Project Title: Demonstration of Reduced Enteric Methane Emissions in Growing/Finishing Beef Cattle Through Dietary Supplementation of 3-Nitrooxypropanol at a Commercial Scale in Alberta

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Feedlot Health Management Services
ALBERTA CATTLE FEEDERS' ASSOCIATION

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Executive Summary

Methane emissions from the ruminant livestock sector represent a significant opportunity to mitigate climate change through innovative approaches. DSM has developed a solution to reduce enteric methane formation in ruminants by over 30% (Vyas, 2016). This investigational product, called 3-nitrooxypropanol and abbreviated 3-NOP, is an organic compound with the formula HOCH2CH2CH2ONO2. It is a breakthrough technology that inhibits methane formation in the rumen of livestock. The inclusion of this highly specific inhibitor in ruminant diets targets the active site of the enzyme methyl-coenzyme M reductase in rumen Archaea, which is responsible for the last step in the methane-forming pathway. At very low effective concentrations in dairy, beef cattle, and sheep diets, previous studies have shown that 3-NOP inhibits the production of methane from archaea while boosting feed efficiency and thus represents a breakthrough technology in suppressing enteric methane emissions (Vyas et al., 2018).

This project demonstrated the commercial viability of feeding 3-NOP in backgrounding and finishing operations in Alberta's beef cattle industry in a large-scale field trial. The project (1) evaluated the relative effects of feeding 3-NOP on methane reduction and feedlot performance, health and carcass quality outcomes in feedlot cattle fed typical North American finishing diets (corn and barley grain based diets) as well as in a high forage, backgrounding diet, (2) evaluated direct measurement techniques for methane emissions in a commercial beef feedlot where 3-NOP was used, and (3) demonstrated the use of 3-NOP in day-to-day practicalities of commercial feedlots.

The study demonstrated there were no significant adverse impact to animal health or performance as a result of including 3-NOP in the diets of corn and barley finished, or backgrounded cattle. In terms of performance, feed conversion rates were improved for cattle fed corn or backgrounding diets supplemented with 3-NOP. Measurements indicated that an average of 70% methane emission reduction was found when 3-NOP was provided in steam-flaked or dry-rolled barley finishing diets at 125 mg/kg of feed dry matter. In steam-flaked corn-based finishing diets, a reduction in the range of 31% - 80% at the 125 mg/kg dosage of 3-NOP was observed. Lastly, in backgrounding diets, increasing the dose of 3-NOP stepwise from 150 to 200 mg/kg decreased the yield of methane by 17%-26% compared with control animals. The study successfully demonstrated that 3-NOP can be included in commercial feedlot diets to reduce methane emissions, without negative effects on animal health and performance parameters and carcass characteristics.

The inclusion of 3-NOP in the diets of cattle has resulted in real, permanent and quantifiable reductions of methane emissions and has broad applications across Alberta's beef and dairy sector. While reductions associated with this project are relatively small in comparison to the footprint of the beef sector in Alberta (for reference: Alberta's enteric methane emissions estimate according to the latest National emissions inventory reporting submitted to the UNFCCC (2017) is 9.4 MT CO2e), the trial by itself generated CO_2e greenhouse gas (GHG) reductions of **1,473 tonnes**. The compound 3-NOP is positioned, upon commercialization, to generate significant reductions in the beef and dairy sector in Canada and globally. This project builds on an existing successful R&D ERA Project (EOI# B140002) entitled "Technologies for Reducing Greenhouse Gas Emissions and Providing Offset Options for the Beef and Dairy Industries" (Beauchemin et al., 2015).

Different methane measurement techniques have been successfully applied and tested throughout this trial to quantify the methane emission and the respective reduction by applying 3-NOP in the different diets. Mobile Greenfeed systems provided a very reliable set of emission data on an individual animal basis in a small pen setting. In addition, we evaluated the application of two micrometeorological

methods, a novel concentration ratio method and an inverse dispersion method, where both were used to measure methane (CH_4) emission reductions in cattle fed a typical backgrounding as well as a typical Canadian barley based as well as a typical US corn finishing diet. Both have proven successful techniques.

Finally, this trial successfully demonstrated the applicability of 3-NOP in its current formulation and helped to understand the day-to-day practicalities of its supply as a feed ingredient in commercial backgrounding and finishing beef-operations. Mixing operations, homogeneity of mixing in feed and the respective intake of 3-NOP by the animals was closely monitored. The application of 3-NOP into the feed via a micromachine proved a convenient and highly reproduceable process to be able to distribute exact small quantities on a day to day basis. No practical concerns were observed during the trial.

The consortium of Viresco Solutions, DSM Nutritional Products, Feedlot Health Management Services and Agriculture and Agri-food Canada would like to thank Emissions Reduction Alberta for its funding and guidance during this project, and Alberta Cattle Feeders Association for its sponsorship.

Introduction

This report meets the obligations set out in Schedule A of the Contribution Agreement dated December 11, 2017 between Emissions Reductions Alberta (ERA) and Viresco Solutions Inc. This report also meets the requirements identified as part of the requirements of the Non-Confidential Final Report. This report includes;

(i) a concise summary of what the project has achieved, including a description of the project and a description of greenhouse gas reductions obtained,
(ii) conclusions and recommendations for further research together with the status of performance of the Project in terms of process, output, outcomes and impact measures,
(iii) sufficient detail to permit readers to use or adapt the results for research and analysis purposes and to understand how the conclusions are arrived at, and
(iv) a communication plan detailing how the Recipients intend to publicly communicate results and outcomes concerning the Project.

Project Overview

This project was a large-scale field trial demonstration of the investigational compound 3-NOP in backgrounding and finishing operations in Alberta's beef cattle industry. The objectives of this study were threefold:

1. To evaluate the relative effects of feeding 3-NOP on methane emissions and feedlot performance, health and carcass quality outcomes in feedlot cattle fed typical North American finishing diets (corn and barley grain) as well as in a backgrounding setting.
2. To use direct measurement techniques (GreenFeed and several micrometeorological techniques) for methane emissions in a commercial beef feedlot where the 3-NOP compound is being used.
3. To demonstrate and understand the day-to-day practicalities of supplying 3-NOP as a feed ingredient in commercial backgrounding and finishing beef-operations.

These 3 objectives are reported on a diet by diet basis.

The study was conducted by a consortium of Viresco Solutions, DSM Nutritional Products, Feedlot Health Management Services (FHMS) and Agriculture and Agri-food Canada. Viresco Solutions Inc. is an environmental consulting firm that helps our clients strategically navigate the complex and evolving world of sustainability. DSM Nutritional Products is a Nutrition and Health company that developed the methane inhibitor 3-NOP. FHMS has established itself as a global leader in animal health and production consulting by providing data-driven approaches and recommendations based on the most current data available.

