426	12,852
Profit, $/year	0	-234	-183	-417	1,455

Results and Discussion: Australian beef farm – nitrate

The analysis of feeding nitrate to the Australian beef farm for methane abatement is shown in Table 5.1.6. Feeding nitrate to the young growing stock only reduced GHG intensity by 1.5%, feeding nitrate to the older growing stock only reduced GHG intensity by 1.9%, feeding nitrate to all growing stock reduced GHG intensity by 3.3% and feeding nitrate to all growing stock plus adult cows reduced GHG intensity by 9.6%. Due to a relatively low net abatement and high dose of nitrate required, feeding nitrate to cattle was not profitable at either carbon price.

Table 5.1.6 Effect of enteric methane mitigation through nitrate feeding on whole farm GHG emissions (t CO_2e) and intensity (kg CO_2/kg live weight), potential gross revenue from GHG abatement using carbon prices of $11.82 and $20.00 per tonne of CO_2e for an Australian beef farm. Nitrate was fed at a rate of 18.7 g/kg DM intake to young growing animals (6-12 mo), older growing animals (2-18 mo), all growing animals (6-18 mo) and all growing animals plus adult breeding cows. Incremental cost of substituting urea with calcium nitrate was assumed to be $0.10/kg of nitrate.

		Nitrate			
	Control	Young growing stock only	Older growing stock only	All growing stock	All growing stock plus cows
t CO_2e/year					
Enteric CH_4	1,581.5	1,549.0	1,541.1	1,508.7	1,374.1
Manure CH_4	2.1	2.1	2.1	2.1	2.1
Direct N_2O	511.5	511.5	511.5	511.5	511.5
Indirect N_2O	0.1	0.1	0.1	0.1	0.1
Energy CO_2	63.9	63.9	63.9	63.9	63.9
Pre-farm emissions	0.0	0.3	0.3	0.5	0.5
Total GHG	2,159.0	2,126.8	2,119.0	2,086.8	1,952.2
Net abatement	0.0	32.2	40.1	72.3	206.8
GHG intensity, t CO_2e/kg beef	15.37	15.14	15.09	14.86	13.90
Gross revenue from GHG reduction/year					
Carbon, $11.82/t	0	381	473	854	2,445
Profit/loss, $/year	0	-529	-625	-1,154	-2264
Carbon, $20/t	0	644	801	1,445	4,137
Profit/loss, $/year	0	-265	-298	-563	-572

General Discussion

The study examined the effectiveness and feasibility of various dietary mitigation strategies to reduce enteric methane from beef and dairy production. The enteric methane reduction technologies investigated were: 1) diet composition (feeding wheat and added fat), and 2) use of feed additives (3-NOP and nitrate). Some of these strategies were examined in combination. The critical findings from the studies are discussed below.

Wheat as a dietary mitigant of enteric methane emissions

Experiment 3.1 showed that in the short term (5 weeks), when lactating dairy cows were fed a diet containing 44% wheat, they produced substantially less methane (g/day) than cows fed similar amounts of either barley or corn and had substantially smaller methane yields (methane adjusted for DM intake) than cows fed similar amounts of barley or corn. Experiment 3.2 confirmed the findings of Experiment 3.1 in that in the short term, i.e. at week 4 of feeding, cows fed a diet containing 45% wheat produced approximately 42% less methane than cows fed a diet containing corn. However, by week 10, the methane mitigation effect was much reduced and by week 16, cows fed the diet containing wheat produced numerically identical amounts of methane as cows fed the diet containing corn. An important finding of this experiment was that over the 16 weeks of the experiment, approximately half of the cows adapted to the wheat diet by steadily increasing their daily emissions of methane, while the remaining cows fed wheat did not adapt to the diet and continued with very low methane emissions out to 16 weeks.

Experiment 3.3 involved late lactation dairy cows fed one of four dietary treatments over a 5 week period. Two of the diets contained approximately 40% of either wheat or corn without any canola oil, while a third diet involved 40% wheat diet plus 0.8 kg/cow/day of canola oil and the fourth diet contained 40% corn plus 0.8 kg/cow /day of canola oil. Unexpectedly, methane emissions and methane yields of cows fed the diets containing wheat were numerically greater than those of cows fed the diets containing corn. The cows in this experiment had all been fed on a high wheat diet for many weeks prior to the experiment. Thus it seems likely the lack of a methane inhibitory effect due to wheat feeding in Experiment 3.3 may be explained by adaptation as was seen at week 16 in Experiment 3.2.

The findings from Experiment 3.1 suggested that wheat feeding to dairy cows had potential as a nutritional strategy for methane mitigation. However, Experiment 3.2 showed that the methane mitigation effect due to feeding wheat to dairy cows is transitory. The findings from Experiment 3.3 support the conclusion that feeding of wheat to dairy cows should not be considered as a reliable strategy for the long-term mitigation of enteric methane emissions from dairy cows. Although this conclusion is disappointing, it is nonetheless, very important because it means that this strategy will not be erroneously adapted by farmers for this purpose, nor will this strategy be erroneously recognised by governments as a strategy that farmers could employ to gain carbon credits. Nevertheless, in both Australia and Canada, the feeding of wheat to dairy cows and feedlot cattle will continue to be a common practice depending upon relative prices of grain. It can be expected that when wheat is fed to dairy cows or feedlot cattle for a short period, methane mitigation will probably occur. Future research could investigate whether the periodic alternation of feeding diets containing entirely forages with diets containing approximately 45% wheat may be a useful strategy to mitigate methane emissions from cattle. The finding in Experiment 3.2 of a cohort of non-adaptive, low-methane emitting cows suggests that the identification of non-adaptive, low-methane emitting dairy cows may be an important area for future methane mitigation research.

Fat as a dietary mitigant of enteric methane emissions

Feeding fat (edible lipids derived mainly from plants) is a recognized methane mitigation approach in Alberta (Government of Alberta, 2016). Thus, the project examined the potential combined effects of feeding fat with the new strategies of wheat (Experiment 3.3) and NOP (Experiment 4.2). In both studies, canola oil was used as the source of fat. In Experiment 3.3 adding fat to a dairy cow diet containing about 3% fat increased the dietary fat content to about 6%. Added fat reduced methane emissions and methane yields of cows fed a diet containing wheat, but not when the diet contained corn. These contrasting results indicate that there are interactions between grain source and added fat. The identification of interactions between grain sources and added fat has not previously been reported, and may have important implications for the quantification protocol for reducing greenhouse gas emissions from fed cattle in Alberta (Government of Alberta, 2016).

Experiment 4.2 showed that the effects of oil and NOP were independent, and that NOP was effective regardless of fat concentration of the diet. Adding 5% fat to the diet reduced methane production by 25% as expected. However, in-depth measurements showed that the reduction in methane was partly attributed to a reduction in fiber digestibility, which is undesirable because it demonstrates a decrease in efficiency of energy use and an increase in manure excretion. Thus, additional energy added to the diet in the form of fat may not lead to an increase in animal performance because of a decrease in the digestible energy concentration of the basal diet.

We conclude that added fat is an effective methane mitigation strategy for wheat-based diets, but adding high levels of fat to a forage-based diet may reduce fiber digestibility in the rumen. Thus, when using fat as a methane mitigation strategy, it is critical to measure the total fat concentration of the diet to ensure that it does not exceed 5% of the diet DM.

3-Nitrooxypropanol to mitigate enteric methane emissions

The research built on the past work that identified NOP as a potential methane mitigation strategy. Previous studies showed NOP reduced methane production in short term studies by about 30 to 50%. One longer term 4-month study had shown that the methane reduction was sustained over time. Experiment 1.1 examined the optimum dose of NOP in feedlot backgrounding and finishing diets. The study showed that 100 to 200 mg/kg DM was effective in decreasing methane production in steers fed high-forage and high-grain diets, without inducing negative effects on DM intake. The results from the study were used to establish the dose rates used in the subsequent feedlot study. Experiment 1.2 was a long-term feeding study that used diets and conditions similar to those of commercial feedlots that background and finish cattle. The dose rates of NOP were (mg/kg DM) 200 in the backgrounding phase and 125 in the finishing phase. In both phases, NOP lowered methane yield by approximately 40%, which is an important finding confirming that the methane reduction reported in previous studies is maintained over the longer term.

Furthermore, the study examined potential interactions between NOP and ionophores (monensin). Monensin is a feed additive that is routinely used in most feedlots in Canada. The study showed that the reduction in methane using NOP was not affected by whether MON was included in the diets.