Project Goals

This study demonstrated that inclusion of 3-NOP in the diets of cattle in a commercial feedlot has resulted in real, permanent and quantifiable reductions of methane emissions. The outcomes presented in Table 5. below show the anticipated project success metrics outlined in the project proposal along with the project achievements. The project successfully completed all the success metrics including:

- Animal Performance – no negative impacts
- Animal Health – no negative impacts
- Methane Reduction – Reductions between 15%-80% (with all finishing diets well above 30%).
- Successful methane measurement of methane in a research setting at a commercial feedlot including publication of results in a peer reviewed journal.

The commercial launch of the product is related to the approval by the authorities (FDA-CVM and Health Canada VDD). The registration process has been started but it is anticipated that the commercial availability is still a few years out.

All studies were located at a commercial feedlot in western Canada. Both the baseline and the project were designed to represent standard industry feeding practices (plus 3-NOP in the project conditions) so that the effectiveness could be explored in an operational commercial setting at large scale. The basic design of the feedlot used in both the baseline and project was representative of standard designs used in western Canada. Open-air, dirt-floor pens were arranged side by side with central feed alleys and 20% porosity wood-fence windbreaks. Each facility had a hydraulic chute equipped with an individual animal scale, a chute- side computer with individual animal data collection and management software (*i*FHMS©, Feedlot Health, Okotoks, Alberta), and separation alleys to facilitate the return of animals to designated pens.

Rations were formulated to meet or exceed the nutritional requirements of feedlot cattle (Nutrient Requirements of Beef Cattle, National Research Council, 2000). Diets were prepared in a modern milling facility equipped with overhead bins. Animals were fed from truck-mounted mixers on load cells. Water was available ad libitum from automatic waterers. Animals were fed diets that contain an ionophore to control coccidiosis and bloat and an antimicrobial to control liver abscesses.

The experimental groups involved in the study (project condition) were:

1) <u>Barley Finishing Diet</u>– Comparison of control and 3-NOP animals that were fed standard *feedlot* <u>barley</u>-based diets, and 3-NOP at a level of 125 mg/kg dry matter (DM) basis.
2) <u>Corn Finishing Diet</u>– Comparison of control and 3-NOP animals that were fed standard *feedlot* <u>corn</u>-based diets and, 3-NOP at a level of 125 mg/kg dry matter (DM) basis.
3) <u>Forage Diet in Backgrounding</u> - Animals were fed standard forage feedlot *backgrounding* diets, and 3-NOP at a level of 200 mg/kg dry matter (DM) basis.

Project Outcomes & Results

The Following sections present the results from the three diets assessed during the project including animal performance and methane reductions.

The project consisted of 2 phases: Pre-trial and Scale-up (Full trial). The study was constructed as such, given that all beef trials to date were conducted at scientific institutes. A first step was therefore to transfer from a scientific to a commercial setting and understand whether any unexpected events or challenges would occur (pre-trial). This phase was successfully completed, and no practical challenges were observed (in line with objective 3 of the project. Post successful completion of the pre-trial, the project was scaled up to the full trial.

All results are described below.

Assessment of 3-NOP on Cattle Fed a Barley Finishing Diet

In summary, it was successfully demonstrated that the application of 3-NOP in a typical Canadian barley based finishing diet realized a substantial saving in enteric methane emissions from beef cattle (70%). The expectations compared to the original project and grant application document were fully met with no negative effect on animal performance and welfare.

As a result of the observed high methane reductions, which were above expectations, we believe this leaves room for further optimization of the daily dose of 3-NOP, considering i) methane reduction, ii) performance improvements and iii) economic benefits.

This part of the trial successfully demonstrated the applicability of 3-NOP in its current formulation and helped to understand the day-to-day practicalities of its supply as a feed ingredient in commercial finishing beef-operations. Mixing operations, homogeneity of mixing and homogeneity in feed and the respective intake of 3-NOP by the animals was closely monitored. The application of 3-NOP into the feed via a micromachine proved an easy, convenient and highly reproduceable process to be able to distribute exact small quantities on a day to day basis. No practical concerns were observed during this part of the trial.

Thereby confirming that 3-NOP can be effectively and easily implemented in commercial feedlot operations.

Pre-Trial

During the pre-trial, approximately 1450 animals were allocated to 3 pilot replicates and fed barley-based finishing diets for an average of 124 days (as typical in commercial operations in North America). Animals fed 3-NOP were supplemented at 125 mg/kg DM. Methane gas fluxes measured using micrometeorological techniques from control and 3-NOP fed animals during the feeding period indicated large reductions (70%) due to the feeding of 3-NOP. These reductions were larger than anticipated and it is believed that these impacts on methane might have depressed intakes. During the pre-trial, 3-NOP animals consumed less feed compared to those in the control group, animals fed 3-NOP also had a small reduction in daily weight gain. However, feed conversion favored cattle in the 3-NOP group. No observed changes in carcass grading or animal health were apparent between the two experimental groups.

Full Trial: Phase 2

Over the trial of 92 days (December 29, 2017 to April 1, 2018), there was, on average, a large methane emission reduction of 70% (±18%) due to the 3-NOP additive. This reduction was found by all micrometeorological methods.

With respect to ancillary production and feedlot performance outcomes, daily dry matter intake was significantly lower (relative decrease 3.75%; $P < 0.040$) in the 3-NOP group than in the CTRL group. On both a live weight basis and a carcass weight basis, there were no significant differences detected in ADG or the dry matter intake to gain ratio between the groups at the $P < 0.050$ level.

With respect to carcass characteristic outcomes, there were less YG Canada 3 carcasses (absolute decrease 6.93%; $P = 0.003$) in the 3-NOP group than in the CTRL group. There were no significant differences detected in any of the other YG or QG variables between the groups at the $P < 0.050$ level.

With respect to animal health outcomes, the lameness treatment rate was higher (absolute increase 2%; $P < 0.001$) in the 3-NOP group than in the CTRL group which mainly seems to be driven by the result of one pen. There were no significant differences detected in any of the other morbidity or mortality outcomes between the groups at the $P < 0.050$ level.

Full Trial: Phases 1 and 3

With respect to ancillary production and feedlot performance outcomes, daily dry matter intake was significantly lower (relative decrease 4.46%; $P < 0.001$) in the 3-NOP group than in the CTRL group. On both a live weight basis and a carcass weight basis, average daily gain (ADG) was significantly lower in the 3-NOP group compared to the CTRL group ($P < 0.050$). There were no significant differences detected in the dry matter intake to gain ratio on either a live weight basis or a carcass weight basis, between the groups at the $P < 0.050$ level. The lower daily dry matter intake in the 3-NOP group did not affect feed conversion, but resulted in lower ADG (relative decrease 3.95% on a carcass adjusted basis). Given the fixed days on trial experimental design used in this study (both experimental groups in a replicate slaughtered on the same day), lower ADG in the 3-NOP group resulted in significantly ($P < 0.050$) lower slaughter weight, weight gain, and carcass weight in the 3-NOP group. In commercial production systems, the inclusion rate of 3-NOP could be further optimized balancing between i) methane reduction, ii) performance improvements and iii) economic benefits.