Another important finding of the study is that NOP improved feed conversion efficiency in both the backgrounding (by 5%) and finishing phases (by 3%), and this improvement in feed conversion was incremental to that of monensin. Thus, the study showed additional revenue from improved cattle performance may be possible when feeding NOP. A 3% improvement in backgrounding phase feed conversion efficiency would result in a gross cost of production savings of approximately \$4/animal using current feed costs, while a 5% improvement in feed conversion efficiency during the finishing period would result in a gross cost of production savings of approximately \$15/animal. As NOP is not commercially available, the price of NOP is unknown, and thus the cost-effectiveness of feeding NOP to reduce methane emissions and improve feed conversion efficiency is unknown. The modeling conducted under Experiment 5 showed that if carbon is priced at \$20/t of CO_2 abated and NOP is priced at \$30/kg, cattle producers that participate in the Carbon Offset Market could make a profit from methane reduction that would be additional to profit from improved animal performance. At the current price of carbon abatement in Alberta (\$15/t) or Australia (\$11.83), the cost of feeding NOP for methane reduction is likely not cost-effective if used strictly for carbon offset. However, at a carbon price of \$20/t, feeding NOP for methane reduction may be profitable, depending upon the price of NOP.

We conclude that NOP is a potent methane inhibitor that can be added to conventional feedlot diets containing MON without incurring negative effects on performance or carcass characteristics. Furthermore, the study suggests that a sustained reduction in methane using NOP may lead to improved feed conversion efficiency. Based on the results from this study, a large scale pilot study to evaluate NOP is in progress in

Alberta. The outcome of that study will be critical for assessing the cost-effectiveness of this technology as well as for registration of the product in Canada, giving Canadian beef producers first access to this methane mitigation technology. On the basis of the present research we conclude that feeding NOP has great potential as a Carbon Offset Protocol.

Nitrate as a methane mitigant

Feeding nitrate (maximum of 3% of the dietary DM) was shown in earlier short-term studies to reduce methane emissions, while providing a source of non-protein nitrogen (similar to urea). In terms of commercial application of using nitrate one major challenge is that it can be toxic if high concentrations are fed or if animals are not adapted beforehand. However, we did not incur any nitrate toxicity in our studies. To prevent toxicity, nitrate was introduced gradually by increasing the dose over an adaptation period. We also showed that encapsulated nitrate slows rate of nitrate availability in the rumen, which reduces the possibility of toxicity. The 10 (finishing phase) to 12% (backgrounding phase) reduction in methane production with nitrate feeding observed in Experiment 2.2 was somewhat less than expected based on short term studies with nitrate (20% reductions observed previously), and the lack of significant effect of nitrate on methane emissions in Experiment 2.1 was completely unexpected. The variability in the results of feeding nitrate to reduce methane highlights the potential inconsistent effects when nitrate is fed over the longer term. It is possible that the rumen microbiome may adapt to nitrate over time thereby lessening it methane mitigation potential.

The modeling studies showed when nitrate replaced urea in the diet of dairy and beef cattle, the additional cost of nitrate would not be compensated for by the revenue from carbon abatement at a carbon price ≤ \$20/t CO_2. However, if encapsulated nitrate was used rather than slow release urea, there would be no additional cost of using this methane abatement strategy, as the price of these two nitrogen sources is about the same. Any additional revenue from improvements in animal performance would further offset the cost of feeding nitrate. In Experiment 2.1 cattle fed the high level of nitrate had a substantial 11% increase in gain:feed ratio, which was confirmed in Experiment 2.2 with a 10% increase in gain:feed ratio. This increase in efficiency may partly be explained by the lack of ionophores or antibiotics used in the diet to control digestive disorders. Additional studies that include nitrate and ionophores would be needed to valid this hypothesis. However, nitrate might be an attractive option in a feeding program that does not use antibiotics.

Feeding nitrate is not recommended for further development as a Carbon Offset Protocol for methane abatement in Alberta because of the relatively small effects on methane mitigation observed in these long-term feeding studies relative to the increased risk management required. However, encapsulated nitrate is an effective means of supplying non-protein nitrogen in cattle diets and increasing feed conversion efficiency of finishing cattle in a natural feeding program that does not use antibiotics. Because the rate of availability in the rumen of encapsulated nitrate is much slower than that of urea, it could be used to partially or fully replace slow-release urea.

Important Lessons Learned

Conducting long term studies using conditions similar to those of commercial production systems is necessary to identify methane mitigation strategies. Short-term studies can be useful as they provide an initial screening, but long term studies are needed to show whether the reduction in methane is maintained over time. Our work shows that with some dietary strategies (e.g., wheat), the mitigation effects can dissipate over time.

There are differences in how individual animals respond to dietary methane mitigation strategies. The intent of feeding studies is to identify strategies that reduce the average methane production from a group of cattle. However, the variation among animals could be explored in the future to understand why some animals produce more/less methane, and why some animals adapt over time while others don't. This knowledge may help scientists better understand the methane production process, and the possibilities for abatement.

Whole farm modeling is helpful for assessing the net impact of a change in diet to reduce methane emissions on the total GHG emissions from producing meat and milk. Including all contributing GHG emissions in the calculations, including those from feed production and digestion, manure, and other on-farm production processes and inputs, helps identify best management practices for methane reduction that don't inadvertently increase emissions elsewhere in the system.

Collaboration between scientists in Canada and Australia was a very effective approach because it brought new perspectives to each team. Due to limited capacity in both countries, we were able to effectively double the number of experiments possible in the timeframe. The project developed future capacity and capability through post graduate students. In addition, the international collaboration led to greater scientific output from both countries.

Greenhouse Gas and Non-GHG Impacts

The reduction in GHG emissions (t of CO_2e) arising directly from the project is estimated at 111 t (Table 6.1). This was calculated based on the number of animals, the duration of feeding, and the net daily reduction in methane measured per animal.

From this project we recommend NOP as a methane mitigation strategy. The reduction in GHG from adoption of NOP by beef feedlots in Canada during backgrounding and finishing phases was calculated using some basic assumptions. The first assumption is that the number of cattle in Canada and in Alberta remains relatively constant in the next 10 years. Table 6.2 shows the estimated number of cattle currently backgrounded (663,300) and finished (1,838,800) in Alberta and backgrounded (1,345,100) and finished (2,974,400) in Canada.

The second assumption is, based on the results from our feeding studies, feeding NOP reduces methane emissions by 40%. For a backgrounding animal the reduction in methane would be about 64 g/d, which is 23.36 kg/year of methane (0.064 kg x 365 d), or 0.584 t CO_2e/year (23.36 kg/year x 25 global warming potential/1000). Similarly, for a finishing animal the reduction in methane would be about 80 g/d, which is 29.2 kg/year of methane, or 0.73 t CO_2e/year. NOP is expected to be commercially available in Canada in 2019. We assumed a 10% adoption rate in the first 5 years and a 20% adoption rate in the following 10 years. It should be noted that the adoption rate may be greatly under- or over-estimated depending upon the commercial price of NOP, which is presently unknown. The net reduction in CO_2e over the 10 year period is in Canada for backgrounding cattle is estimated at 1,178,308 t (0.584 t CO_2e/year x 1,345,100 x 0.10 x 5 years) + (0.584 t CO_2e/year x 1,345,100 x 0.20 x 5 years). For finishing cattle the net reduction in CO_2e over the 10 year period is 3,256,968 t CO_2e. Thus total reduction for Canada over the 10 years is estimated at 4,435,276 t CO_2e. For Alberta, the total net reduction is 2,594,537 t CO_2e. Using an average cost of \$30 t CO_2e, the return on investment (\$1,377,523) from NOP for the project by Emissions Reduction Alberta is 4,435,276 t CO_2e x \$30 t = 97:1.

Table 6.1. Emissions reduction associated with the project

Experiment No.	Avg. Methane reduction (g/d)	No. head on study	No. head on methane reduction treatment	No days	Methane reduction (kg/study)	Total CO_2e (t/study)
1.1 (backgrounding)	38	15	9	56	19.2	0.48
1.1 (finishing)	58	15	9	56	29.3	0.73
1.2 (backgrounding)	139	240	120	100	1,668	41.7
1.2 (finishing)	130	240	120	120	1,872	46.8
2.1(backgrounding)	27	138	105	100	283	7.09
2.1(finishing)	0	138	105	100	0	0

2.2(backgrounding)	64	88	44	100	282	7.04
2.2 (finishing)	39	88	44	100	172	4.29
3.1 (dairy)	146	32	8	35	40.9	1.02
3.2 (dairy)	171	21	10	28	47.9	1.20
3.3 (dairy)	68	32	8	35	19.0	0.48
4.2 (backgrounding)	72	8	6	28	12.1	0.30
Total						111.13

Table 6.2. Number of cattle in backgrounding and finishing operations in Alberta (Source: CANSIM 2016)

Alberta	At Jan. 1	At July 1	Average	Total Assuming Two Turns/Year
Alberta				
Backgrounding	139,800	523,500	331,650	663,300
Finishing	878,200	960,600	919,400	1,838,800
Canada				
Backgrounding	326,400	1,018,700	672,550	1,345,100
Finishing	1,397,900	1,576,500	1,487,200	2,974,400

Overall Conclusions

Combining scientific expertise from teams in Canada and Australia was a highly effective method of simultaneously assessing a number of methane mitigation strategies for the beef and dairy sectors. The enteric methane reduction technologies investigated were: 1) diet composition (feeding wheat and added fat), and 2) use of feed additives (3-NOP and nitrate). The major conclusions from the research are:

Wheat as a dietary mitigant of enteric methane emissions

Feeding of wheat is not a reliable strategy for long-term mitigation of enteric methane emissions. Reductions in enteric methane due to wheat feeding may occur in the short-term, but the reductions may not be maintained over a long-term feeding period.