With respect to carcass characteristic outcomes, there were no significant differences detected in any of the yield grade (YG) or quality grade (QG) variables between the groups at the $P < 0.050$ level.

The results of the current study generally agree with previous work by Vyas et al. (2016) showing a tendency for lower dry matter intake with the use of 3-NOP. However, improved feed conversion was not observed in the current study and this differs from the previous work by Vyas et al. (2018) and the results observed using corn-based finishing diets in a large-pen study conducted at the same time as the current study. This difference may be due to the shorter exposure to 3-NOP in the current study (< 120 days of 3-NOP exposure) compared to both the previous work by Vyas et al. (2016 and 2018) where animals were exposed to 3-NOP in both the backgrounding and finishing phases for more than 200 total days and the concurrent corn-based finishing diet large-pen study (~160 days of 3-NOP exposure). Additional large-scale research is required to understand the impact of the 3-NOP exposure period on feed conversion by starting animals from backgrounding through finishing and to determine the most cost-effective feeding regimes for 3-NOP in feedlot cattle fed barley-based finishing diets.

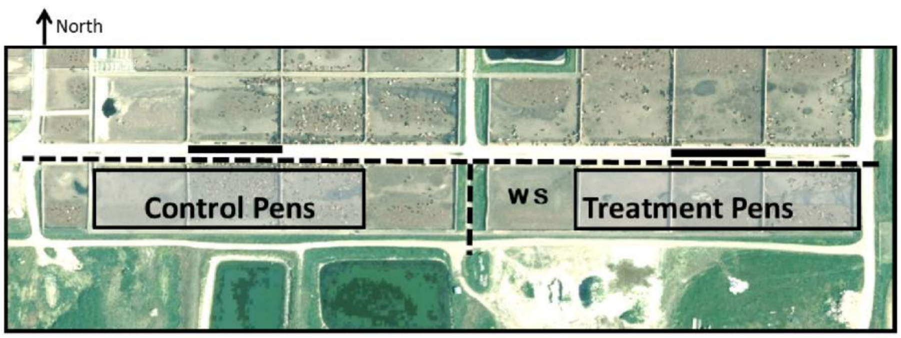
Methane Measurement Techniques

During the barley trial, we evaluated the application of three micrometeorological methods, a novel concentration ratio method using full plume or partial plume measurements and an inverse dispersion method, to measure methane emission reductions in cattle fed 3-NOP (125 mg/kg Dry Matter Intake) compared with cattle fed just the basal diet (steam-flaked barley grain based diet).

Over the trial of 92 days (December 29, 2017 to April 1, 2018), on average, a large methane emission reduction of 70% (±18%) was observed due to the supplementation of 3-NOP. This reduction was consistently observed by all micrometeorological methods.

Emissions were calculated from 1344 cattle that were located in six measurement pens (222 animals per pen). Three adjacent pens to the east and three to the west were designated as the treatment (TRT) and control (CTRL) blocks, respectively (Figure 1). Underlying the concentration ratio method was the measurement symmetry between the TRT and CTRL blocks in the feedlot, and the assumption of homogeneous wind conditions over the two blocks. Three open-path lasers (OPL) were set up in two configurations (A and B, Figure 1) to monitor methane concentrations upwind and downwind of the pen blocks. Meteorological equipment was setup in the feedlot to measure the wind and turbulence.

Emission reduction was also assessed by another method in a short study that used an open-path Fourier Transform Infrared sensor (OP-FTIR) to measure CH_4, CO_2, H_2O, N_2O and NH_3 gases. During this study there was a 62% reduction in methane emissions from TRT group compared to CTRL group. The CO_2 reduction was only 3%: expected given the number of respiring animals in the TRT and CTRL groups were similar. For NH_3, where the emission source is the quick conversion of urinary urea to NH_3, there was no difference in emissions between TRT and CTRL groups. Although pen manure is the same source for NH_3 and N_2O, the slower release of the N_2O resulted in a 17% emission reduction.



W-location of the weather station; S - location of the sonic anemometer.

Figure 1 Schematic diagram with the location of equipment and pens on the southern perimeter of the feedlot. The short and solid lines are path Configuration A and the dashed lines are path Configuration B

A scientific paper was published on the 3 different ways to derive emission reduction (McGinn et al., 2019). An average of 70% methane emission reduction was found. During this Milestone an abstract and accompanying poster was presented at the Greenhouse Gases and Animal Agriculture (GGAA 2019) conference in Brazil (held from August 4th to 10th 2019, Foz do Iguassu, Brazil).

Assessment of 3-NOP on Cattle Fed a Corn Finishing Diet

In summary, it was successfully demonstrated that the application of 3-NOP in a typical corn-finishing diet realized a substantial saving in enteric methane emissions from beef cattle (38%-80%). The expectations compared to the original project and grant application document were fully met with no negative effect on animal performance and welfare. Carcass characteristics and quality tended to be improved.

We believe there is further optimization potential in the step-up protocol and recommend to consider this in future studies.

This part of the trial successfully demonstrated the applicability of 3-NOP in its current formulation and helped to understand the day-to-day practicalities of its supply as a feed ingredient in commercial finishing beef-operations. Mixing operations, homogeneity of mixing and homogeneity in feed and the respective intake of 3-NOP by the animals was closely monitored. The application of 3-NOP into the feed via a micromachine proved an easy, convenient and highly reproduceable process to be able to distribute exact small quantities on a day to day basis. No practical concerns were observed during this part of the trial. Thereby confirming that 3-NOP can be effectively and easily implemented in commercial feedlot operations.

Pre-Trial

Understanding the effect of 3-NOP dosage in corn-based diets is important as all previous work conducted at Lethbridge has been studied in barley-based diets. Thus, 75 animals from each group were partitioned from the 1 large-pen replicate to facilitate a dose step-up study, while maintaining the integrity of the large pen replicate, with an equal number of animals from each group housed in smaller pens. Cattle in the dose step-up study were supplemented with increasing target doses of 3-NOP in the basal ration. Analytical results as well taking into account the dilution through additional feed intake from the Greenfeed system covered an inclusion range of 3-NOP of 0, 85.6, 107.6 and 124.5 mg/kg. The study duration was 112 days, comprised of 28 days of adaptation followed by three 28-d periods. The cattle were housed in small pens (25 head per pen) equipped with individual intake (GrowSafe feed bunks) and methane emission (GreenFeed) monitoring devices. Meanwhile, cattle in the large pen replicate were fed 125 mg/kg 3-NOP or CTRL. Methane reductions favored cattle in the 125 mg/kg group, compared to the other groups.

Compared with control (10.78 g/kg DM intake), 3-NOP decreased ($P < 0.001$) methane yield (i.e., g methane/kg DMI) by 45%, 81%, and 55%, for 85.6 , 107.6, and 124.5 mg 3-NOP/kg DM, respectively in the small pens.

For the corn pre-trial, methane was measured using the GreenFeed system and individual animal DMI were recorded using automated feed bunks. Compared to control (11.34 kg/day), DMI decreased ($P < 0.001$) by 6.4%, 0%, and 11.5% for 85.6, 107.6 and 124.5 mg 3-NOP/kg DM, respectively.

Data from the large pen cohorts fed 125 mg/kg 3-NOP revealed an improvement in feed conversion (3.6%) compared with CTRL. Thus, based on both the improvement in feed conversion and the methane reduction, the project team decided that all 9 remaining replicates were to be allocated at 125 mg/kg.

Overall, the pre-trial indicated that supplementation of corn-based finishing diets with 3-NOP, at an optimum dose of 125 mg/kg, is an effective methane mitigation strategy for commercial beef feedlots.

Full Trial

During this part of the study, methane was measured over 96 days and a substantial reduction in methane was measured. With the measurement techniques as described below an average emission reduction of 38% was measured on average, with a range from 31 to 45% (using the concentration ratio technique); the Inverse Dispersion technique resulted in a reduction of 67%.

With respect to ancillary production and feedlot performance outcomes, daily dry matter intake was significantly lower (relative decrease 5.97%; $P < 0.001$) in the 3-NOP group than in the CTRL group. On both a live weight basis and a carcass weight basis, average daily gain (ADG) was significantly lower and the dry matter intake to gain ratio was significantly improved in the 3-NOP group compared to the CTRL group ($P < 0.050$). The lower daily dry matter intake in the 3-NOP group resulted in improved feed conversion (relative improvement 2.12% on a carcass weight basis), but it also resulted in lower ADG (relative decrease 3.85% on a carcass adjusted basis). Given the fixed days on trial, the experimental design used in this study (both experimental groups in a replicate slaughtered on the same day), lower ADG in the 3-NOP group resulted in significantly ($P < 0.050$) lower slaughter weight, weight gain, and carcass weight in the 3-NOP group. In commercial production systems, the step-up rate of 3-NOP could be further optimized balancing between i) methane reduction, ii) performance improvements and iii) economic benefits.

With respect to carcass characteristic outcomes, there were significantly more yield grade (YG) Canada 1 carcasses (absolute increase 5.07%; $P = 0.032$) and significantly less YG Canada 3 carcasses (absolute decrease 5.07%; $P = 0.003$) in the 3-NOP group than in the CTRL group. With respect to quality grade (QG), there were significantly less QG Canada AAA carcasses (absolute decrease 5.13%; $P = 0.037$) and significantly more QG Canada AA carcasses (absolute increase 5.60%; $P = 0.018$) in the 3-NOP group than in the CTRL group. There were no significant differences in any of the other YG or QG variables detected between the groups at the $P < 0.050$ level. The carcasses in the 3-NOP group were lighter (13.2 lb) than in the CTRL group, which explains at least part of the difference in YG and QG between the experimental groups.

With respect to animal health outcomes, there were no significant differences detected in overall mortality, bovine respiratory disease mortality, other disease mortality, or any of the morbidity outcomes between the groups at the $P < 0.050$ level.,

Methane Measurement Techniques

Methane emissions in the full corn study were measured from cattle in six large pens (one block of 3 pens for control, one block of 3 pens for 3-NOP, as previously described for the barley-finishing study). The cattle were fed the corn finishing diet and emissions were measured using the same micrometeorological techniques (Concentration Ratio and Inversion Dispersion Modelling) that were used in the barley trial (Milestone 4; barley finishing diet). The arrangement of cattle and the equipment setup was similar to the aforementioned barley trial, with approximately 1500 cattle allocated into two blocks, where each block consisted of three adjacent pens (approximately 250 animals per pen).

Briefly stated, there were two instrumentation configurations considered (referred to as Method A and B). More specifically, Method A sampled a small portion of the dispersed methane plume from each group, using two short laser paths deployed along the centre portion of the Control and Treatment pen blocks. Method B sampled the entire plume being emitted from the two pen blocks, by using long laser paths. In each method the laser recorded the average methane concentration (ppm) each second, along a narrow beam between the laser and a retroreflector. It was assumed that measuring a subsample of the plume (Method A) would be sufficient to characterize emission differences from the two pen blocks, and was comparable to the full plume measurements (Method B). The use of two different measurement methods provided both a check on the accuracy of the emission measurements, but also a level of redundancy. For example, if one of the lasers in Method A or B became misaligned, a backup measurement was available.

In addition to comparing control and 3-NOP pens of cattle, we calculated whole-feedlot methane emission rate using two alternative techniques, to those reported herein. In technique 1, a fixed tower centrally located in the middle of the feedlot recorded the eddy covariance data (a micrometeorological method based on measuring the rapid vertical transfer of methane and wind speed). In technique 2, were used a drone to sample methane along pre-determined flight paths, and those concentrations were used with the Inverse Dispersion Modeling technique (WindTrax) to calculate emissions.

Methane Concentration Measurements

Both the Concentration Ratio and Inverse Dispersion Method rely on accurate laser concentration data in order to accurately estimate methane emissions. Following quality-control filtering of the concentration data, we observed that the background methane level of “fresh air” coming into the feedlot (Figure 2) was close to 2 ppm, as expected. There are a few outliers that we speculate are the result of stable atmospheric periods, which are associated with complex wind patterns that increase the risk of methane contamination from nearby animal pens. Figure 3 shows the methane concentrations downwind of the Control (upper graph) and Treatment (lower graph) pen blocks. The greater concentration above background is due to emissions from the cattle in the pens. The lesser concentration of methane above background concentration for the 3-NOP pens compared with Control pens, indicates a reduction in methane emission due to 3-NOP.