Fat as a dietary mitigant of enteric methane emissions

Feeding fat (lipids derived from plants) is a recognized methane mitigation approach in Alberta and Australia. Feeding fat was shown to reduce methane emissions of cows fed a diet containing wheat, but not when the diet contained corn indicating that the effectiveness of fat feeding may depend on the ingredients in the diet. Adding fat to a high forage diet to reduce methane production lowered fiber digestibility, and therefore methane mitigation using fat may not improve animal performance. When using fat as a methane mitigation strategy, it is critical to measure the total fat content of the diet to ensure that it does not exceed 6% of the diet DM.

Methane inhibitor 3-nitrooxypropanol

The methane inhibitor NOP lowered methane production in beef cattle fed high-forage and high-grain diets by 30 to 40%, with the response dose dependent. Optimum dose rate is 100 to 200 mg/kg DM, depending on diet composition (lower range for high grain diets, higher range for high forage diets). The reduction in methane is not affected by whether monensin ionophore or fat is included in the diets. The study suggests a link between sustained reduction in methane and improved feed conversion efficiency, which may encourage cattle producers to adopt this methane mitigation approach. 3-Nitrooxypropanol improved feed conversion efficiency by 3 to 5%, with no negative effects on carcass characteristics. We conclude that NOP is a potent methane inhibitor that can be added to conventional feedlot diets in Alberta. The information from this report will contribute to the dossier needed to register the product with Health Canada. Additionally, the results from this research form the basis for a large scale evaluation of NOP at a commercial feedlot in Alberta (Project #0160164. Demonstration of Reduced Enteric Methane Emissions in Growing/Finishing Beef Cattle Through Dietary Supplementation of 3 Nitrooxypropanol at a Commercial Scale in Alberta).That study will demonstrate the day-to-day practicalities of supplying NOP as a feed ingredient in commercial backgrounding and finishing beef-operations including the establishment of a Carbon Offset Protocol.

Nitrate

Encapsulating calcium nitrate slowed its release rate in the rumen and helped minimize possible toxicity effects. The effects of nitrate on methane abatement were variable in backgrounding and finishing diets (from 0 to 12% reduction) evaluated in longer-term studies. However, feeding encapsulated improved feed conversion efficiency by 10% in cattle fed high grain diets that did not include ionophores or antibiotics.

Scientific Achievements

Experiment 1.1 Dose Response to Methane Inhibitor (AAFC)

Vyas, D., S. M. McGinn, S. M. Duval, M. K. Kindermann and K. A. Beauchemin. 2016. Optimal dose of 3-nitrooxypropanol for decreasing enteric methane emissions from beef cattle fed high-forage and high-grain diets. Anim. Prod. Sci. published online 26 May 2016 http://dx.doi.org/10.1071/AN15705

Vyas, D., S. McGinn, S. Duval, M. Kindermann and K. Beauchemin. 2016. Determining the optimal dose of 3-nitrooxypropanol for reducing enteric methane emissions from beef cattle fed a high forage diet. Poster 73. 6th Greenhouse Gas and Animal Agriculture Conference, Feb 14-18, Melbourne, Australia

Experiment 1.2 Methane Inhibitor and Feedlot Cattle Performance (AAFC)

Vyas, D., A. W. Alemu, S. M. McGinn, S. M. Duval, M. Kindermann and K. A. Beauchemin. 2018. The combined effects of supplementing monensin and 3-nitrooxypropanol on methane emissions, growth rate, and feed conversion efficiency in beef cattle fed high forage and high grain diets. J. Anim. Sci. (submitted)

Vyas, D., A. W. Alemu, S. M. McGinn, S. M. Duval, M. Kindermann and K. A. Beauchemin. 2018. The combined effects of supplementing monensin and 3-nitrooxypropanol on methane emissions, growth rate, and feed conversion efficiency in beef cattle fed high forage and high grain diets. The 10th International Symposium on the Nutrition of Herbivores, Clermont-Ferrand, France, September 2 to 6 (abstract submitted).

Experiment 2.1 Alternative Hydrogen Sink (Nitrate) (AAFC)

Lee, C., R. C. Araujo, K. M. Koenig and K. A. Beauchemin. 2017. Effects of encapsulated nitrate on growth performance, nitrate toxicity, and enteric methane emissions in beef steers: backgrounding phase. J. Anim. Sci. 95:3700-3711 doi:10.2527/jas.2017.1460

Lee, C., R. C. Araujo, K. M. Koenig and K. A. Beauchemin. 2017. Effects of encapsulated nitrate on growth performance, carcass characteristics, nitrate residues in tissues, and enteric methane emissions in beef steers: finishing phase. J. Anim. Sci. 95:3712-3726. doi:10.2527/jas2017.1461

Lee, C., R. C. Araujo, K. M. Koenig and K. A. Beauchemin. 2017. Effects of encapsulated nitrate on growth performance, nitrate toxicity, and enteric methane emissions in feedlot beef steers: backgrounding phase. ASAS-CSAS Annual Meeting & Trade Show, Baltimore, Maryland, July 8-12 (Abstr. 568)

Lee, C., R. C. Araujo, K. M. Koenig and K. A. Beauchemin. 2017. *In situ* and *in vitro* evaluation of a slow release form of nitrate for ruminants: nitrate release rates, rumen nitrate metabolism and production of methane, hydrogen, and nitrous oxide. ASAS-CSAS Annual Meeting & Trade Show, Baltimore, Maryland, July 8-12 (Abstr. 579)

Lee, C., R. C. Araujo, K. M. Koenig and K. A. Beauchemin. 2017. Effects of encapsulated nitrate on growth performance, carcass characteristics, nitrate residues in tissues, and enteric methane emissions in feedlot beef steers: finishing phase. ASAS-CSAS Annual Meeting & Trade Show, Baltimore, Maryland, July 8-12 (Abstr. 580)

Experiment 2.2 Alternative Hydrogen Sink (Nitrate) (AAFC)

Alemu, A. W., A. Romero-Pérez, R. C. Araujo and K. Beauchemin. Effect of slow release nitrate and essential oil (Activo® Premium) on animal performance and methane emissions from feedlot cattle fed high forage diet. J. Anim. Sci. (in preparation)

Romero-Pérez, A., A. W. Alemu, R. C. Araujo and K. A. Beauchemin, Effect of slow release nitrate and essential oil (Activo® Premium) on animal performance and methane emissions from feedlot cattle fed high grain diet. J. Anim. Sci. (in preparation)

Experiment 3.1 Alternative Starch Source (DEDJTR and U of M)

Moate, P. J., S. R. O. Williams, J. L. Jacobs, M. C. Hannah, K. A. Beauchemin, R. J. Eckard and W. J. Wales. 2017. Wheat is more potent than corn or barley for dietary mitigation of enteric methane emissions from dairy cows. J. Dairy Sci. 100:1-15. https://doi.org/10.3168/jds.2016-12482

Experiment 3.2. Rate of Dietary Starch Degradation and Methane/Milk Production, Long-term Effects (DEDJTR and U of M)

Moate, P. J., J. L. Jacobs, M. C. Hannah, G. L. Morris, K. A. Beauchemin, P.S. Alvarez Hess, R. E. Eckard, Z. Liu, S. Rochfort, W. J. Wales and S. R. O. Williams. 2018. Adaptation responses in milk fat yield and methane emissions of dairy cows when wheat was included in their diet for 16 weeks. J. Dairy Sci. (submitted)

Experiment 3.3. Starch Sources and Oilseeds Effects on Methane/Milk Production (DEDJTR and U of M)

Alvarez Hess, P.S., S. R. O. Williams, J. L. Jacobs, M. C. Hannah, K. A. Beauchemin, R. E. Eckard, W. J. Wales, G. L. Morris and P. J. Moate. Effect of dietary fat supplementation on methane emissions from dairy cows fed wheat or corn. J. Dairy Sci (in preparation)

Alvarez Hess, P.S., P. J. Moate, S. R. O. Williams, J. L. Jacobs, M. C. Hannah, K. A. Beauchemin, R. E. Eckard and W. J. Wales. 2017. Effect of basal diet on the methane mitigation effect of dietary fats. 13th Chilean Buatrics Conference (XIII Congreso Chileno de Buiatria), Osorno, Chile. Nov. 23-25.