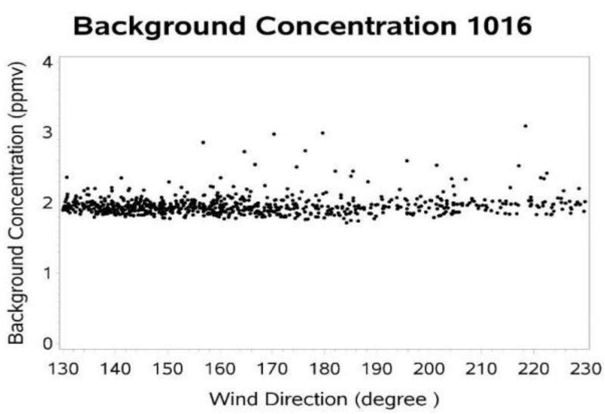


Figure 2: Variability in the background methane concentration as a function of wind direction. At the edges of the wind direction there was no undesirable spikes, which indicates no contamination of the concentration from adjacent pens of animals. The number 1016 indicates the laser number.

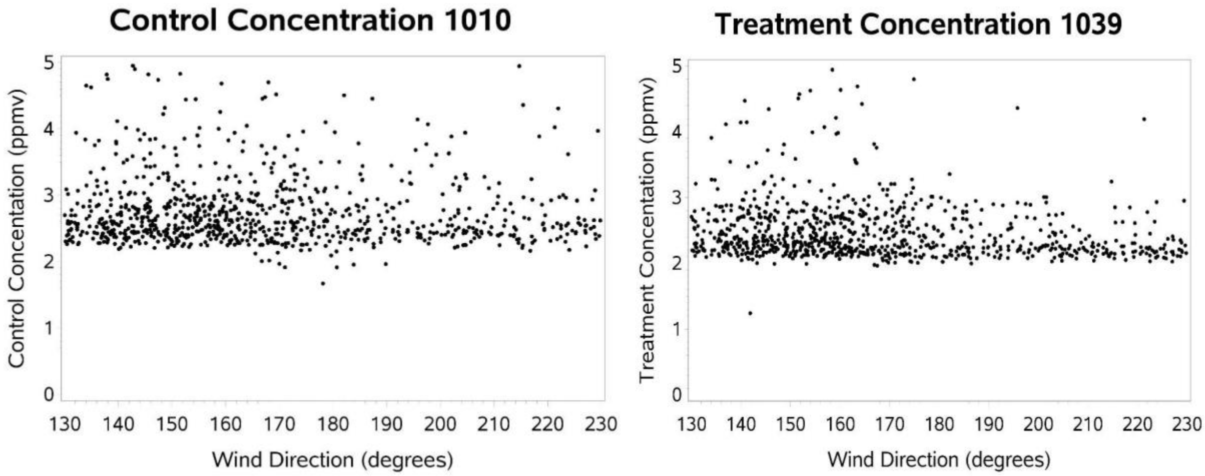


Figure 3: Methane concentrations downwind of the Control (left graph) and Treatment (right graph) pen block showing greater concentrations above background due to enhancement from the cattle. The numbers 1010 and 1039 indicate the laser number.

Emission Reduction using Concentration Ratio

The emission reduction due to 3-NOP was calculated using the novel Concentration Ratio (CR) technique and the Inverse Dispersion Method (IDM) technique. We used two independent measurement approaches (Method A and B), and normalized the emission ratios based on animal related differences between the treatment and control blocks, resulting in a simple ratio, a ratio normalized on animal numbers, and ratio normalized on feed intake. Thus, we have six different estimates of the emission reduction (**Error! Reference source not found.**). Over the 96 days of this study, the six different c alculations give an average 38% emission reduction, with a range from 31% to 45%. We interpret this range to reflect the uncertainty in our calculations – an uncertainty due to sensor uncertainty, differences in the number of animals being sampled by the two laser methods, imperfect sampling of the diel and long-term emission reduction patterns, and so forth.

Table 1: The average concentration ratio (treatment/control) values for methane using a subsample and full sample of the methane gas plume emitted from the Treatment and Control block pens where each sample type includes three ways of normalizing the concentration in Corn Finishing Diet

Sub sampling (Method A)	Number of 30 min Periods	Average over Period
Ratio Normalization		
1-ratio only	672	0.68
2-animal number	672	0.69
3-feed intake	672	0.64
Average		0.67
Full sampling (Method B)		
Ratio Normalization		
1-ratio only	754	0.55
2-animal number	753	0.59
3-feed intake	753	0.55
Average		0.56

Emission Reduction for Inverse Dispersion Method

As an alternative to the analysis of the emission reduction ratios (described above), we are in the process of using the Inverse Dispersion Method (WindTrax) to analyze the laser concentration data. The objective of this analysis is to calculate absolute methane emission rates (g animal^{-1} day^{-1}) for the control and treatment animals, from which we then calculate emission reduction. In principle this should give a better estimate of total emission reductions as compared to the simpler analysis of emission reduction ratios (i.e., this would account for the impact of any correlation between the diel trend in animal emissions, and a diel trend in the 3-NOP emission reductions.

Using laser data associated with the sub-sample configuration (Method 'A') in the WindTrax analysis, we calculated an average emission rate of 170 (Control) and 56 (Treatment) g animal^{-1} day^{-1}. This represents an emission reduction of 67% from the treatment animals. This reduction is greater than average of 38% calculated using the earlier Concentration Ratio analysis.

Implications of Results

There was a large difference between the emission reduction (72%) for the steam-flaked barley-based diet compared to 38% reduction for steam-flaked corn diet. The difference may be due to differences in the basal feed between the two trials, where the 3-NOP was more effective in reducing methane for more rapidly digestible feed (barley grain versus corn grain). The difference may also be due to weather, with the measurements for barley diet conducted in the winter compared to corn diet conducted in the spring. Weather conditions can influence feed intake, with greater dry matter intake in winter coinciding with greater methane emissions and the potential for mitigation.

In a Corn Finishing Diet investigated the impact of 3-NOP on methane emissions that are generated from a commercial beef cattle feedlot. The micrometeorological methods used were non-intrusive with regard to the daily operations of the feedlot. The measurements show that 3-NOP reduced methane emissions from cattle in open feedlots by 38%-44% at the 125 mg/kg dosage of 3-NOP (corn finishing diet). More information on the long-term emissions rates, and the dose and resulting emission reduction are needed to better evaluate the impact of 3-NOP as a greenhouse gas strategy in the red meat sector.

Assessment of 3-NOP on Cattle Fed a Backgrounding Diet

In summary, it was successfully demonstrated that the application of 3-NOP in a typical backgrounding diet realized a substantial saving in enteric methane emissions from beef cattle (up to 28%). Methane reduction response was slightly below expectations, feed efficiency was significantly improved by 2.5% while other animal health and performance parameters were not affected.

This part of the trial successfully demonstrated the applicability of 3-NOP in its current formulation and helped to understand the day-to-day practicalities of its supply as a feed ingredient in commercial finishing beef-operations. Mixing operations, homogeneity of mixing and homogeneity in feed and the respective intake of 3-NOP by the animals was closely monitored. The application of 3-NOP into the feed via a micromachine proved an easy, convenient and highly reproduceable process to be able to distribute exact small quantities on a day to day basis. No practical concerns were observed during this part of the trial. Thereby confirming that 3-NOP can be effectively and easily implemented in commercial feedlot operations.