Experiment 4.1 *In vitro* Assessments of Additivity and Synergy of Mitigation Strategies (AAFC, DEDJTR, and U of M)

Alvarez Hess PS, S. R. O. Williams, J. L. Jacobs, P. J. Moate and R. J. Eckard. The comparative ruminal *in sacco* degradation and *in vitro* gas and methane production of barley, corn and wheat (in preparation)

Alvarez Hess, P. S., R. J. Eckard, P. J. Moate, S. R. O. Williams and J. L. Jacobs. 2016 Comparison of different cereal grains for their *in vitro* total gas and methane production. Australian Dairy Science Symposium. Sydney, Australia Nov 16-18.

Alvarez Hess, P. S., P. A. Giraldo, S. R. O. Williams, J. L. Jacobs, M. C. Hannah, P. J. Moate and R. J. Eckard. The use of total gas collection for measuring methane production in vented *in vitro* systems. Anim. Sci. J. (submitted)

Alvarez Hess, P. S., J. L. Jacobs, M. C. Hannah, P. J. Moate and R. J. Eckard. 2018. Comparison of five methods for the estimation of methane production from vented in vitro systems. J. Sci. Food Agric. (submitted).

Alvarez Hess, P. S., J. L. Jacobs, M. C. Hannah, P. J. Moate and R. J. Eckard. 2016. Analysis of two methods for the estimation of *in vitro* CH_4 production from vented bottles. 31st Conference of the Australian Society of Animal Production. Adelaide, Australia. July 5-7.

Alvarez, P.S., P. J. Moate, J. L. Jacobs, K. A. Beauchemin and R. E. Eckard. 2017. Effects of basal diet on *in vitro* methane inhibition by 3 nitrooxypropanol and nitrate. 13th Chilean Buatrics Conference (XIII Congreso Chileno de Buiatria), Osorno, Chile. Nov. 23-25.

Alvarez Hess P. S., P. J. Moate, S. R. O. Williams, J. L. Jacobs, K. A, Beauchemin, Z. Durmic, J. Vadhanabhuti and R. J. Eckard. The effect of donor animal diet and forage to grain proportion in the substrate on *in vitro* methane production from wheat and corn. J. Sci. Food Agric. (in preparation).

Alvarez Hess, P. S., P. J. Moate, S. R. O. Williams, J. L, Jacobs, K. A. Beauchemin, Z. Durmic, J. Vadhanabhuti and R. J. Eckard The effect of substrate on the *in vitro* methane mitigation effect of 3-nitrooxypropanol, nitrate or fat. J. Sci. Food Agric (in preparation).

Alvarez Hess, P. S., P. J. Moate, S. R. O. Williams, J. L. Jacobs, K. A. Beauchemin, Z. Durmic, J. Vadhanabhuti and R. J. Eckard. 2018. Effects of basal diet on in vitro methane inhibition by 3-nitrooxypropanol. 10th International Symposium on the Nutrition of Herbivores, Clermont-Ferrand, September 2-6.

Vyas, D. and K. A. Beauchemin. *In vitro* dose response of adding 3-nitrooxypropanol to cattle diets (in preparation)

Lee, C., R. C. Araujo, K. M. Koenig and K. A. Beauchemin. 2017a. *In situ* and *in vitro* evaluations of a slow release form of nitrate for ruminants: rumen nitrate metabolism

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Experiment 4.2 Additivity and Synergy of Mitigation Strategies (AAFC)

Smith, M. L. 2017. Assessing the potential of a novel feed additive and an unsaturated fat alone and in combination to lower methane emissions from cattle and reduce their contribution to climate change. PhD dissertation, University of Delaware.

Smith, M. L., S. M. Duval, M. Kindermann, K. A. Beauchemin and L. Kung Jr. 2017. Assessing the potential of 3-nitrooxypropanol and canola oil alone and in combination to lower methane emissions from cattle and reduce their contribution to climate change. American Dairy Science Association Annual Meeting, Pittsburgh, PA June 25-28. (Abstr. 256, Oral presentation)

Smith, M., D. Vyas, L. Kung, S. Duval, M. Kindermann, and K. Beauchemin. 2018. The combined effects of supplementing 3-nitrooxypropanol and lipids on emissions of methane and hydrogen, digestibility, and rumen fermentation in beef cattle fed a forage based diet. The 10th International Symposium on the Nutrition of Herbivores, Clermont-Ferrand, France, September 2-6 (abstract submitted)

Guyader, J., E. M. Ungerfeld and K. A. Beauchemin. 2017. *In vitro* modification of metabolic hydrogen production and consumption with methanogenesis inhibitors. American Dairy Science Association Annual Meeting, Pittsburgh, PA June 25-28. (Abstr. 206, Poster presentation)

Guyader, J., E. M. Ungerfeld and K. A. Beauchemin. 2017. Redirection of metabolic hydrogen by inhibiting methanogenesis in the rumen simulation technique (RUSITEC). Frontiers in Microbiol. 8:393. doi: 10.3389/fmicb.2017.00393

Experiment 5.1 Conduct modeling studies to determine the broader potential for GHG reductions using low methane diet technologies, as applicable to Alberta and Victorian dairy farms (U of M and AAFC)

Alemu, A., S. Little, X. Hao, D. Thompson, A. Iwaasa, V. Baron, K. Beauchemin, H. Janzen and R. Kröbel. 2017. Assessment of grazing management on farm greenhouse gas intensity of beef production systems in the Canadian Prairies using life cycle assessment. Agric. Syst. 158:1-13. doi.org/10.1016/j.agsy.2017.08.003

Alvarez Hess, P., S. Little, P. Moate, J. Jacobs, K. Beauchemin and R. Eckard. 2018. A partial life cycle assessment of the greenhouse gas mitigation potential of feeding 3-nitrooxypropanol and nitrate to cattle (in preparation)

Alvarez Hess, P. S., S. Little, P. J. Moate, J. L. Jacobs, K. A. Beauchemin and R. J. Eckard. 2018. A partial life cycle assessment of the greenhouse gas mitigation potential

of feeding 3-nitrooxypropanol in two Australian dairy farms. 10th International Symposium on the Nutrition of Herbivores, Clermont-Ferrand, September 2-6.

Guyader, J., S. Little, R. Kröbel, C. Benchaar and K. A. Beauchemin. 2017. Comparison of greenhouse gas emissions from corn- and barley-based dairy production systems in Eastern Canada. Agic. Syst. 152:38-46. doi.org/10.1016/j.agsy.2016.12.002

Guyader, J., S. Little, R. Kröbel, C. Benchaar and K. A. Beauchemin. 2017. Carbon footprint of dairy production systems in Québec: Barley versus corn silage. American Dairy Science Association Annual Meeting, Pittsburgh, PA June 25-28. (Abstr. 110, Poster presentation)

Little S. M., C. Benchaar, H. H. Janzen, R. Kröbel, E. J. McGeough, and K. A. Beauchemin. 2017. Demonstrating the effect of forage source on the carbon footprint of a Canadian dairy farm using whole-systems analysis and the Holos model: alfalfa silage vs. corn silage. Climate 5:87 doi:10.3390/cli5040087

Next Steps

The immediate next steps are to complete and publish scientific manuscripts resulting from the research. Publishing the findings in peer reviewed scientific manuscripts will confirm the scientific quality and validity of the work, and provide the underpinning evidence required to develop an offset method. A related follow-up project from the research is the ERA project #O160164, Demonstration of Reduced Enteric Methane Emissions in Growing/Finishing Beef Cattle Through Dietary Supplementation of 3-Nitrooxypropanol at a Commercial Scale in Alberta. The findings from the current research formed the basis for this new study. The new project is considered a Near Commercial Pilot Demonstration (TRL 7) Project. At this stage of development, 3-nitrooxypropanol is ready to demonstrate for a first time at a commercially-relevant scale. Viability will be assessed in an operational environment. Completion of ERA project #O160164 is key to demonstrating the viability of NOP in a commercial feedlot setting. The present study along with the new demonstration study will form the basis of a Carbon Offset Protocol and will contribute to the dossier needed for regulatory approval of the product in Canada.

Communications Plan

A feature article is in development by Agriculture and Agri-Food Canada, Public Affairs Branch. The article will be posted in English and French on the web site, http://www.agr.gc.ca/eng/news/scientific-achievements-in-agriculture/?id=

1379013177194. Agriculture and Agri-Food Canada will distribute the article to national media and national industry organizations in Canada, in March 2018. The Australian Department of Environment and Primary Industries will distribute the article to their media and industry contacts.

Once the article is released, reporters will have the opportunity to either place the article verbatim in their respective newspaper or will follow-up and request an interview with a scientist working on the project to write his/her own story.

A Media pitch (condensed version of the feature article) will also be developed for distribution to local media (Alberta and other regions of interest) following the release of the feature article. The media pitch will be for additional exposure and opportunity for media pick-up.

The feature article content will include the following project areas: 1) methane inhibitor (3-nitrooxypropanol), 2) alternative hydrogen sink (nitrate), 3) alternative starch source (wheat), and 4) modeling outcomes.

Each project within the article will outline the key findings, the importance of doing the research, the process used and next steps. The article includes quotes from the individual scientists working on the project area.

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