Pre-Trial

For the large pen study, the experimental groups were considered homogenous ($P > 0.050$) with respect to the baseline variable average initial weight and hip height. There were no differences detected in animal health, ancillary production, or feedlot performance outcomes between the feeding programs at the $P < 0.050$ level. At the $P < 0.100$ level, dry matter intake tended ($P = 0.060$) to be less in the 3-NOP group, which also resulted in a tendency ($P = 0.073$) for improved feed conversion in the 3-NOP group. The tendencies for lower dry matter intake and improved feed conversion agree with previous work (Vyas et al., 2018). Differences in magnitude of response to 3-NOP in this study may be partially due to differences in cattle adaptation to 3-NOP under large pen commercial production conditions as compared to small pen research conditions.

Full Trial

The addition of 3-NOP to the backgrounding TMR resulted in decreases in methane yield (g/kg DMI) at all doses. The doses of 150, 175 and 200 mg/kg reduced methane yield by 17.5%, 25.6%, and 21.4%, respectively.

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<u>Methane Measurement Techniques</u>

In the full trial, methane measurements were conducted using the GreenFeed system (no micrometeorological methods were used). Fifty animals (328 ± 28 kg) were allocated to the methane portion of the study; 25 animals fed a control backgrounding total mixed ration (TMR, basal diet), and 25 animals fed a backgrounding TMR supplemented with increasing doses of 3-NOP over time. The animals were on the study for a total of 127 days.

At the initiation of the backgrounding study the animals were fed a TMR containing barley silage. However, due to a shortage in barley silage during the study the animals were adapted to corn silage, with 50:50 barley: corn silage fed from July 9 to 15, and corn silage fed from July 16 to September 6. Due to this change in diet an additional two weeks of measurement at 175 mg/kg 3-NOP was required. The resulting measurement periods were adaptation (May 3 – May 30, 2018, barley silage in diet), 150 mg/kg 3-NOP (May 31 – June 27, 2018, barley silage in diet), 175 mg/kg (June 28 – August 8, 2018; corn silage in diet), and 200 mg/kg (August 9 – September 6, 2018, corn silage in diet). The measurements that were collected during the adaptation from barley silage to corn silage were removed from the data that is summarised in this report.

In the adaptation and 150 mg/kg 3-NOP feeding periods the actual concentration of 3-NOP measured in the TMR (basal diet, not including pellet offered in the Greenfeed system) was slightly lower than the desired concentration (131 and 125 mg/kg, respectively). Contrarily, average 3-NOP concentration in the TMR during the 175 and 200 mg/kg measurement periods was greater than desired (193 and 227 mg/kg, respectively). The reason for this discrepancy is not clear but could be due the analysis, mixing of 3-NOP into the ration, or most likely, the difficulty of obtaining a representative sample.

Total dry matter intake (DMI) was measured daily by combining the intake of the TMR basal diet offered in the GrowSafe system and the intake of the pellet offered in the GreenFeed system. Increasing the target dose of 3-NOP in the TMR from 150 mg/kg to 175 and 200 mg/kg resulted in larger decreases in dry matter intake compared to the control animals (Table 2).

Table 2: Average dry matter intake (basal diet plus GreenFeed pellet) per animal CH4 measurements in Backgrounding Diet.

	Control	3-NOP	SEM	P-value
Phase 1: Low (150 mg/kg DM)	10.03	9.74	0.248	0.255
Phase 2: Medium (175 mg/kg DM)	11.85	11.27	0.265	0.036
Phase 3: High (200 mg/kg DM)	12.37	11.72	0.371	0.089

Methane measurements were measured using a GreenFeed system. Because there was only one GreenFeed system, animals were rotated between pens weekly, resulting in bi-weekly measurements of GHG emissions for each treatment. Two bi-weekly measurements from each treatment were averaged to determine methane emissions during the measurement periods. The animals were allowed to adapt to the GreenFeed system for four weeks (May 3 – 30, 2018), during which animals on the 3-NOP treatment were also adapted to 150 mg/kg 3-NOP in the TMR.

Table 3: Emissions of enteric methane (CH4) and hydrogen (H2) production for beef steers fed a forage-based diet supplemented without (control) or with increasing doses of 3-nitrooxypropanol (3-NOP) in Barley Backgrounding Study

Item	Control	3-NOP	SEM	P-value
Phase 1: Low dose (150 mg/kg basal diet DM)[a]				
CH_4 production				
g/day	224.8	185.5	8.04	0.007
g/kg DM intake	22.9	18.9	1.12	0.023
% Gross energy intake	5.99	4.85	0.287	0.004
CO_2 production				
g/day	7179	7482	145.2	0.093
g/kg DM intake	730.0	765.1	17.04	0.114
H_2 production				
g/day	0.37	1.29	0.091	< 0.001
g/kg DM intake	0.04	0.14	0.007	< 0.001
Phase 2: Medium dose (175 mg/kg basal diet DM)[a]				
CH_4 production				
g/day	259.7	184.8	6.37	< 0.001
g/kg DM intake	22.3	16.6	0.66	< 0.001
% Gross energy intake	5.83	4.42	0.176	< 0.001
CO_2 production				
g/day	7833	7947	183.4	0.573
g/kg DM intake	675.1	711.8	10.88	0.001
H_2 production				
g/day	0.41	1.64	0.076	< 0.001
g/kg DM intake	0.03	0.14	0.006	< 0.001
Phase 3: High dose (200 mg/kg basal diet DM)[a]				
CH_4 production				
g/day	275.7	198.4	8.45	0.001
g/kg DM intake	22.4	17.6	0.65	< 0.001
% Gross energy intake	5.88	4.79	0.175	< 0.001
CO_2 production				
g/day	8312	8412	169.9	0.600
g/kg DM intake	675.9	742.7	16.27	< 0.001
H_2 production				
g/day	0.39	1.55	0.058	< 0.001
g/kg DM intake	0.03	0.13	0.005	< 0.001
Overall (Phases 1 to 3)				
CH_4 production				
g/day	255.3	189.6	6.89	0.001
g/kg DM intake	22.5	17.6	0.64	0.001
% Gross energy intake	5.89	4.66	0.173	0.001
CO_2 production				
g/day	7814	7967	140.5	0.348
g/kg DM intake	690.5	738.1	8.453	< 0.001
H_2 production				
g/day	0.39	1.50	0.054	< 0.001
g/kg DM intake	0.03	0.14	0.005	< 0.001

[a] 3-Nitrooxypropanol was added only to the basal diet.

The addition of 3-NOP to the backgrounding diet resulted in decreases in daily emissions of methane at all doses (Table 3). Administering 3-NOP in doses of 150, 175 and 200 mg/kg led to significant CH_4 yield (g/kg DMI) reductions of approximately 17.5%, 25.6%, and 21.4%, and 21.8% overall.

Achievements

GHG Benefits Arising From this Project

Overall, the project reduced emissions by 1473 CO_2e MT (Table 4) which was above the anticipated 1276 CO_2e MT as indicated in the project application.

Offsets were quantified for 4 areas of the project: 1) cattle finished with barley-based diets at 125 mg/kg (7 replicates, Phases 1 and 3), 2) cattle finished with barley-based diets at an increasing dosage from 75 mg/kg to 125 mg/kg (3 replicates, Phase 2), cattle finished with corn based diets at 125 mg/kg (10 replicates), cattle backgrounded on a high-forage diet (8 replicates). Greatest amounts of offsets per animal were generated in the barley diets due to model sensitivity to an 70% decrease in the methane emission factor. Negative offset tonnage for N_2O in the barley finishing portions was observed and reflects the impact of poorer ADG and lack of response on feed conversion and associated greater manure output due to greater days to be finished per unit of carcass weight produced. Cattle fed corn had lower offsets per animal fed 3-NOP than barley due to a smaller reduction in methane, but this was partially balanced by an improvement in feed conversion and a smaller impact on ADG. Backgrounding resulted in the lower amount of offsets per head, due to the smallest impact on methane reduction and limited change in performance. The amount of offset generation per animal due to the feeding of 3-NOP in this study was approximately 3-4x what is normally generated in other GHG offset projects previously registered with the Alberta Emissions Offset Registry by Trimble and FHMS.

Table 4: Greenhouse gas quantification from the project

Quantification of Expected Annual GHG Benefits

Study	Barley Finishing Phases 1 & 3	Barley Finishing Phase 2	Corn Finishing	Backgrounding	Total Offsets Generated by the project
Emission Factor, % GEI in CTRL	4.00	4.00	4.00	6.50	
Emission Factor, % GEI for 3-NOP	1.20	1.20	2.38	5.51	
N_2O Offsets, CO_2e MT	-2	-9	26	10	**25**
CH_4 Offsets, CO_2e MT	515	233	542	159	**1448**
Total Offsets, CO_2e MT	513	224	567	169	**1473**
Offsets per animal fed 3-NOP, CO_2e MT	0.3065	0.2867	0.2309	0.0837	

Quantification was completed using project data and a proprietary GHG calculator developed by Trimble.[1] This calculator is based on the Reducing Greenhouse Gas Emissions from Fed Cattle Protocol developed for the Alberta Offset System and uses animal performance and diet information to estimate emissions. This protocol quantifies enteric emissions as well as methane and nitrous oxide (N_2O) emissions generated from manure storage and handling within the operations. The protocol quantifies reductions through a scientifically valid methodology and emission factors in the finishing stages of beef cattle at feedlots in Alberta. Unique to this project was the available data on emission reductions (from methane), which normally are unchanged, except for feeding diets with added fat. The average reported reduction from each portion of the project was incorporated into the model to determine what the estimated offset tonnage would be.

Immediate and Potential Future non-GHG Benefits

Increasing feed use efficiency in beef cattle means more efficient use of feed energy and less production of methane due to enteric fermentation. This means less feed needs to be grown, and less energy is consumed in transportation, which both benefit the GHG footprint. Further, generally less manure is excreted, resulting in reduced manure emissions. Both of these mechanisms result in emission reductions per kilogram of beef produced, resulting in more efficient production of beef and fewer greenhouse gas emissions. Although enteric and manure-based emissions were produced by cattle during the project condition, just as they were during the baseline condition, the incorporation of 3-NOP into the diet will lower the volume of greenhouse gases emitted per kilogram of live weight gain during backgrounding and/or per kilogram of hot carcass weight gain in the feedlot.

Use of 3-NOP has been shown to increase feed efficiency (Vyas et al. 2018). As a result, there are feed savings that the producer may realize.

Use of 3-NOP will not only reduce enteric based and other greenhouse gas emissions but potentially generate emission offsets eligible in Alberta's Offset System. Alberta's Fed Cattle Protocol could be modified to allow the inclusion of feed additives that reduce enteric fermentation. This could generate potential revenue for the producer and offset the cost of feeding the compound.

Additionally, the use of 3-NOP will reduce enteric based and other greenhouse gas associated with beef production. There may be a consumer demand for more sustainably produced beef thereby creating branding opportunities for the beef industry. Also globally, we increasingly see beef producers targeting low/zero emissions beef projects (e.g. Brazil has a carbon zero and a low carbon beef label, Australian beef is targeting being net zero by 2030, and already today some beef brands are selling a carbon neutral product). On the export markets it will be important that Canada can also offer a product with a low footprint. Until this is a broadly adopted practice, there will be marketing/product differentiation options.

[1] https://agriculture.trimble.com/product/carbon-credits/

Publications

The current project will lead to a number of publications, some of which have already appeared, and others for which manuscripts are being drafted.

Refereed Scientific Papers

Wilson J.D., T.K. Flesch, S.M. McGinn. 2018. The Wind Field in a Cattle Feedlot: Measurements and Simulations. Agricultural and Forest Meteorology 256–257: 84–92 https://doi.org/10.1016/j.agrformet.2018.02.024

McGinn, S. M., T. K. Flesch, K. A. Beauchemin, and M. Kindermann. 2019. Micrometeorological Methods for Measuring Methane Emission Reduction at Beef Cattle Feedlots: Evaluation of 3-Nitrooxypropanol Feed Additive. J. Environ. Qual. doi:10.2134/jeq2018.11.0412

Alemu, A. W., A. Shreck, C. W. Booker, S. M. McGinn, M. Kindermann, and K. A. Beauchemin. 2020. Use of 3-nitrooxypropanol in corn-based finishing diets to decrease enteric methane emissions from cattle in a commercial feedlot. J. Anim. Sci. (in review)

Alemu, A. W., L. Pekrul, A. L. Shreck, C. W. Booker, S. M. McGinn, M. Kindermann and K. A. Beauchemin. 2020. Feeding 3-Nitrooxypropanol to backgrounding cattle in a commercial feedlot decreased enteric methane production: Implications for sustainability of beef cattle production. (to be submitted)

Posters and Conference Proceedings

Alemu A.W., C.W. Booker, A. Shreck, S. M. McGinn, S. N. Williams, M. Kindermann and K. A. Beauchemin. 2019. Sustained Decrease in Enteric Methane Production by Beef Cattle in a Commercial Feedlot Fed a Forage-based Backgrounding Diet Supplemented with Increasing Doses of 3-Nitrooxypropanol. 7th Greenhouse Gas and Animal Agriculture Conference, Foz do Igaussu Falls, Brazil, August 4-10 (Abstract and oral presentation).

McGinn, S.M., T.K. Flesch, K.A. Beauchemin, A. Shreck and M. Kindermann. 2019. Commercial-Scale Evaluation of fed 3-NOP in Reducing methane from Cattle Feedlots Using Micrometeorological Methods. 7th Greenhouse Gas and Animal Agriculture Conference, Foz do Igaussu Falls, Brazil, August 4-10 (Abstract and Poster Presentation).

Alemu, A. W., A. Shreck, C. W. Booker, S. M. McGinn, M. Kindermann, and K. A. Beauchemin. 2020. 3-Nitrooxypropanol decreased enteric methane emissions from feedlot cattle fed corn-based diets. European Association of Animal Production Annual Meeting 2020, Porto, Portugal (Abstract and oral presentation)

Next Steps

Currently, DSM is in the process of applying for product approval in the dairy sector in North America (the United States and Canada), and in several markets globally (EU, LatAm, China, Australia and New Zealand). Where possible this authorization will be sought for all ruminants. In a number of cases, depending on local legislation, a dairy and a beef authorization will be sought consecutively, as is the case in North America.

Furthermore, this project has highlighted a strong performance on methane reduction, without negative effects on the animal, and without facing any practical issues when transferring to a commercial scale operation. It did highlight the need for some further research on dose and step-up protocol under various diet combinations.

The outcomes of this project will be presented in scientific publications that will be prepared over the next 12 months and submitted to peer-reviewed scientific journals. Additionally the project will be reported in line with ERA requirements. Provided that the product is still under review by regulatory authorities (FDA-CVM and VDD) any other communication will occur post market authorization

Literature Cited

Beauchemin, K.A., T.A. McAllister, and S.M. McGinn 2009. Dietary mitigation of enteric methane from cattle. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources. 4 (No. 035), 18 pp.

Beauchemin, K.A., E.M. Ungerfeld, R. Eckard, and M. Wang. 2019. REVIEW: Fifty years of research on rumen methanogenesis - lessons learned and future challenges for mitigation. Animal (in press)

Environment and Climate Change Canada. 2019. National inventory report: greenhouse gas sources and sinks in Canada. http://publications.gc.ca/site/eng/9.816345/publication.html

Dijkstra, J., A. Bannink, J. France, E. Kebreab, and S. van Gastelen. 2018. Short communication: Antimethanogenic effects of 3-nitrooxypropanol depend on supplementation dose, dietary fiber content, and cattle type. Journal of Dairy Science 101, 9041–9047. doi:10.3168/jds.2018-14456

Duin, E.C., T. Wagner, S. Shima, D. Prakash, B. Cronin, D.R. Yáñez-Ruiz, S. Duval, R. Rümbeli, R.T. Stemmler, R.K. Thauer, and M. Kindermann. 2016. Mode of action uncovered for the specific reduction of methane emissions from ruminants by the small molecule 3-nitrooxypropanol. Proceedings of the National Academy of Sciences 113, 6172–6177. doi:10.1073/pnas.1600298113

Gerber, P.J., H. Steinfeld, B. Henderson, A. Mottet, C. Opio, J. Dijkman, A. Falcucci, and G. Tempio 2013. Tackling climate change through livestock – A global assessment of emissions and mitigation opportunities. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy. http://www.fao.org/3/a-i3437e.pdf.

Hristov, A.N., J. Oh, C. Lee, R. Meinen, F. Montes, T. Ott, J. Firkins, A. Rotz, C. Dell, B. Adesogan, W. Yang, J. Tricarico, E. Kebreab, G. Waghorn, J. Dijkstra, and S. Oosting 2013. Mitigation of greenhouse gas emissions in livestock production – A review of technical options for non-CO2 emissions. In FAO Animal Production and Health Paper No. 177 (ed. PJ Gerber, B Henderson and HPS Makkar), pp 1-226. FAO, Rome, Italy. www.fao.org/3/i3288e/i3288e.pdf.

Hristov, A.N., J. Oh, F. Giallongo, T.W. Frederick, M.T. Harper, H.L. Weeks, A.F. Branco, P.J. Moate, M.H. Deighton, R.O. Williams, M. Kindermann, and S. Duval. 2015. An inhibitor persistently decreased enteric methane emission from dairy cows with no negative effect on milk production. Proceedings of the National Academy of Sciences of the United States 112, 10663–10668. doi:10.1073/pnas.1504124112

Intergovernmental Panel on Climate Change (IPCC). 2014. AR5 Synthesis Report: Climate Change 2014, https://www.ipcc.ch/report/ar5/syr/synthesis-report/

Muller, R.A., and E.A. Muller 2017. Fugitive methane and the role of atmospheric half-life. Geoinformatics & Geostatistics: An Overview 5: 3. doi:10.4172/2327-4581.1000162

Romero-Perez, A., E.K. Okine, S.M. McGinn, L.L. Guan, M. Oba, S.M. Duval and K.A. Beauchemin. 2014. The potential of 3-nitrooxypropanol to lower enteric methane emissions from beef cattle. Journal of Animal Science 92, 4682-4693. doi:10.2527/jas.2014-7573

Romero-Perez, A., E.K. Okine, S.M. McGinn, L.L. Guan, M. Oba, S.M. Duval, M. Kindermann, and K.A. Beauchemin. 2015. Sustained reduction in methane production from long-term supplementation of 3-nitrooxypropanol to a beef cattle diet. Journal of Animal Science 93, 1780-1791. doi:10.2527/jas2014-8726

Thiel, A., R. Rümbeli, P. Mair, H. Yeman, and P. Beilstein. 2019a. 3-NOP: ADME studies in rats and ruminating animals. Feed and Chemical Toxicology 125, 528-539.

Thiel, A., A.C.M. Schoenmakers, I.A.J. Verbaan, E. Chenal, S. Etheve and P. Beilstein. 2019b. 3-NOP: mutagenicity and genotoxicity assessment. Feed and Chemical Toxicology 123, 566-573. doi:/10.1016/j.fct.2018.11.010

Ungerfeld, E.M. and R.A. Kohn 2006. The role of thermodynamics in control of ruminal fermentation. Pages 55–85 in Ruminant Physiology. Digestion, Metabolism and Impact of Nutrition on Gene Expression, Immunology and Stress. Sejrsen, K., Hvelpund, T. and Nielsen, M.O., ed. Wageningen Academic Publishers, Wageningen, The Netherlands.

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. Animal Production Science 58, 1049-1055 http://dx.doi.org/10.1071/AN15705

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 diet. J. Anim. Sci. 2018.96:2923–2938. doi: 10.1093/jas/sky174.